Oral Abstract Session: Basic Science: Influenza - Adaptive immune response to infection

Irina Alymova - AOXI0008

Low glycan occupancy of N-linked glycosylation sites on hemagglutinin is sufficient to divert adaptive immune responses to A(H3N2) influenza virus

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Background

Hemagglutinin (HA), a major surface antigen of influenza A viruses (IAV), is heavily N-glycosylated in recent A(H3N2) isolates. The presence of glycans can alter host antibody responses by shielding or modifying immunodominant and immunosubdominant antigenic sites on the globular head and stem region of HA, as well as by interacting with the innate immune response. While nine of the 12 N-glycosylation sites of 2013 A(H3N2) IAV HA have high site occupancy by glycans (from 70% to 100%), the occupancy of sites at residues N45, N144, and N165 is only 32.6%, 22.5%, and 21.3%, respectively. Adding or removing existing HA N-glycosylated sites has been shown to alter antibody-mediated neutralization of influenza viruses, but there is no information on how N-glycosylation site occupancy affects antibody titers and virus neutralization. Here, we investigate the effects of the low N-glycosylation site occupancy at residues N45 and N144 of HA of 2013 A(H3N2) IAV on generation of adaptive immune responses against the virus.

Method

We constructed reverse-genetics IAV lacking N-glycosylation sites at residues N45, N144, or N45/N144 of 2013 A(H3N2) HA. The ability of these concentrated purified BPL inactivated glycosylation mutant viruses to generate adaptive immune responses was assessed by immunization of naïve mice with two intramuscular injections of 10 µg of HA, followed by measurement of serum antibody titers by hemagglutination inhibition assays at post-immunization (p.i.) days 35, 43, 49, and 67, and ELISA tests at p.i. days 35 and 43. Antibody responses generated by glycosylation mutant viruses were compared to those of the wild-type virus (containing glycosylation at both N45 and N144).

Result

In ELISA, the reverse genetics glycosylation mutant viruses induced significantly higher homologous and heterologous serum antibody responses than did the parent wild-type 2013 A(H3N2) IAV. Similarly, titers of the antibodies that are correlated with protection against influenza disease, measured by hemagglutination inhibition assays, were 4 to 8 times higher in mice immunized with glycosylation mutant viruses than in those immunized with fully glycosylated virus, with the strain lacking glycans at residue N45 inducing the highest response in hemagglutination inhibition tests.

Conclusion

Our data suggest that even low level of site occupancy by N-linked glycosylation is sufficient to alter the interactions of influenza virus with the immune system and leads to reduced overall and protective adaptive immune responses.

Oral Abstract Session: Basic Science: Influenza - Adaptive immune response to infection

Sophie Valkenburg - AOXI0024

Influenza ADCC-antibody responses in seasonal vaccination and pandemic infection of children as a correlate of protection

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Background

Influenza viruses are extremely diverse and vaccine mediated protection by current whole virion inactivated vaccines elicit strain specific neutralising antibodies as the main protective function. Seasonal influenza vaccination in children in 2009 resulted in 47% vaccine effectiveness against H1N1pdm influenza virus. Some classes of antibodies can cross-react between seasonal, pandemic and avian influenza viruses and may have a protective role against limiting acquisition or severity of influenza virus infection. Antibodies can mediate effector functions such as antibody dependent cellular cytotoxicity (ADCC), directing immune cells to kill infected cells, or engulf them by phagocytosis (ADCP), which are respectively mediated by FcgRIIIA and FcgRIIA engagement on B cells, Natural Killed (NK) cells and Macrophages.

Method

In this study, we utilised a large biobank of immune serum from a randomised control trial of seasonal influenza vaccination in children at the onset of the 2009 H1N1 pandemic, and tracked over the subsequent 5 years. To quantify influenza-specific antibodies before and after vaccination, and pandemic infection, we used a systems serology approach using bead multiplex approach which coupled FcR dimer proteins, a diverse HA proteins including HA-stem constructs, and antibody subclasses (IgG1/2/3) and isotypes (IgG/A1).

Result

We found that vaccination increased HA-specific antibodies, in terms of magnitude and FcR effector functions, even to the pandemic virus H1/2009 HA and NA proteins, which declined within one year post vaccination and then remained stable as per other viral proteins. Total H1/2009 HA IgG, and IgG2, was higher in children who were not vaccinated and uninfected compared to unvaccinated infected children, suggesting a protective role of cross-reactive HA antibodies. However, whilst vaccination increased IgG1 response against vaccine and related proteins, it did not impact infection status, possibly masking baseline protective effects seen in unvaccinated uninfected children.

Conclusion

The antibody effector response is boosted by vaccination however vaccine breakthrough infection occurs despite these increases, therefore other immune parameters may account for pandemic virus protection.

Oral Abstract Session: Basic Science: Influenza - Adaptive immune response to infection

Katina Hulme - AOXI0277

Increasing HbA1c levels reduces the CD8 T cell response to influenza virus in a TCR-dependent manner in individuals with diabetes mellitus

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Background

Globally, diabetes mellitus is on the rise. Diabetes is a known susceptibility factor for severe influenza virus infections. However, the mechanisms by which diabetes increases the severity of an influenza virus infection are yet to be fully defined. Diabetes mellitus is hallmarked by high glucose concentrations in the blood, known as hyperglycaemia. An indication of glycaemic control over the preceding three-month period can be obtained by measuring the percentage of glycosylated haemoglobin (HbA1c). We hypothesised that high glucose levels directly affect the functionality of CD8+ T cells, which play a key role eliminating virus infected cells and decrease influenza severity.

Method

To study the effect of hyperglycaemia on CD8+ T cell function, we collected peripheral blood mononuclear cells (PBMCs) from donors with and without diabetes mellitus (both type 1 and 2). Constant glucose measurements over a two-week period were obtained with patient consent. PBMCs were stimulated ex vivo with either i) A/H3N2 (HKx31), ii) an influenza virus peptide pool, iii) CD3/CD28-coated beads, or iv) PMA/ionomycin. After stimulation, cells were assessed for functionality (as defined by expression of IFN-γ, TNF-α, MIP-1β and CD107a) using flow cytometry.

Result

Increasing HbA1c levels correlated with a reduction in TNF-α production by CD8+ T cells in response to both T cell receptor (TCR)-specific stimulation (H3N2 infection and influenza virus peptide pool) and non-specific TCR stimulation (CD3/CD28-coated beads). This was not associated with any changes in the phenotypic composition of the CD8+ T cells. These results were exacerbated in PBMCs from individuals with diabetes and wide fluctuations in blood glucose levels, known as glycaemic variability. Non-TCR-specific stimulation (PMA/ionomycin) was able to overcome the hyperglycaemia-dependent deficiency, suggesting hyperglycaemia has a direct effect on the ability of the TCR complex to initiate the signalling cascade.

Conclusion

This study demonstrated for the first time that hyperglycaemia may increase influenza severity by dampening the CD8+ T cell response. We hypothesize that this could in turn impair the development of adaptive immunity to influenza viruses by reducing CD8+ T cell function. Given the hyperglycaemia-driven hyporesponsiveness is specific to the TCR complex, these results could hold implications for other viral infections in individuals with diabetes. By identifying the role of hyperglycaemia and glycaemic variability in anti-viral immunity, the results explain one piece of the puzzle as to why individuals with diabetes may be more susceptible to severe influenza.

Oral Abstract Session: Basic Science: Influenza - Adaptive immune response to infection

So Young Chang - AOXI0268

Influenza virus infection induces high levels of CD52 expression on effector CD8 T cells in the infected lung

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Background

CD8+ T cells provide broadly cross-reactive immunity against distinct influenza viruses, however key markers associated with optimal effector function of influenza-specific CD8+ T cells remain unclear. Using single cell RNAseq, we have identified CD52 as the top marker of effector tetramer-specific CD8+ T cells during human influenza virus infection. We investigated the novel role of CD52 expression on influenza-specific CD8+ T cells in mice to understand mechanisms underpinning generation of optimal effector CD8+ T cells.

Method

C57BL/6 (B6) mice were infected with influenza A virus (IAV) A/HKx31 (H3N2) to define kinetics of CD52 expression on endogenous CD4+/CD8+ and influenza-tetramer+CD8+ T cells in vivo. Naïve OT-I cells were adoptively transferred into B6 mice 1 day prior to infection with 104 PFU A/HKx31-OVA intranasally. On days 8-10 post-infection, different tissues were analysed for CD52 expression, together with other activation markers by flow cytometry. To assess the role of CD52 on the functional and memory profiles of OT-I cells, CD52hi and CD52lo OT-I cells were sorted from different tissues during the effector phase for bulk RNAseq. In addition, CRISPR/Cas9 gene knock-out and co-transfer assays were used to investigate the role of CD52+ and CD52- OT-I cells during the effector phase following X31-OVA infection. Our findings were also verified using Semliki Forest virus (SFV) and LCMV mouse models of infection.

Result

From our human single-cell RNAseq, we discovered that Cd52 was highly expressed on influenza-tetramer+CD8+ T cells in the blood of a seasonal IBV-infected patient at 14 dpi. In mice, CD52 expression was also expressed at high levels on tetramer+CD8+ T cells recruited to the site of infection during the effector phase in IAV- (lung) and SFV-infected (brain) B6 mice. Functional, transcriptomic, and proteomic analyses of CD52hi OT-I cells at the effector phase revealed signatures of recently-activated and exhausted-memory-like features, whereas CD52lo OT-I cells displayed features of resting-memory CD8+ T-cells and exhibited a more superior recall capacity at the memory stage (d30-60). Co-transfer assays with CD52+ OT-I and CD52- OT-I cells showed enhanced proliferation of CD52- OT-I cells in comparison to CD52+ OT-I cells at 8 dpi. Moreover, co-expression of the activation marker, CD38, was higher on CD52- OT-I cells than CD52+ OT-I cells at the site of infection, but not in the draining lymph node and spleen.

Conclusion

Our findings demonstrate for the first time how CD52 expression is tightly regulated within CD8+ T-cell immune responses during influenza virus infection, thereby revealing novel mechanisms that underpin optimal CD8+ T cell immunity against influenza and other viruses.

Oral Abstract Session: Basic Science: Influenza - Adaptive immune response to infection

Julianna Han - AOXI0435

Revealing epitope hierarchies in human polyclonal antibody responses to antigenically drifting seasonal influenza A viruses

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Background

Dissecting how human immune pressure at each hemagglutinin (HA) epitope of antigenically drifting seasonal H3N2 viruses selects for variants that evade human immunity is crucial for limiting vaccine escape and informing design of vaccines that focus immune responses on conserved epitopes. Immunodominant antibody responses to seasonal H3N2 viruses focus on the highly mutable major antigenic sites A-E on the HA head.

Method

Here, we evaluate the dynamics of bulk polyclonal antibody (pAb) responses of 50 human subjects to H3N2 viruses after 2017-2018 seasonal influenza vaccination, through 4 subsequent years of mutating H3N2 strains until 2022. Using electron microscopy polyclonal epitope mapping (EMPEM) and functional assessment of >100 mAbs, we further disentangle antigenic hierarchies from 7 individuals.

Result

While baseline pAb responses almost exclusively targeted conserved central stem and RBS epitopes, at days 7, 14, and 180 post-vaccination pAbs expanded to cover variable epitopes on the HA head. Moreover, pAb and neutralizing mAb responses to major antigenic sites A, B, and D, correlating with HA mutations at these sites, were least able to cross-react with subsequent H3N2 strains from 2018-2022. Indeed, high resolution cryo-electron microscopy of a neutralizing mAb:HA complex revealed that a single point mutation within antigenic site D allowed subsequent H3N2 strains to evade protection offered by this mAb.

Conclusion

Our results demonstrate that, although HA accumulates mutations throughout the head domain, mutations in antigenic sites A, B, and D result in the most strain-specific pAb and mAb responses, potentially driving immune pressure for H3N2 variants. To limit immune evasion and increase cross-reactive protection, vaccines should aim to bias antibody responses away from antigenic sites A, B, and D and focus responses on conserved sites.

Oral Abstract Session: Basic Science: Influenza - Innate immune response to infection

Sang-Uk Seo - AOXI0028

Lung epithelial cell injury increases resistance to influenza virus infection in a type I interferon-dependent manner

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Background

Acute respiratory distress syndrome (ARDS) is a fatal pulmonary disease characterized by pulmonary fibrosis, hypoxemia, and infectious complications that are triggered by acute lung injury (ALI). Patients with ARDS are reported to be vulnerable to bacterial complications, but the incidence of respiratory virus infection in patients with ARDS remains unknown.

Method

ice treated with bleomycin (BLM) to induce ALI and housed for 14 days until their body weight returned to normal. Groups of mice were then infected with influenza virus (A/PR/8/34).

Result

Mice treated with BLM were more resistant to influenza virus infection and exhibited higher levels of interferon-I (IFN-I) transcription during the early infection period. BLM-treated mice also exhibited a lower viral burden and reduced pro-inflammatory cytokine production and neutrophil levels. In contrast, BLM-treated IFN-I receptor 1 (IFNAR1)-knockout mice failed to show this attenuated phenotype, indicating that IFN-I is key to the antiviral response in ALI-induced mice. The STING/TBK1/IRF3 pathway was found to be involved in IFN-I production and the establishment of an anti-viral environment in the lung. The depletion of plasmacytoid dendritic cells (pDCs) reduced the effect of BLM treatment against influenza virus infection, suggesting that pDCs are the major source of IFN-I and are crucial for defense against viral infection in BLM-induced lung injury.

Conclusion

Overall, this study showed that BLM-mediated ALI in mice induced the release of double-stranded DNA, which in turn potentiated IFN-I-dependent pulmonary viral resistance by activating the STING/TBK1/IRF3 pathway in association with pDCs.

Oral Abstract Session: Basic Science: Influenza - Innate immune response to infection

Helena Aagaard Glud - AOXI0173

Analysis of mucosal innate immune responses to human and swine adapted influenza A viruses using swab samples

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Background

The mucosal surface of the respiratory tract function as the first line of defence against respiratory pathogens such as influenza A virus (IAV). Upon infection of respiratory epithelial cells, the local cytokine milieu change from a steady to an antiviral state within hours after infection. The innate antiviral immune response to IAV infection involves activation of the viral pattern recognition receptors (PRRs) and type I and III interferons (IFNs), further stimulating a wide range of interferon-stimulated genes (ISGs), that will protect the infected cell and neighbouring cells from viral infection and spread. Even though more than 90% of all infections are assumed to be recognized and controlled by the innate immune system, local viral recognition and immune response in the nasal mucosa is poorly understood.

Method

Groups of 12 pigs were inoculated with 10^7 TCID50/ml of swine- (swH1N1pdm09) or human-adapted (huH1N1pdm09) influenza virus strains. Nasal mucosal swab samples were collected before viral infection and at days 1, 2, 3, 4, 7, 10, and 14 after inoculation. RNA was isolated from nasal swab samples, converted into cDNA, and analyzed by microfluidic qPCR (Biomark, Fludigm) targeting several PRRs, IFNs, ISGs, and mucins.

Result

Type I and type III IFNs, as well as several ISGs, such as OAS1, MX1, IFIT1, and ISG20, were highly upregulated after infection with fold changes ranging between 2 and 59. Peak expression varied between the swine- and human-adapted strains. Expression of seven different membrane-bound and secreted mucins were investigated. The excreted mucin MUC5AC was downregulated at several time points after infection. This regulation was most significant after infection with the swine-adapted strain. More innate immune factors found to be significantly regulated upon infection in swab samples were likewise regulated in nasal mucosal tissue of the same pigs at necropsy at day 3.

Conclusion

Non-invasive and cost-efficient swab sampling was successfully used to compare the innate mucosal immune response to a human-adapted and a swine-adapted strain of the influenza A H1N1pdm09 virus in pigs. Host factors centrally involved in early orchestration of the antiviral immune response were regulated in the nasal mucosa after infection. Expression levels and dynamics after infection differed between the two strains and might be linked to mechanisms important for host tropism and thereby zoonotic transmission.

Oral Abstract Session: Basic Science: Influenza - Innate immune response to infection

Andrew Mehle - AOXI0286

Sensing of self DNA amplifies innate immune responses to suppress influenza virus replication

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Background

Innate sensing of nucleic acids is a key strategy for host cells to detect and control viral infections. RNA viruses such as influenza virus (IAV) are detected by germline-encoded sensors, with effectors establishing an antiviral state including upregulation of interferon-stimulated genes (ISGs). DNA- and RNA-sensing pathways do not always act independently, often sharing functional components or substrates. Furthermore, they rely on common up- and downstream regulators, ripe for exploitation by viruses.

Method

To uncover host factors with uncharacterized pro-viral functions during IAV infection, we developed a novel "self driving" competition-based screen where virally encoded RNAs program Cas9-mediated activation of cellular genes (i.e., CRISPRa). Viruses that activate pro-viral factors gain a replicative advantage compared to the genome-wide pool and quickly dominate the viral population.

Result

The virus itself did the heavy lifting to pinpoint the most critical cellular regulators of infection. A major hit from our experiments was the DNA exonuclease, TREX1. TREX1 degrades cytosolic DNA and is a down-regulator of host self-sensing, with mutant alleles conferring autoimmunity in affected individuals. We validated the ability of TREX1 to enhance gene expression and replication for IAV and multiple other RNA viruses, a function dependent on its exonuclease activity. A role for DNA sensing during RNA virus infection was unexpected, therefore we investigated the cGAS-STING DNA sensing pathway during IAV infection. Cells lacking STING supported higher IAV replication, whereas cells treated with a STING agonist resisted infection. Disabling DNA sensing also enhanced replication in vivo. IAV infection stimulated release of mitochondrial DNA into the cytosol, a known immunogenic substrate of cGAS. This was exacerbated in TREX1 knockout cells but reversed by TREX1 complementation. Cytosolic DNA isolated from infected cells stimulated ISG expression when introduced into naïve cells. Further, cells lacking TREX1 also induced higher levels of ISG expression in response to both IAV infection and re-introduction of self-DNA. This heightened antiviral state supported lower levels of progeny virus production.

Conclusion

We have developed a fitness-based, programmable screening platform applicable to any virus that can deliver a targeting RNA. Using this, we identified TREX1 as a pro-viral cellular factor restricting flux through the cGAS-STING pathway. These experiments revealed that self-DNA is deployed to amplify host innate sensing during RNA virus infection and suppress viral replication.

Oral Abstract Session: Basic Science: Influenza - Innate immune response to infection

Sarah Londrigan - AOXI0327

Release of influenza A virus vRNPs by macrophages during abortive infection may shape innate responses

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Background

Airway epithelial cells and macrophages (MΦ) represent the cellular targets of infection by influenza A virus (IAV). Epithelial cells support productive replication as defined by the release of infectious viral particles. However, our previous studies in murine MΦ showed that seasonal IAV is blocked at the late stages of replication through abortive infection, such that newly synthesised viral particles are not released. Herein, we further characterise IAV replication in MΦ during abortive infection.

Method

urine MΦ were infected with IAV and the absence of infectious virus in cell-free supernatants (SN) was confirmed by plaque assay or TCID50. The expression and cellular localisation of viral proteins were assessed using flow cytometry, confocal microscopy and immuno-blotting. qRT-PCR was used to quantitate levels of newly synthesised vRNA and mRNA in infected cells as well as vRNA present in SN. Presence and abundance of all vRNA segments in SN was determined by next-generation sequencing. Electron microscopy (EM) and co-immunoprecipitation techniques enabled visualisation and characterisation of vRNPs released from infected MΦ.

Result

All IAV structural proteins were expressed in MΦ, and HA, NA and M2 localised to the cell surface. Despite the lack of infectious virions released from infected MΦ, newly synthesised viral RNA (represented by M, PB1, NA and PA gene segments) and NP protein, were detected in SN. The release of vRNA from IAV-infected MΦ occurred independently of apoptosis and necroptosis, as vRNA levels were not reduced when pan-caspase and RIP3-kinase inhibitors were included during infection. EM analysis of IAV-infected MΦ SN revealed the presence of vRNA structures that were associated with viral NP as visualised by immunogold labelling. Moreover, immunoprecipitation of IAV NP in association with IAV polymerase proteins from MΦ SN indicated that vRNP complexes were packaged and released during abortive infection. Finally, vRNPs released from MΦ elicited potent proinflammatory responses when exposed to uninfected human monocytes/macrophages and epithelial cells.

Conclusion

We provide novel mechanistic insight into how MΦ shape innate immune responses during IAV infection. While MΦ represent a dead-end for IAV infection (abortive replication), we show that infected MΦ trigger proinflammatory and antiviral responses in neighbouring uninfected cells through the release of vRNPs.

Oral Abstract Session: Basic Science: Influenza - Innate immune response to infection

Aisha Souquette - AOXI0645

Integrated drivers of basal immunity and acute responses to influenza infection in diverse human populations

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Background

Prior studies have identified genetic, infectious, and biological associations with immune competence and influenza disease severity; however, there have been few integrative analyses of these factors and study populations are often limited in demographic diversity.

Method

Utilizing samples from 8 populations in 5 countries, we examined putative determinants of immune profiles, including: single nucleotide polymorphisms, ancestry informative markers, herpesvirus (HV) status, age, and sex. We modeled the effects of these factors on immune outcomes in vitro and in vivo, at baseline and in response to immune challenge.

Result

In healthy subjects, we found significant differences across populations in basal cytokines, leukocyte phenotypes, and gene expression. The transcriptional immune response post in vitro challenge varied by cohort and was dependent on, in order of prevalence, ancestry, the baseline transcriptional profile, HVs, age, and sex. Analysis of 94 SNPs shows genetic differences by opposing major alleles in immune related genes, with potential for transcriptional effects, as 38 were identified as expression quantitative trait loci. In subjects with acute influenza, we found two distinct disease severity cytokine profiles, largely driven by age. Analyzing acute cytokine levels with a regression model to account for age, sex, genetics, and HVs, we found that HVs have unique and interactive effects on cytokines that are high in magnitude, result in distinct signatures of immune modulation, and are specific to anatomical location during infection. Additionally, 11 cytokines had significant genetic influence during flu infection, each of which were identified as a correlate of severity in these studies.

Conclusion

These results, comprising 123,178,117 data points from 1,705 individuals, provide novel insight into the scope of basal and acute immune heterogeneity across diverse groups, and the influence of the basal setpoint on acute immune responses. Ancestry was the strongest determinant for basal immunity. In response to immune challenge,

while accounting for the baseline immune profile, ancestry and HVs were the major determinants, with distinct effects from those at baseline. The observation that these effects occur in correlates of severity, highlights how variation in these factors can lead to disparate immunophenotypes during infection and the potential impact on illness outcome. Collectively, this underscores the need for future studies to search for correlates of protection or severity across diverse populations and to consider the factors which contribute to their variation in order to identify the number of severe immune profiles, and to optimize therapeutic selection for robust, broadly applicable treatments.

Oral Abstract Session: Basic Science: Influenza - Pathogenesis

Andrés Pizzorno - AOXI0050

Autoantibodies against type I IFNs in patients with critical influenza pneumonia

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Background

Autoantibodies (auto-Abs) neutralizing type I interferons (IFNs) can underlie critical COVID-19 pneumonia and yellow fever vaccine disease. We previously showed that these auto-Abs are common in the general population, being present in 1% of individuals <70 years old, 2.3% of those aged between 70 and 80 years, and 6.3% of those >80 years old. These auto-Abs are the second most common determinant of COVID-19 death after age. We therefore hypothesized that auto-Abs neutralizing type I IFNs might also underlie life-threatening influenza pneumonia.

Method

We recruited 279 patients from Belgium, Greece, Spain, Israel, and France who had been hospitalized for critical influenza pneumonia between 2012 and 2021. Thirty-two of the 279 patients died and 247 survived. We searched for blood auto-Abs neutralizing type I IFNs in luciferase-based neutralization assays and compared the prevalence of auto-Abs against type I IFN between patients with life-threatening influenza and the general population. We then titrated the neutralization capacity of auto-Abs present in patient plasma in both the A549 respiratory epithelial cell line and in reconstituted human airway epithelia (HAE) infected with influenza and pretreated with IFN-a2. We finally evaluated the functional impact of auto-Abs on the expression of a series of ISG following influenza infection or IFN-a2 treatment.

Result

We report here 13 patients harboring autoantibodies neutralizing IFN-a2 alone (five patients) or with IFN-w (eight), among 279 patients (4.7%) aged six to 73 years with critical influenza pneumonia. Nine and four patients had antibodies neutralizing high and low concentrations, respectively, of IFN-a2, and six and two patients had antibodies neutralizing high and low concentrations, respectively, of IFN-w. The patients' autoantibodies increased influenza A virus replication in both A549 cells and reconstituted human airway epithelia. The prevalence of these antibodies was significantly higher than that in the general population for patients under (5.7% vs. 1.1%, p=2.2x10-5), but not over (3.1% vs. 4.4%, p=0.68) 70 years of age. The risk of critical influenza was highest in patients with antibodies neutralizing high concentrations of both IFN-a2 and IFN-w (OR=11.7, p=1.3x10-5), especially those under 70 years old (OR=139.9, p=3.1x10-10). We also identified nine patients in additional influenza patient cohorts.

Conclusion

Autoantibodies neutralizing type I IFNs account for ~5% of cases of life-threatening influenza pneumonia in patients under 70 years old.

Oral Abstract Session: Basic Science: Influenza - Pathogenesis

Lisa Kercher - AOXI0202

The use of telemetry and whole-body plethysmography for acquiring real-time physiological data for improved host response analysis during an influenza virus infection in ferrets.

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Background

The development of influenza vaccines and antivirals rely on evaluation in animal challenge models. The US NIAID/NIH strategic plan for development of a universal influenza vaccine specifically identifies improved animal model development as an area where significant improvements are needed. For example, contemporary seasonal A(H3N2) viruses cause little to no obvious clinical signs in ferrets, even at high doses, limiting the value of the model for assessing vaccine or antiviral impact. We hypothesized that influenza viruses currently regarded as asymptomatic in ferrets do cause clinical symptoms and result in robust immune responses when measured with more sensitive methods. To better characterize the clinical and immunological course of infection, we optimized unique and state-of-the-art animal monitoring systems and used them to determine the impact of A(H3N2) virus infection on physiologic and immunologic properties of the upper respiratory and circulatory compartments.

Method

Ferrets were implanted with telemeters and infected with A/Hong Kong/45/2019 A(H3N2). Concurrent data was collected on internal temperature, activity, blood pressure, heart rate, ECG, and respiration rate. Direct lung function was measured using whole body plethysmography chambers. Peripheral bloods were collected from the same animals during the infection course as were nasal washes which were used to measure viral titers. Flow cytometry analysis was performed on PBMCs and populations of immune cells were analyzed for CD4, CD8, and CD11b.

Result

Real-time physiological and plethysmography data during influenza virus infection showed significant changes in temperature (peak fever at 48 hours post infection), activity, blood pressure, and tidal volumes in infected ferrets compared to uninfected ferrets. Nasal wash titers peaked at day 2 post infection (Log10 5.5 TCID50/ml), and gradually decreased until they were undetectable at day 9 post infection. T cell populations isolated from PBMCs were indicative of increased CD11b, CD4 and CD8 positive cells as early as 48 hours post infection.

Conclusion

Wireless physiological monitoring and whole-body plethysmography has been optimized to measure discrete clinical outcomes in ferrets infected with relevant human seasonal influenza viruses that do not show overt symptoms in the ferret model. The system generated more robust information from the animals and increased the granularity of measuring clinical disease and immune correlates. The real-time data allowed for longitudinal sampling of the infected ferrets to make the influenza challenge model more broadly implementable across challenge viruses.

Oral Abstract Session: Basic Science: Influenza - Pathogenesis

Isabelle Foo - AOXI0275

Exacerbated disease severity and perturbed immune responses directed towards influenza viruses following arbovirus co-infection

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Background

Relative to a single virus infection, prior infection with an unrelated virus, could affect antiviral responses, tissue pathology and the clinical severity of disease. Limited information exists on how prior infection with a non-respiratory virus affects a subsequent influenza virus infection, or vice versa. As influenza is prevalent worldwide, its geographical distribution overlaps with that of many arboviruses and we have investigated sequential infections of an influenza virus and an encephalitic arbovirus.

Method

We established a C57BL/6 mouse model of co-infection using Influenza A virus (IAV) and Semliki Forest Virus (SFV), a neurotropic arbovirus. Adult C57BL/6 mice were infected with IAV only (respiratory infection); SFV only (neurotropic infection); or sequentially co-infected on day 8 post-primary infection (either SFV→IAV or IAV→SFV). Viral, inflammatory and immunological analyses were performed on pre-selected time points following either single infection (IAV) or co-infection (SFV→IAV; IAV→SFV).

Result

In the SFV→IAV sequential co-infection group, we observed markedly exacerbated disease severity than IAV alone. This was linked to delayed IAV viral clearance from the lungs, an excessive production of cytokines in the lungs, and more severe lung pathology. Moreover, intriguingly we found altered trafficking of IAV-specific CD8+ T cells being redirected to the brain in SFV→IAV co-infection. Using ovalbumin-specific T-cells as measurable system, we observed suboptimal OT-I T cell proliferation in the mediastinal lymph nodes of SFVï‚®IAV-OVA infected mice at day 3.5; with a lower percentage and number of proliferating CD8+CD45.1+ OT-Is cells. Further investigation showed that in SFV-only infection at day 8, the time at which the IAV infection is given, there was upregulation of Ly6A, a marker associated with dendritic cell paralysis.

Conclusion

These results provide new insights into how a prior unrelated virus infection can alter the immune response and disease outcome of influenza infection. Improved fundamental knowledge on how viral infections interact to affect the course of immune responses, could be of a direct relevance to improved disease management programs, specialist treatments and optimisation of vaccination strategies.

Oral Abstract Session: Basic Science: Influenza - Pathogenesis

Douglas Reed - AOXI0448

Seasonal influenza vaccination protects macaques against lethal respiratory disease following inhalation of small particle aerosols containing H5N1 influenza.

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Background

Highly pathogenic avian influenza viruses can cause rapid, severe, and often fatal acute respiratory distress syndrome in humans. H5N1 viruses, which can be found across the globe, have a 60% case fatality rate in humans. While incidence of human disease is low and primarily among poultry workers with little or no human-to-human transmission, there is concern that these viruses could evolve to be more readily transmissible in humans, leading to a new pandemic with high mortality like the 1918 pandemic. It has been suggested that prior exposure to seasonal human influenza viruses may impact survival against avian influenza viruses. We developed the first lethal model of H5N1 infection in cynomolgus macaques resulting from inhalation of the virus in a small particle aerosol.

Method

Cynomolgus macaques were infected with small particle aerosols containing highly pathogenic avian influenza H5N1 (A/Vietnam/1203/2004). Radiotelemetry was used to monitor changes in fever and activity. Respiratory function was monitored by plethysmography. For a proof-of-principle vaccine study, a group of macaques was vaccinated with an adjuvanted seasonal influenza vaccine followed by lethal challenge. Mock-vaccinated macaques were used as a comparison.

Result

We determined that H5N1 lethality after inhalation in a small particle aerosol is only seen at doses ≥10e5 pfu. Macaques typically succumb within 3-7 days after exposure due to acute respiratory distress syndrome. Repeated vaccination with adjuvanted seasonal human influenza vaccines in macaques (n=6) conferred significant protection against lethality after challenge with 5.4x10e5 pfu of aerosolized H5N1 while four of six mock-vaccinated controls succumbed at this dose between 3-4 days after challenge. Vaccinated macaques did develop a fever after challenge, but the severity of the fever was significantly lower (>0.5ºC in average elevation) in vaccinated macaques. An increased respiratory rate over baseline was noted at day 2 for vaccinated macaques which was half of what was seen in unvaccinated controls although this difference was not statistically significant. Radiographs taken during the study also suggested lower levels of viral pneumonia in vaccinated macaques.

Conclusion

These studies demonstrate that the lethality of aerosolized H5N1 is a function of the inhaled dose, and that vaccination can protect against morbidity and mortality resulting from aerosol H5N1 challenge in the macaque model.

Oral Abstract Session: Basic Science: Influenza - Pathogenesis

Anke Huckriede - AOXI0591

Levels of virus-binding and virus-neutralizing antibodies to historic strains of influenza virus are birthyear-dependent and evolve with different dynamics

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Background

The high genetic and antigenic variability of influenza virus and the repeated exposures to the virus account for the human immune responses toward this pathogen to continuously evolve during an individual's lifespan. Influenza-specific immune memory to past strains has been shown to affect the immune responses to subsequent influenza strains and in turn to be changed itself through the new virus encounter. However, exactly how and to what extent this happens remains unclear.

Method

We studied pre-existing immunity against influenza A virus (IAV) in 60 adolescents, 60 adults, and 60 elderlies by assessing virus-binding (IgG) and virus-neutralizing antibodies to 5 different IAV strains (A/Puerto Rico/8/34 (H1N1/34), A/Aichi/1/68 (H3N2/68), A/New Caledonia/20/99 (H1N1/99), A/Perth/16/2009 (H3N2/09), and A/California/7/2009 (H1N1 pdm09). Plasma samples were obtained from the Lifelines cohort study run in Groningen, Netherlands, and were taken at a 5-year interval.

Result

In each age cohort, the highest IgG titres were seen against recent virus strains but the biggest increase in titre occurred against older strains. In contrast, the highest neutralizing antibody titres were seen for a virus strain that circulated early in the participants' lives but the highest increase in titre was observed for the most recent virus strains. Significant increases in neutralizing antibody titres against a newly encountered virus strain were observed in all age cohorts demonstrating that pre-existing immunity did not hamper antibody induction.

Conclusion

Our results indicate that the evolution of influenza-specific humoral immunity differs for rather cross-reactive virus binding antibodies and more strain-specific neutralizing antibodies. Nevertheless, in general, our observations lend support to the antigenic seniority theory according to which the antibody response to influenza is broadened with each virus encounter, with the earliest encountered strain taking in the most senior and thus dominant position.

Oral Abstract Session: Basic Science: Influenza - Transmission

Xiangjie Sun - AOXI0116

A naturally occurring HA stabilizing amino acid (HA1-Y17) in a low pathogenic influenza A A(H9N2) virus contributes to virus airborne transmission

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Background

Airborne transmissibility is a prerequisite for a pandemic influenza virus, and a better understanding of how zoonotic influenza viruses evolve to acquire a transmissible phenotype is essential for pandemic preparedness. Select contemporary avian influenza A viruses of the A(H9N2) subtype have exhibited transmission capability by the airborne route in the ferret model; therefore, it is of great importance to identify viral factors that contribute to enhanced transmission to better inform strategies for influenza surveillance and pandemic preparedness.

Method

A(H9N2) viruses deposited in the GISAID possess either a Tyrosine (Y) or Histidine (H) residue at amino acid position HA1-17 and the A/Anhui-Lujiang/39/2019 A(H9N2) virus with a moderate capacity for airborne transmission, bears a Y at this position. The recombinant A(H9N2) viruses with either HA1-Y17 or H17 were rescued, and viral replication and airborne transmission were assessed in a ferret model. Furthermore, fitness of these two recombinant viruses were evaluated in a ferret co-infection competition experiment where virus identity in nasal wash and tissue specimens as well as exhaled air were analyzed by next generation sequencing. Lastly, laboratory aerosolization performance of both viruses was compared.

Result

The residue at HA1-17 modulates the pH threshold for HA fusion of A(H9N2) virus, and the recombinant H9N2 virus harboring HA1-H17 exhibited a higher pH threshold for fusion and lost the ability to transmit by air between ferrets despite similar replicative efficiency compared to the virus with HA1-Y17. Furthermore, the A(H9N2) HA1-Y17 virus displayed a higher abundance in large particles (>4μm) in the air exhaled by the infected animals than the HA1-Y17H virus, and was also the dominant species in exhaled air, nasal wash and tissue specimens during ferret co-infections. Lastly, considerable differences in aerosolization performance were noted between HA1-Y17 and HA1-H17 viruses.

Conclusion

The A(H9N2) virus with the enhanced ability to transmit by the air possesses a key residue at HA1-Y17, which confers virus acid stability and contributes to virus airborne transmission. The higher virus abundance released into the air from infected animals and better fitness in the host as well as the difference in aerosolization state associated with the A(H9N2) virus harboring the HA1-Y17 residue may all contribute to enhanced airborne transmission in mammalian hosts. Taken together, our study highlights the requirement for an acid stable HA in efficient airborne transmission for a zoonotic influenza virus; the underlying mechanism may be multifactorial including both virus fitness in the host and virus aerosol stability in the air.

Oral Abstract Session: Basic Science: Influenza - Transmission

Ndongo Dia - AOXI0185

Epidemiology and molecular analyses of Influenza B viruses in Senegal from 2010 to 2019.

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Background

Influenza viruses' type A and B are responsible of acute viral infections that affect annually 1 billion people, with 290,000 to 650,000 deaths worldwide. In this study, we investigated the circulation of influenza B viruses over a 10-years period (2010-2019).

Method

Specimens from patients suspected of influenza infection were collected. Influenza detection was performed following RNA extraction and real-time RT-PCR. Genes coding for the hemagglutinin (HA) and the neuraminidase (NA) of influenza B viruses were partially sequenced, and phylogenetic analyses were carried out subsequently.

Result

During the study period, we received and tested a total of 17,276 specimens. Influenza B virus was detected in 1,397 (8.1%) specimens. The mean age of influenza B positive patients was 10.9 years. We noted that women were more likely to be infected with influenza than men with a crude odds ratio OR=1.14 (p<0.001). The most reported symptoms in patients were fever (92.6%) and cough (81.9%), followed by rhinorrhea (42.3%), headache (17.1%), sore throat (15.8%) and myalgia (13.9%). When compared to reference viruses, HA genes from Senegalese circulating viruses showed deletions in the HA1 region. Phylogenetic analysis highlighted the co-circulation of B/Victoria and B/Yamagata lineage viruses with reassortant viruses. Inside the B/Vic lineage, we identified two clades: the minority clade 1B which was exclusively detected in 2010 and the clade 1A (66/68) which circulated from 2012 to 2019. Regarding B/Yam, Senegalese strains clustered with clades 2 and 3, with a high predominance of the B/Yam clade 3. Interestingly, analysis of the NA showed that some IBV strains appeared to be reassortants between B/Yam and B/Vic viruses. Indeed, 12 (11 in 2015 and one in 2016) strains belonging to the B/Yam clade 3 based on the HA tree and clustered with the B/Vic clade 1A based on the NA gene phylogeny. We also detected one strain in 2018 that belonged to B/Vic lineage based on the HA gene and to the B/Yam lineage based on the NA gene. In addition, we observed intra-lineage reassortment cases between B/Vic strains. We also noted a clear seasonal pattern of circulation of the influenza B in Senegal.

Conclusion

The present study highlights the epidemiology and evolution dynamics of IBV strains that circulated in Senegal between 2010 and 2019. More studies are needed to estimate the disease burden as well as the severity associated to influenza infections in Senegal, in different age group and populations. During our study, we detected inter-lineage as well as intra-lineage reassortants. It would be interesting to assess the impact of such reassortment on the disease presentation and transmission rate of these viruses.

Oral Abstract Session: Basic Science: Influenza - Transmission

Irina Glas - AOXI0199

Impact of acidic pH in respiratory aerosol on the inactivation times of influenza and coronaviruses

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Background

Transmission via aerosols is key for the spread of influenza A virus (IAV) and SARS coronavirus 2 (SARS-CoV-2) but the processes governing virus inactivation in aerosols are incompletely understood. Aerosol particles tend to become more acidic when exposed to ambient relative humidity (RH). In fact, ambient aerosol particles can acquire a pH as low as 0 throughout their lifetime in the atmosphere, while pH of respiratory aerosols remains unknown. Therefore, we herein combine a computational model predicting pH in respiratory aerosols with inactivation dynamics of influenza and coronaviruses at acidic pH to investigate inactivation times in indoor environments.

Method

Inactivation dynamics of one IAV strain (A/WSN/33) and two different types of coronaviruses (HCoV-229E-Ren and SARS-CoV-2) were determined in bulk solutions assuming a first order kinetic. To resemble conditions in respiratory aerosols, we used synthetic lung fluid (SLF) and nasal mucus harvested from human primary airway cells and compared it to inorganic buffer as bulk matrix. Our biophysical model simulating aerosol pH is based on SLF properties measured with an electrodynamic balance (EDB). In addition, it considers ambient air composition such as RH and trace gas concentrations.

Result

According to our model, small respiratory aerosol particles in indoor air at 50 % RH acidify to a pH of roughly 4 inactivating IAV in a time course of minutes. Coronaviruses, in contrast, remain infectious for days due to their high resistance to acidic pH. Further, we found that air composition impacts pH of respiratory aerosol: Decreasing ammonia, for example, will accelerate the pH drop in drying aerosol particles and therefore decrease influenza inactivation times, but has no effect on coronaviruses. Adding 50 ppb of nitric acid (considered non-hazardous) into room air, on the other hand, decreases aerosol pH by 2 units leading to efficient inactivation of IAV and also SARS-CoV-2 within seconds to a few minutes.

Conclusion

Here we show that respiratory viruses differ vastly in their sensitivity to acidic pH and thus vary in their stability in aerosols. We show that the surrounding air plays a crucial role for aerosol pH and affects IAV stability in aerosols substantially as well as SARS-CoV-2 stability in some cases. Our study sheds light to the implications of air composition concerning transmission risk of airborne respiratory viruses.

Oral Abstract Session: Basic Science: Influenza - Transmission

Yuan Liang - AOXI0281

Pathogenesis and infection dynamics of high pathogenicity avian influenza virus (HPAIV) H5N6 (Clade 2.3.4.4b) in pheasants and onward transmission to chickens

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Background

Since 2014, clade 2.3.4.4 H5Nx high pathogenicity avian influenza viruses (HPAIVs) have spread worldwide via wild bird migration and have severely affected commercial poultry flocks resulting in large economic losses. Clade 2.3.4.4 incursions have also occurred in many wild and farmed pheasants, from which infection could then spread into commercial poultry. Defining the transmission dynamics within different avian species is key to understanding incursion risks into different populations.

Method

A group of pheasants (n=6) were directly-infected with a wild pheasant-origin clade 2.3.4.4b H5N6 HPAIV (A/pheasant/Denmark/12106-3/2018 (H5N6-DEN-2018)). The directly-infected pheasants served as donors to establish a transmission chain. Four additional groups (n=6 in each) of contact pheasants were introduced stepwise through four cohousing stages and subsequently two contact chicken groups. A second transmission chain, beginning with directly-infected pheasants with onwards transmission through two successive contact chicken groups was also investigated. Buccal and cloacal swabs were collected daily from each bird for the detection of viral shedding. Organs were harvested at necropsy for virology and pathology evaluation. Sera was collected to assess for seroconversion.

Result

In the first transmission chain, directly-infected and contact pheasants in each group shed viral RNA following exposure, confirming infection. After four passages of passage in pheasants, H5N6-DEN-2018 was transmitted inter-species to a contact group of chickens with 6/6 becoming infected. However, onward transmission to another contact group of chickens was limited with only 2/6 becoming infected. The remaining chickens did not shed virus, with no evidence of viral replication or seroconversion. Similar results were obtained in the second, shorter transmission chain where directly-inoculated pheasants transmitted H5N6-DEN-2018 inter-species to all chickens in the first contact group, but onward transmission to a second contact chicken group was unsuccessful. The pathogenesis of infected pheasants and chickens manifested as a typical HPAIV infection, although apparent species differences in mean death time and tissue tropism were observed.

Conclusion

We demonstrated that H5N6-DEN-2018 can be sustained efficiently intra-species within pheasants and also that they are able to act as a bridging host between wild birds and farmed chickens. The onward spread from chicken to chicken was only limited which could reflect a lower level of virus adaptation in this poultry host.

Oral Abstract Session: Basic Science: Influenza - Transmission

JIE ZHOU - AOXI0477

The Mechanisms of Hemagglutinin and Neuraminidase Adaptation in the Emergence of the 1968 H3N2 Pandemic Virus

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Background

The 1968 H3N2 pandemic was the most recent pandemic resulting from adaptation of an avian influenza virus to humans. The emerged virus differs from the putative avian precursor (AP) by seven substitutions (R62I, D81K, N92K, A144G, N193S, Q226L and G228S) in HA segment and one substitution (P126H) in NA segment. However, the evolution pathway from the avian precursor to the fully adapted human seasonal influenza virus is still unclear.

Method

We rescued recombinant viruses of A/Aichi/2/1968 virus carrying various combinations in the HA restoring the ancestral avian-like amino acids coupled with either NA 126P or 126H. The recombinant viruses were characterised in vitro by receptor binding, neuraminidase activity, and pH stability assays. We also infected ferrets to evaluate transmission and in vivo evolution. Nasal wash samples were collected daily for virological analysis and next-generation sequencing (NGS).

Result

ceptor binding assays showed that the avian precursor H3 HA preferentially binds to 3'SLN, Q226L alone switched HA binding from 3'SLN to 6'SLN matching the profile of Aichi H3N2 virus, while G228S alone enhanced HA binding to 6'SLN but still maintained binding to 3'SLN. The P126H in NA slightly decreased enzyme activity. The HAs of Aichi68, avian precursor and S5 (5 compensatory mutations) had similar acid stability, while S2 (Q226L and G228S) was more sensitive to low-pH treatment, indicating that human-specific Q226L and G228S substitutions decreased acid stability and that compensatory mutations alleviated this problem. Aichi68 virus was passed between ferrets through at least two chains of transmission, including some transmission by respiratory droplet exposure. NGS did not detect any obvious mutations in nasal washes from either donors or sentinels. When ferrets were inoculated with avian precursor virus with either 126P or 126H NA, all donors shed infectious virus in nasal wash samples starting from 1dpi. Virus was transmitted to 2/4 and 1/4 co-housed sentinels in the avian precursor with 126P and 126H NA groups respectively. The NGS data showed that both Q226L and G228S appeared in nasal washes from the donors inoculated with either avian precursor, while G228S showed higher proportion and was detected in sentinel animals.

Conclusion

Our results suggest a route of adaptation whereby the H3 avian precursor HA acquires G228S first, which enables dual receptor-binding properties. We hypothesize that Q226L might be acquired later for fully adapting to binding to the human receptor, while 126H NA and further compensatory HA mutations re-balanced the HA with substitutions G228S and Q226L in the receptor binding domain which reduce the stability of HA.

Oral Abstract Session: Basic Science: Influenza - Virus-host cell interactions

Cindy Spruit - AOXI0197

Binding of H7 influenza A virus to N-glycolylneuraminic acid and sialyl-LewisX on N-glycans

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Background

Influenza A virus (IAV) infection is initiated by the binding of the viral hemagglutinin (HA) to glycans with terminal sialic acids on the cell surface. The binding specificity is affected by modifications of the sialic acid, sialic acid linkage, and glycan structure. H7 IAV is often highly pathogenic and is present in many species, including ducks, chickens, and humans, thus posing a high zoonotic risk. Most H7 influenza viruses bind α2,3-linked N-acetylneuraminic acid (NeuAc) and the only known naturally occurring IAVs that exclusively bind N-glycolylneuraminic acid (NeuGc) are extinct highly pathogenic equine H7N7 viruses.

Method

We observed a high similarity between the crystal structures of an equine H7 HA in complex with NeuGc and an avian H7 HA in complex with NeuAc. To determine the molecular basis for NeuAc and NeuGc specificity, we performed systematic mutational analyses, based on the structural insights, on avian H7 and H15 HAs. The binding was analyzed using a glycan microarray, hemagglutination assay, and tracheal epithelium tissue staining.

Result

We found that mutation A135E is key for binding α2,3-linked NeuGc but does not abolish NeuAc binding. Additional mutations S128T, I130V, T189A, and K193R converted the specificity from NeuAc to NeuGc on the glycan array, although binding to chicken erythrocytes and epithelium tissue, which lack NeuGc, was maintained. This phenotype was conserved in H15 HAs. Novel tri-antennary N-glycan structures with sialyl-LewisX (sLeX) epitopes were chemoenzymatically synthesized and binding of both wild-type and mutant HAs was observed, whereas no binding was observed to linear glycans with sLeX.

Conclusion

The results demonstrate that genetically distinct H7 and H15 HAs can be switched from NeuAc to NeuGc binding after the introduction of several mutations, providing insights into the adaptation of H7 viruses to (potentially archaic) NeuGc receptors. However, apparently sLeX specificities are as important, which were only observed with complex tri-antennary N-glycans with sLeX epitopes. These novel glycans allow for further investigation of the fine receptor specificity of IAV and the potential role of sLeX as an (intermediate) receptor.

Oral Abstract Session: Basic Science: Influenza - Virus-host cell interactions

Ian York - AOXI0214

Aberrant Cellular Glycosylation Modifies the Influenza Glycome Without Genomic Changes Allowing Virus Evasion of Host Immune Responses

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Background

People with cancer, autoimmune disease, diabetes, or obesity often have metabolic dysregulation of cellular glycosylation, and also have tend to more severe influenza virus (IV) disease, with a poor immune response to the virus and low vaccine efficacy. Host cells are responsible for glycosylation of IV hemagglutinin (HA) and neuraminidase (NA), and glycosylation is important for interactions of these proteins with the immune system. IV isolated from people with metabolic disorders do not show consistent genomic variations. However, IV may have improper glycosylation that is not reflected by their genomic sequence. We investigate the consequences of aberrant cellular glycosylation for the glycome and biology of and immune responses to IV.

Method

Aberrant N-linked glycosylation (NLG) in cultured cells was induced with a non-toxic dose (10 µM) of an oligosaccharyltransferase inhibitor, NGI-1. Replication of tested IV in NGI-1-treated NHBE and MDCK cells was evaluated with 0.01 or 1.0 MOI. Morphologic and glycomic changes of NGI-1-treated IV were determined by electron microscopy and mass spectrometry-based analyses respectively, 24 hours after infection of MDCK cells with 1.0 MOI. Binding of NGI-1-treated virus to red blood cells and to the innate immunity respiratory tract collectin surfactant protein D (SP-D) was assessed by hemagglutination and hemagglutination inhibition assays, respectively. The NA activity of NGI-1-treated virus was measured in fluorometric and enzyme-linked lectin assays with MUNANA or fetuin as a substrate. The ability of NGI-1-treated HA to generate immune responses was assessed by immunization of naïve mice with two 10 µg HA doses followed by measurement of serum antibody titers by HI and ELISA tests. Inhibition of NGI-1-treated virus growth by SP-D and mouse serum antibodies was determined in virus neutralization assays.

Result

Treatment of cells with NGI-1 lowered infectious and genomic IV titers up to 1000 folds, resulting in live virus with reduced NLG site occupancy of HA and NA, but with an unmodified genome and morphology, that was able to infect cells with normal glycosylation. This IV had less agglutinating ability per µg and required higher rhSP-D concentrations for HA inhibition and virus neutralization than virus with normal glycan occupancy. HA and NA with reduced glycan occupancy generated lower total and protective antibody responses in mice than those with normal glycosylation.

Conclusion

Imbalanced cellular glycosylation can lead to sequence-neutral changes in the IV glycome, and these glycome-modified viruses may be less well recognized by the host innate and adaptive immune system resulting in a severe influenza and reduced IV vaccine efficacy.

Oral Abstract Session: Basic Science: Influenza - Virus-host cell interactions

Nathalie AJ Verzele - AOXI0326

Silencing pulmonary sensory neurons increases influenza disease severity

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Background

During influenza A virus (IAV) infection, the driver of disease severity is often an aberrant inflammatory response with various cell types contributing to the pathogenesis. The lungs are densely innervated by pulmonary sensory neurons, critical for monitoring the environment within the airways and lungs. We recently showed that during IAV respiratory viral infection these pulmonary sensory neurons undergo transcriptional changes and take on an antiviral inflammatory phenotype, characterized by increased expression of Irf9. However, to date, the pulmonary sensory neurons have not been investigated as a potential therapeutic target in IAV infections.

Method

In a murine model (C57B6/J mice, 8-10 weeks) of IAV respiratory infection (Auckland/1/09 H1N1), disease pathogenesis was compared with and without inhibiting pulmonary sensory neuron activity through daily treatment with QX-314 (300µM by nebulisation), a charged sodium channel inhibitor. The role of IAV-induced upregulated vagal ganglia Irf9 expression was assessed in disease pathogenesis by knocking down ganglia expression using targeted delivery of an adeno-associated viral vector encoding Irf9 short hairpin RNA (AAV-IRF9shRNA). Pathogenesis was measured using whole body plethysmography, clinical scoring, ganglia gene expression, viral titre and immune cell infiltration into the lungs and ganglia.

Result

QX-314 treated animals demonstrated more severe weight loss and increased severity of clinical symptoms compared to vehicle treated IAV infected mice. This was accompanied by increased lung total cell counts, with a decrease in CD8+ T-cells. In the vagal ganglia, IAV-induced Irf9 expression was significantly lower in QX-314 treated animals compared to vehicle treated controls. IAV-infected AAV-IRF9shRNA animals similarly presented with increased clinical symptoms and functional measures of increased airflow obstruction compared to blank vector treated controls.

Conclusion

These data indicate that the vagal sensory system plays an important role in regulating IAV pathogenesis. Modulating their activity may therefore be a novel therapeutic approach to reduce the severity of respiratory viral infections.

Oral Abstract Session: Basic Science: Influenza - Virus-host cell interactions

Catherine Isel - AOXI0366

High-throughput droplet-based analysis of influenza A virus genetic reassortment by single-virus RNA sequencing

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Background

The segmented RNA genome of influenza A viruses (IAVs) enables viral evolution through genetic reassortment after multiple IAVs co-infect the same cell, leading to viruses harboring combinations of eight gene segments from distinct parental viruses. Existing data indicate that reassorment is not random and that genotypes are not equiprobable. However, the low throughput of available virology techniques does not allow quantitative analysis nor understanding of the rules underlying genetic reassortment.

Method

Here we report a high-throughput single-cell droplet microfluidic system allowing single virus sequencing and analysis, through a customized bioinformatics pipeline. Briefly, our workflow consists of co-infecting cells with distinct IAV strains at a high m.o.i. to generate a viral stock containing potential reassortant viruses. This is followed by infection of fresh cells at a low m.o.i. with the newly generated stock, to recover cells infected with a single viral particle. Such infected cells are isolated upon fluorescence-activated cell sorting, and are then encapsulated in droplets using a microfluidic device. Within droplets, the genomic vRNA is reverse transcribed to cDNA through usage of customized barcoded primers for targeted viral RNA sequencing, enabling subsequent Illumina RNA-seq library preparation and NGS. Over 18,000 viral genotypes resulting from co-infection with two circulating human H1N1pdm09 and H3N2 IAVs were analyzed over six distinct replicates.

Result

were highly reproducible, confirmed that genetic reassortment is far from random and allowed accurate quantification of reassortants, including rare events. In cells where the eight segments were detected, 159 out of the 254 possible reassortant genotypes were observed, with widely varied prevalence (from 0.038% to 8.45%). Importantly, all 112 possible pairwise co-occurences of segments were observed. Including data from single cells where less than eight segments were detected allowed analysis of pairwise co-occurences between segments with very high confidence. Direct Coupling Analysis accurately predicted the fraction of pairwise segments and full genotypes.

Conclusion

Overall, our results indicate that a large fraction of all possible genotypes emerge upon co-infection and can be detected and quantified, over a wide range of frequencies, pointing to our experimental and bioinformatic pipeline as a powerful system for systematic and exhaustive monitoring of the reassortment potential of IAVs.

Ana Villamil - AOXI0497

Developing an assay for nucleic acid exposure to probe influenza membrane fusion.

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Background

Influenza virus enters cells by fusing with endosomal membranes. We seek to understand the physical determinants of this process; in this case how endosome curvature and deformability control fusion and how these might constitute restriction factors for infection. In recent work, we showed that lipid mixing kinetics between X-31 influenza virus and endosomal membranes are largely identical to lipid mixing kinetics between X-31 influenza and small synthetic liposomes but that the deformability of the membranes does play a key role in controlling kinetics. Here, we extend this approach by developing an assay to measure influenza viral genome exposure as part of the fusion process.

Method

We use single-virus microscopy of influenza virus binding and fusing to synthetic or endosomal membranes. Fluorescent probes are chosen to report on state changes of the virus-either lipid-phase probes that increase fluorescence when they diffuse out of the virus and into a target membrane or content probes that increase fluorescence either upon diffusion through a fusion pore or, new to this work, binding to viral genomic RNA. The basic parameters of these single-virus fusion measurements are well described in previous work; here, we employ synthetic liposomes or bilayer-encapsulated nanoparticles rather than the supported lipid bilayers that were initially described in single-virus fusion experiments. All experiments performed here used X-31 influenza virus as a model system (H3N2; HA and NA from A/Aichi/68).

Result

Genome exposure assays show that influenza RNA is accessible shortly after fusion pore opening, likely reflecting early stages of disassembly. Rates of pore opening and genome exposure do not depend on liposome size, indicating that endosomal curvature has a minimal effect on the rate-limiting steps of influenza membrane fusion. As in our previous experiments, reducing membrane deformability does measurably slow influenza viral entry.

Conclusion

We have developed a sensitive assay for real-time single-virus detection of RNA virus genome exposure. We find early accessibility of influenza genomic RNA after fusion pore formation. The membrane factors controlling influenza entry likely include membrane deformability, which is known to be altered by host restriction factors such as IFITM-3, but not membrane curvature.

Oral Abstract Session: Basic Science: SARS-CoV-2 - Novel treatment and prevention strategies

Melanie Wu - AOXI0093

Modulation of the renin-angiotensin system to reduce immunopathology in SARS-CoV-2 and influenza A infection

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Background

SARS-CoV-2 and influenza virus infections are associated with pronounced pro-inflammatory responses and immunopathology. Angiotensin-converting enzyme 2 (ACE2) and the Mas receptor (MasR) are key components of the renin-angiotensin system (RAS) and play a significant role in the protective ACE2/Ang(1-7)/MasR axis. Activation of ACE2 and/or MasR induces anti-inflammatory and anti-fibrotic responses including vasodilation, vasoprotection, and hypotension. However, no studies to date have investigated the therapeutic potential of modulating RAS to reduce the immunopathology associated with respiratory virus infection. Here, we studied how activation of ACE2 and/or MasR affects SARS-CoV-2 and influenza virus replication and virus-induced inflammatory pathways.

Method

To explore the potential of ACE2 and/or MasR activation in reducing virus immunopathology, primary human nasal epithelial cells or Calu-3 cells were treated with a novel ACE2 activator (peptide 2A; derived from snake venom) or commercially available MasR agonists (AVE0991 and Ang(1-7)) pre- or post-infection with SARS-CoV-2 or influenza virus. RNA was harvested for quantitative polymerase chain reaction analysis of viral and host inflammatory gene expression. Viral titres from supernatant were quantified by plaque assay.

Result

Pre-treatment with the ACE2 activator peptide 2A prevents SARS-CoV-2 infection and the associated inflammatory response in vitro. Peptide 2A treatment also reduced the pro-inflammatory response to SARS-CoV-2 and influenza virus infection in vitro (as determined by IL-6 expression). We further demonstrate the anti-inflammatory effects of MasR agonists, Ang(1-7) and AVE0991 in vitro to respiratory virus infections (as determined by IL-6, TNFα, and IL-8 expression and production).

Conclusion

ACE2 activator peptide 2A and MasR agonists (AVE0991 and Ang(1-7)) have shown potential in both lowering inflammation from SARS-CoV-2 and influenza virus infection in vitro. Together, these data provide the first evidence that modulating of RAS may represent a novel approach to prevent the immunopathology associated with SARS-CoV-2 and influenza virus infection.

Oral Abstract Session: Basic Science: SARS-CoV-2 - Novel treatment and prevention strategies

Sebastian Beck - AOXI0241

CYP19A1 mediated sex hormone metabolism promotes severe SARS-CoV-2 disease outcome in males.

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Background

Male sex belongs to one of the major risk factors for severe COVID-19 outcome. However, underlying mechanisms that could affect sex dependent disease outcome are yet unknown.

Method

In this study, we used the golden hamster model for SARS-CoV-2 infection in combination with targeted therapy to investigate the influence of sex hormones on sex disparity in COVID-19 disease manifestation and progression.

Data derived from the animal model were then translated to humans using lung tissue from deceased patients. Finally, a genetic approach was applied to a large COVID-19 patient cohort to detect gene alterations significantly associated with disease severity and outcome.

Result

Upon viral infection, transcription of the testosterone-to-estradiol metabolizing enzyme CYP19A1 (alias aromatase) is elevated in the lung of male golden hamsters correlating with reduced testosterone and increased estradiol levels. Dysregulated circulating sex hormone levels in male golden hamsters are associated with reduced lung function compared to females. Treatment of SARS-CoV-2 infected hamsters with letrozole, a clinically approved CYP19A1 inhibitor, supported recovery of dysregulated plasma sex hormone levels and was associated with improved lung function and health in male but not female animals compared to placebo controls. Whole human exome sequencing data analysis using a Machine Learning approach revealed a CYP19A1 activity increasing mutation being associated with the development of severe COVID-19 for men. In human autopsy-derived lungs, CYP19A1 was expressed to higher levels in men who died of COVID-19, at a time point when most viral RNA was cleared.

Conclusion

Here, we identified the CYP19A1 gene as a male abundant host factor that contributes to worsened disease outcome in males.

Our findings highlight the role of the lung as a yet unrecognized but critical organ regulating metabolic responses upon respiratory virus infection. Furthermore, inhibition of CYP19A1 by the clinically approved drug letrozole may pose a new therapeutic strategy to reduce poor long-term COVID-19 outcome.

Oral Abstract Session: Basic Science: SARS-CoV-2 - Novel treatment and prevention strategies

Alexandre Le Vert - AOXI0230

OVX033, T-CELL BASED VACCINE TARGETING THE NUCLECAPSID PROVIDES BROAD-SPECTRUM PROTECTION AGAINST SARS-COV-2 VoC IN HAMSTER CHALLENGE MODEL

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Background

Vaccines eliciting neutralizing antibodies against Spike are very effective against COVID-19 in the absence of antigenic variations. However, variants of concerns escape humoral responses, thus reducing vaccine efficacy. Beyond antibodies, T-cell responses are involved in viral clearance and disease prevention. More specifically CD8+ T-cells against the Nucleocapsid (N) antigen - highly conserved across variants - correlate with protection.

OVX033 is a recombinant N protein fused to oligoDOM®, OSIVAX' self-assembling nanoparticle technology, specifically designed to trigger T-cell responses. We assessed the immunogenicity of OVX033 with and without SQ adjuvant, a squalene-in-water-emulsion containing cholesterol and saponin QS21 developed by the Vaccine Formulation Institute (VFI), in Golden Syrian hamsters and evaluated efficacy against SARS-CoV-2 challenges.

Method

Naïve hamsters were vaccinated twice 28 days apart with OVX033 ± SQ, or NaCl 0.9%. Animals were sacrificed 28 days later to evaluate N-specific humoral and cellular responses (n=5); or challenged (n=12/variant) by intranasal instillation of 3 different SARS-CoV-2 variants (B.1 Europe, Delta, Omicron). Animals were monitored daily for body weight and sacrificed 4 or 7 days post-challenge, for lungs viral load and histopathological analyses.

Result

Immunogenicity: OVX033 induced high anti-N IgG titers (ELISA) and N-specific IFNγ T-cell response in spleen and lungs (ELISpot) of all OVX033 vaccinated hamsters.

Protection from original strain (B.1): Weight loss following challenge was significantly reduced ($p \leq 0.0001$) for animals vaccinated with OVX033 ± SQ, compared to controls. Lung viral loads at D4 (TCID50) were reduced by 1 to 1.5 log, respectively in OVX033 ($p \leq 0.05$) and OVX033+SQ groups ($p \leq 0.01$). Histopathological evaluations revealed a strong reduction of the pneumonia area at D7 post-challenge ($p \leq 0.01$) in both OVX033 ± SQ groups.

Protection from Delta and Omicron variants: Weight loss was reduced for the OVX033+SQ group after Delta challenge ($p \leq 0.001$). Omicron challenge induced very limited weight loss in all groups. Lung viral loads at D4 (TCID50) were reduced by 1.5 to 2 (Delta) or 1.1 log (Omicron) in OVX033 ± SQ groups. Histopathological analyses will be available at the time of the congress.

Conclusion

OVX033 demonstrated protective efficacy against (i) weight loss, (ii) lung viral load and (iii) pneumonia caused by SARS-CoV-2challenge. SQ adjuvant tends to increase the immune responses and protection. These results warrant further development of OVX033 T-cell based vaccine with and without adjuvant to assess its ability to provide durable and broad-spectrum protection against both current and future Sarbecoviruses.

Oral Abstract Session: Basic Science: SARS-CoV-2 - Novel treatment and prevention strategies

Michael D'Agostino - AOXI0461

Development and application of a next-generation single-dose mucosal COVID-19 vaccine

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Background

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has infected over 500 million people and claimed over 6 million lives globally to date. Notably, most authorized first-generation vaccines are designed to elicit antibodies against the spike protein of ancestral SARS-CoV-2 following intramuscular injection. However, due to the high global incidence of SARS-CoV-2 infections, several variants of concern (VoC) have emerged with substantially mutated spike proteins are more transmissible, and more immune evasive than ancestral strains. Together, these have reduced the effectiveness of first-generation vaccines. Therefore, the need for next-generation vaccines that generate broad immunity against diverse VoC (and potentially other coronaviruses) is dire. Several strategies may increase the protective breadth of SARS-CoV-2 vaccines, including multivalent vaccine designs, and mucosal vaccine delivery. Together, these strategies provide multiple targets for the immune system, and draw vaccine-derived immunity to the respiratory tract. To this end, we developed a mucosal next-generation trivalent chimpanzee adenovirus (ChAd) vectored vaccine (Tri:ChAd) encoding two conserved internal viral antigens in addition to SARS-CoV-2 spike.

Method

Using a murine model of vaccination and SARS-CoV-2 infection, we characterized the immunogenicity and efficacy of Tri:ChAd vaccination against multiple SARS-CoV-2 VoC.

Result

piratory mucosal Tri:ChAd vaccination induced more potent immune responses than intramuscular immunization. Critically, the route of respiratory mucosal vaccination was essential for the induction of tripartite immunity within the airway, consisting of tissue resident memory T cells, class-switched antibody-producing B cells, and trained airway macrophages. Each of these components of the immune response, and each of the viral antigens, were important for the breadth of protection observed.

Conclusion

Altogether, our work demonstrates that single-dose intranasal Tri:ChAd vaccination is markedly superior to intramuscular vaccination, and the resultant pulmonary immunity when combined with immunity against conserved viral antigens is protective against SARS-CoV-2 VoC. These encouraging pre-clinical results are currently being validated in a phase I human clinical trial (NCT05094609).

Oral Abstract Session: Basic Science: SARS-CoV-2 - Novel treatment and prevention strategies

Kent Kirshenbaum - AOXI0472

CLAROMERS: Synthetic oligomer mimics of antimicrobial peptides for treatment and prevention of SARS-CoV-2 and other respiratory viruses

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Background

New antiviral agents are urgently needed that can effectively address current SARS-CoV-2 strains and retain activity against future Variants of Concern. Antimicrobial peptides (AMPs) represent a novel source of potential antiviral agents, as they can inactivate enveloped viruses through disruption of their membrane structures. For example, the human cathelicidin peptide LL-37 exhibits broad spectrum activity against bacteria, fungi and viruses. LL-37 is virucidal for influenza and KHSV through disruption of the viral envelope. However, clinical introduction of AMPs as antimicrobials is hampered by many factors, particularly the susceptibility of peptides to proteolytic degradation in vivo. To circumvent this problem, we are developing N-substituted glycine peptoid oligomers, also termed CLAROMERs™. In addition to being resistant to proteases, CLAROMERS also exhibit increased membrane permeability. We previously reported initial results establishing that CLAROMERS act directly on the virion, in pre-attachment phase, and can inactivate both HSV-1 and SARS-CoV-2 (Diamond et al., Pharmaceuticals, 2021). Here we examine the potential of CLAROMERS to treat and prevent SARS-CoV-2 infections through a series of pre-clinical in vitro and in vivo studies.

Method

In vitro efficacy assays were conducted by preincubation of CLAROMERs with a panel of respiratory viruses, including coronaviruses such as SARS-CoV-2, followed by plaque quantification in Vero E6 cells. In vivo studies of prophylactic and therapeutic efficacy were performed in a Syrian Hamster model of SARS-CoV-2 infection, quantifying weight loss and lung virus titer.

Result

Certain CLAROMER compounds exhibited favorable in vitro efficacy against a panel of coronaviruses, with EC50 values in the nanomolar concentration range. In vivo studies demonstrated potent efficacy at preventing weight loss in Syrian Hamsters both when administered prior to and also subsequent to challenge with SARS-CoV-2 virus. Additional mechanism of action studies suggest that the presence of negatively charged phospholipids within the viral envelope are a critical feature to enable selectivity towards viral membranes versus host cell structures.

Conclusion

The data establish that CLAROMERs represent a promising new class of broad-spectrum antiviral agents for prophylaxis or treatment of SARS-CoV-2 and also suggest their potential as therapeutics for other enveloped respiratory viruses. CLAROMERs exhibit selectivity toward targeting virus membranes and are well tolerated in vivo. The mechanism of action involves engaging viral membrane lipid constituents, suggesting that CLAROMERS may retain activity against emerging SARS-CoV-2 variants, even as ongoing mutations create extensive variations in viral protein structures.

Oral Abstract Session: Basic Science: SARS-CoV-2 - Pathogenesis & Transmission

Alba Escalera - AOXI0044

Mutations in SARS-CoV-2 variants of concern link to increased spike cleavage and virus transmission

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Background

Since 2019, SARS-CoV-2 lineages have diverged into highly prevalent variants termed Variants of Concern (VOCs). These VOCs harbor different mutations in the spike (S) protein that may have an impact on efficient spike cleavage, viral transmission, pathogenesis and escape from antibodies induced by infection and vaccination. Here, we investigated the role of arising S polymorphisms in vitro and in vivo to understand the emergence of SARS-CoV-2 variants.

Method

SARS-CoV-2 WA1 (USA-WA1/2020) encoding for S amino acid (aa) mutation H655Y was obtained after passaging in mink, VeroE6 cells or directly from nasal swabs of infected patients. VOCs were obtained from BEI resources. SARS-CoV-2 isolates were used to study viral kinetics, spike cleavage efficiency and syncytia formation in VeroE6, Vero-TMPRSS2 and human pneumocyte-like cells. Mass-spectrometry was also used to quantify abundance furin-cleaved S peptide. WA1-655Y was used to determine transmission in viral competition experiments in Syrian golden hamsters and human pneumocyte-like cells. Cell-cell fusion assays were performed to quantify syncytia formation. Finally, phylogenetic analysis was performed to understand high prevalent aa changes within and surrounding the furin cleavage of the S protein of current circulating SARS-CoV-2 VOCs.

Result

First, we showed that the S:655Y is selected after in vivo replication in the mink model. This mutation is present in the Gamma and Omicron VOCs, but it also occurred sporadically in early SARS-CoV-2 human isolates. To better understand the impact of this polymorphism, we analyzed the in vitro properties of a panel of SARS-CoV-2 isolates containing the S:655Y in different lineage backgrounds. Results demonstrated that this mutation enhances viral replication, spike protein cleavage and syncytia formation. Moreover, viral competition experiments showed that S:655Y substitution was transmitted more efficiently than its ancestor S:655H in the hamster infection model and was able to overcome S:655H in the human airway epithelial system. Finally, we investigated the cleavage efficiency and fusogenic properties of selected VOCs containing different mutations in their spike proteins. Results showed that novel circulating VOCs have independently acquired mutations associated with a gain in spike cleavage and syncytia formation. Taken together, our study shows a link between an increased spike processing and virus transmission due to the spike mutations present in SARS-CoV-2 variants that become epidemiologically more prevalent in humans.

Conclusion

The S:655Y is an important adaptative mutation that enhances viral cell entry, transmission, and host susceptibility. Moreover, SARS-CoV-2 VOCs showed a convergent evolution that promotes the S protein processing and syncytia formation.

Oral Abstract Session: Basic Science: SARS-CoV-2 - Pathogenesis & Transmission

Oyahida Khatun - AOXI0218

SARS-CoV-2 ORF6 protein targets TRIM25 mediated ubiquitination of RIG I to mitigate Type I Interferon Signalling

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Background

SARS-CoV-2 ORF6, a small protein consisting of 61 amino acids, inhibits the interferon (IFN) induction and signaling. Its primary mode of action has been described as inhibition of the nuclear import of transcription factors like IRFs, and STATs. These factors are downstream of viral detection by RIG I. The action of ORF6 on the upstream event of viral detection by RIG I has not been described and the molecular basis of the same was unclear.

Method

We screened the Type I IFN-antagonistic ability of SARS-CoV-2 proteins using an IFN beta promoter-driven dual-luciferase assay which revealed ORF6 as one of the most potent inhibitors of both IFN induction and signaling. The IFN-antagonistic activity of ORF6 was mapped through domain deletion and mutagenesis studies, and the effect of the same on nuclear import IRF3 was tested. Next, immunoprecipitation was performed to examine the interaction of ORF6 with key mediators of the IFN pathway. Furthermore, the impact of ORF6 on RIG I ubiquitination through TRIM25 was examined.

Result

ORF6 directly interacts with RIG I and blocks its ubiquitination. More specifically, ORF6 restricts K63-linked ubiquitination of RIG I, which regulates its activation and stability. It does so by targeting TRIM25 E3 ligase for proteasomal degradation and the C-terminal cytoplasmic tail of ORF6 is crucial for this activity.

Conclusion

SARS-CoV-2 ORF6 inhibits K63-linked ubiquitination of RIG I by E3 ligase TRIM25, which leads retards the downstream signaling leading to type I IFN induction. This activity is mapped to the C-terminal cytoplasmic domain of ORF6.

Oral Abstract Session: Basic Science: SARS-CoV-2 - Pathogenesis & Transmission

Li Wang - AOXI0458

Diminished capability of SARS-CoV-2 Omicron variant to replicate at febrile temperature

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Background

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has spread globally and the resultant COVID-19 pandemic has caused an unprecedented health crisis. SARS-CoV-2 replicates efficiently in the upper and lower respiratory tracts, which exhibit different temperatures. Although most infections are asymptomatic or only result in mild symptoms, many infections cause severe illness or even death. Here we evaluated the replication kinetics of multiple SARS-CoV-2 strains circulating from early 2020 to late 2021 at a wide range of temperatures (28 to 40°C, representing low temperature in the nasal valve area to high febrile temperature) and compared their replication profiles with influenza viruses and common cold respiratory viruses.

Method

Wild type SARS-CoV-2 and influenza viruses were isolated at the US CDC laboratories from clinical specimens. SARS-CoV-2 fluorescent reporter viruses were generated by reverse genetics. Endemic human coronaviruses (229E, OC43, NL63) and rhinoviruses were obtained from the International Reagent Resource and ATCC. Cells were infected with SARS-CoV-2, influenza viruses, and endemic coronaviruses and rhinoviruses and were incubated at a range of temperatures. Supernatants were collected at different time points post infection and virus titers were determined by TCID50. Cytopathic effect and fluorescence signals (for SARS-CoV-2 fluorescent reporter viruses) were observed and recorded at different time points post infection. All experiments involving SARS-CoV-2 were performed in BSL-3 laboratories.

Result

The SARS-CoV-2 progenitor virus and most variants replicated only slightly less efficiently at 40°C compared to 37°C, while the replication of the Omicron subvariants, BA.1 and BA.2, was severely inhibited at 40°C. The spike protein was the main viral factor responsible for the growth attenuation of the Omicron variant at 40°C but non-spike genes also contributed. Characterization of other respiratory viruses revealed that the replication kinetics of the influenza A(H1N1)pdm09 virus was similar to the SARS-CoV-2 progenitor at various temperatures. In contrast, influenza A(H3N2) and influenza B viruses, human coronaviruses (229E, OC43, and NL63), and human rhinovirus species A and B all displayed attenuated replication at 39 to 40°C.

Conclusion

This study demonstrated that pre-Omicron SARS-CoV-2 successfully replicated in a broader range of clinically relevant temperatures compared to other respiratory viruses, which may contribute to the disease severity of COVID-19. The impaired replication of the BA.1 and BA.2 Omicron subvariants at 40°C may contribute to the attenuation of this variant in vivo and may represent a milestone in the evolution of SARS-CoV-2 toward a seasonal, influenza-like or common cold-like virus.

Anika Singanayagam - AOXI0578

Viral emissions into the air and environment after SARS-CoV-2 human challenge

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Background

Non-pharmaceutical interventions (NPIs) contribute substantially to slow the advance of the COVID-19 pandemic. The effective implementation of NPIs relies on identifying and isolating contagious individuals, which requires understanding of the timing and duration of their contagiousness. Whilst viral load in upper respiratory swabs has been commonly used to infer potential infectiousness, measuring viral emissions into the air and the consequent environmental contamination may be more accurate to indicate the chance of onward transmission and to identify likely routes. SARS-CoV-2 human challenge studies provide opportunities to sample early, daily and through whole course of infection, including the incubation period.

Method

36 healthy, young, immunologically-naïve participants were intranasally inoculated with 10 TCID50 of SARS-CoV-2 (D614G wild-type virus). Nose and throat swabs were collected daily. Emissions were collected daily from the air (using a Coriolis air sampler and directly into adapted facemasks) and the surrounding environment (via surface and hand swabs). The amount of SARS-CoV-2 RNA and viable virus was quantified, as well as human housekeeping genes to indicate total airborne emissions.

Result

18 of 36 participants (50%) became infected, resulting in protracted high viral loads in the nose and throat following a short incubation period, with mild symptoms (Killingley et al. Nature Medicine 2022). No viral contamination was detected in the breath, air or rooms of uninfected participants. All 18 infected participants exhaled virus-laden particles into the air and/or contaminated the surrounding environment; however, the extent, timing and route of contamination was very heterogeneous. Several individuals emitted virus in the absence of, or before, symptoms. Virus shedding into the environment and air was more strongly correlated with virus replication in the nose than in the throat, suggesting that the infected nasal mucosa may be the source. Individuals who reported the highest symptoms score were not those who emitted most virus. Finally, we observed a small subset of infected individuals (2/18, 10%) who emitted disproportionately higher amounts of virus into the air (90%), giving support to the notion of superspreaders. These individuals showed early high nasal shedding of viral RNA, at the time of airborne virus emission, but not higher output of human markers, and reported few symptoms.

Conclusion

Human challenge captured the earliest events in the course of SARS-CoV-2 infection, demonstrating that presymptomatic shedding likely contributes to the difficulty in controlling spread of respiratory viruses. Our data implicates the nasal mucosa as a key source of onwards infection.

Oral Abstract Session: Basic Science: SARS-CoV-2 - Pathogenesis & Transmission

Andrew Bowman - AOXI0648

SARS-CoV-2 in white-tailed deer indicates establishment of the species as a wildlife reservoir in North America

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Background

The role of animals in SARS-CoV-2 epidemiology and ecology is unknown; however, white-tail deer have become an increasing concern as a potential wildlife reservoir because they have been experimentally and naturally infected. Our report of SARS-CoV-2 in free-ranging white-tailed deer in northeast Ohio in early 2021 was the first reported detection in this species globally. Since then, we expanded our surveillance beyond our initial study area to explore the spread and persistence of SARS-CoV-2 in white-tailed deer.

Method

Between October 2021 and March 2022, we collected 1522 nasal swabs from free-ranging white-tailed deer across Ohio. Our sampling covered 83 of Ohio's 88 counties, with all samples coming from deer harvested by hunting or deer population management programs.

Result

We detected SARS-CoV-2 RNA in 10.6% (n=161) of the samples that represented 48 counties. We were able to generate high-quality sequence data for 82 of the positive samples. The vast majority (n = 73) of the viruses belonged to the Delta lineage (B.1.617 and 13 sub-lineages). Each lineage/sub-lineage represents an independent human-to-deer transmission event and some lineages were introduced multiple times from humans to deer. Additionally, nine alpha (B.1.1.7) viruses were identified in white-tailed deer in four counties in southern Ohio. A time-scaled maximum clade credibility inferred using Bayesian methods for B.1.1.7 viruses identified in deer and humans in Ohio indicated these 9 deer viruses cluster into two clades that represent two independent introductions from humans into deer. The MCC tree for one of the clusters estimates that this introduction occurred during April - June 2021, when the virus was widespread in humans, and was maintained in deer until our sampling in late 2021.

Conclusion

The widespread, repeated human-to-deer introductions and subsequent deer-to-deer transmission that sustained the virus over > 6 months indicates white-tailed deer have become a wildlife reservoir of SARS-CoV-2.

Oral Abstract Session: Basic Science: Zoonotic Influenza

Charlotte Kristensen - AOXI0128

Preparing for the next pandemic: Impact of pigs challenged with pre-pandemic, swine-adapted and human-adapted influenza A H1N1pdm09 viruses

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Background

Influenza A virus (IAV) causes respiratory disease in a variety of mammals and due to exchanges of IAVs between humans and pigs, the risk of creating new viruses with pandemic potential is present. It is unclear why some IAV strains that evolve in swine can infect and transmit between humans while others seem to be swine specific. This study aimed to compare the infection dynamic and pathogenesis of a presumed pre-pandemic, swine-adapted and human-adapted H1N1pdm09 virus.

Method

A total of 42 seven-week-old Danish Landrace pigs were challenged with 4 ml of 10^7 TCID50/ml of either swine-adapted H1N1pdm09 (n=12), human-adapted H1N1pdm09 (n=12), a presumed pre-pandemic H1N1pdm09 (n=12) or mock (n=6). Nasal swabs, body weight measurements and rectal temperatures were obtained during the study. The control group and eight pigs from each virus-challenged group were euthanized three days post-inoculation (DPI), whereas four pigs from each virus-challenged group were euthanized 14 DPI. Three different areas of lung tissue were collected at necropsy. Quantification of IAV in nasal swabs and lung tissues was investigated by reverse-transcriptase qPCR. The total viral load 0-3 DPI was calculated as the median under the curve (AUC). The clinical impact was evaluated based on observations of respiratory disease symptoms, the proportion of days with rectal temperatures above 40°C and body weight gains.

Result

The highest viral shedding was found in the swine-adapted H1N1pdm09 group at all DPIs until 10 DPI. At 14 DPI, none of the swine-adapted H1N1pdm09 pigs tested positive for IAV, whereas one pig from the human-adapted H1N1pdm09 group and one pig from the pre-pandemic H1N1pdm09 group tested positive for IAV. The total viral load 0-3 DPI was significantly higher in the swine-adapted H1N1pdm09 group compared to the human-adapted H1N1pdm09 group. The highest clinical impact was found in the pre-pandemic H1N1pdm09 group while the lowest clinical impact was found in the swine-adapted H1N1pdm09 group.

Conclusion

A high viral load combined with low clinical impact found in the swine-adapted group and a low viral load combined with high clinical impact found in the human-adapted group demonstrate the importance of host adaptation concerning viral fitness and virulence. This suggests that spillover events of swine-adapted IAVs to humans could potentially result in more severe disease outcome compared to infections with human-adapted IAVs. Further studies investigating antiviral immune responses and viral sequencing are required to determine host factors important for the host adaptation.

Oral Abstract Session: Basic Science: Zoonotic Influenza

Pia Ryt-Hansen - AOXI0099

Rapid surge of the H1pdmN1av reassortant in Danish swine and its zoonotic potential

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Background

The intensification of swine production over the last few decades has resulted in influenza A virus becoming an enzootic prevalent pathogen circulating all-year-round in the swine herds. Consequently, several subtypes of swine influenza virus are circulating and the risk of reassortment events has increased and novel influenza A viruses with unknown zoonotic potential are being generated continuously, posing a potential threat to the public health.

Method

Denmark has had a passive surveillance program of influenza A viruses in swine since 2010, which has allowed the discovery of novel reassortants occurring in Danish swine herds. Using multiplex real time RT-PCR, the HA and NA lineages were determined for 33-77 % of the influenza A virus positive submissions in the surveillance program between 2010-2021. Each year, whole genome sequencing and phylogenetic analysis were performed on selected samples representing the different lineages identified. In addition, antigenic characterization of H1pdmN1av isolates was performed.

Result

In 2018, one submission contained a novel reassortant termed "H1pdmN1av" as it contained an HA gene of H1N1pdm09 origin and an NA gene of Eurasian avian-like swine H1N1 origin. This reassortant increased rapidly in prevalence in the Danish herds, and by 2021, it accounted for 24 % of all submissions that had the HA and NA lineage determined. Whole genome sequencing of more isolates revealed that the H1pdm09N1av virus had two different internal gene constellations; one with an internal cassette of H1N1pdm09 origin, and a similar one, but with an NS gene of Eurasian avian-like swine origin. Phylogenetic analysis revealed that especially the HA gene of these reassortants was genetically distant to other H1N1pdm09 viruses circulating in Danish swine. In addition, the antigenic characterization confirmed that this virus was antigenically distant to other swine and human H1N1pdm09 isolates. In 2021, this H1pdmN1av of swine origin led to the second human zoonotic transmission documented in Denmark.

Conclusion

The surveillance program was successful in detecting the novel H1pdmN1av reassortant and documented the rapid spread of the reassortant in Danish swine herds. The genetic and antigenic analysis combined with the recent spillover event to human, emphasize that this reassortant should be of concern as limited immunity and vaccine effect is expected in the human population.

Ghazi Kayali - AOXI0175

Cross-species spill-over potential of the H9N2-like bat influenza virus

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Background

Influenza A viruses (IAVs) spread among a variety of different hosts and cross species barriers. While waterfowl have long been the only relevant natural reservoir for IAVs, infections in mammalian hosts pose a great risk for human infections. Bats carry viruses asymptomatically and are natural reservoirs for multiple zoonotic viruses. In 2009-2010, two novel IAV subtypes (H17N10 and H18N11) were detected molecularly in South American bats. In 2017, we isolated a novel H9N2-like IAV (A/bat/Egypt/381OP/2017) in Egypt from Egyptian fruit bats. This virus was successfully propagated in MDCK cells and embryonated chicken eggs and was found to be able to infect and transmit within bats in laboratory colonies. Here, we conducted natural infection experiments and several biological assays to understand the cross-species spill-over potential of this virus.

Method

DBA/2J mice were experimentally infected with the bat virus and its most similar H9N2 avian virus and monitored for signs of morbidity and mortality. A similar experiment was conducted in ferrets and included the addition of direct contact and aerosol contact ferrets to assess transmissibility. Ducks and mallards were infected to assess replication and transmissibility. The virus was inoculated in ex-vivo human bronchus cells. HA stability and NA activity were assessed. Glycan binding affinity was tested.

Result

In mice, the virus replicated readily in nasal turbinates and lungs. Pathological findings indicated that the virus was highly pathogenic to respiratory epithelium in the nose, trachea, bronchi, and bronchioles. Body weight loss and mortality were observed. In ferrets, the virus replicated in lungs, trachea, nasal turbinates, and brains. The virus transmitted to direct and aerosol contact animals. The virus did not replicate in chickens and mallard ducks. Ex-vivo human bronchus cultures were readily infected by the virus. HA stability results indicated that the bat virus was resistant to inactivation by exposure to extracellular pH similar to human-adapted IAVs. The virus had NA activity similar to known NA subtypes. Higher affinity to α2,3 sialic acid receptors was noted.

Conclusion

The virus crossed the species barrier to infect mammalian species without the need for prior adaptation. Though the virus did not replicate in avian species, it was able to grow in chicken eggs suggesting that cross-species transmissibility may occur but adaptation is needed. The H9N2-like bat IAV poses a risk to humans and other mammalian species and may also transmit to avian species. Surveillance for bat

influenza viruses is needed to understand their diversity and zoonotic potential.

Oral Abstract Session: Basic Science: Zoonotic Influenza

MARIA OGRZEWALSKA - AOXI0520

Influenza A (H11N2) detection in fecal samples from Adelie (Pygoscelis adeliae) and chinstrap (Pygoscelis antarcticus) penguins, Penguin Island, Antarctic.

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Background

Although wild birds play a role in the transmission and ecology of Avian Influenza Viruses (AVI) across the globe, there are significant gaps in our understanding of the worldwide distribution of these viruses in polar environments. Previous serological studies show evidence that AIV infection is widespread and prevalent in Antarctic birds, however the pathogenicity and genetic characterization of AIV in these populations remains limited.

Method

Fecal samples of penguins were collected throughout November and January/February of the 2019/2020 breeding season in Antarctic summer season, in the South Shetland Islands, close to the Antarctic Peninsula. Fecal material was preserved in viral transport medium and stored in -800C. Influenza A virus was detected using a TaqMan®-based quantitative one step real time RT-PCR assay that covers all subtypes of influenza A virus via detection of a conserved matrix (M) gene region. For influenza A virus whole genome sequencing, the viral RNA was used in a Multi-segmented reverse transcription PCR protocol, using influenza A universal primers. The cDNA library was constructed using the Nextera XT DNA Library Preparation Kit (Illumina) and submitted to sequencing by the Illumina MiSeq System. The obtained high-quality sequences were used to for phylogenetic analyses.

Result

In the present study, a total of 97 environmental and avian fecal samples collected in the seven locations in Antarctica, were tested by RT-PCR. Five out of seven samples collected in Penguin Island were positive; four of these were collected in the environment from the colony of P. antarcticus and one sample collected of one isolated P. adeliae. AIV whole and partial genomes were recovered from four samples. The phylogeographic analysis revealed, the clusterization of obtain sequences with all available Antarctic AIV H11N2, in a highly supported cluster. The TMRCA inferred for the Antarctic cluster in this analysis showed the continued circulation of the H11N2 subtype in the continent at least since the beginning of the second decade of the XXI century. In all trees but in the HA one, the detected cluster had a common ancestor outside Antarctica in North America.

Conclusion

This identification suggests the persistence of Influenza A(H11N2) in penguins' colonies in South Shetland Islands once it was first identified on 2014 in P. adeliae living around the Antarctic Peninsula. Our results reinforce the need for continuous surveillance of avian influenza in the Antarctic continent adding new data about to the natural history and ecology of AIV.

Oral Abstract Session: Basic Science: Zoonotic Influenza

Mathilde Richard - AOXI0623

Mapping the molecular basis of antigenic evolution of H5 highly pathogenic avian influenza viruses to support the design of broadly reactive vaccines.

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Background

Avian H5 influenza viruses from the A/goose/Guangdong/1/96 (GsGd) lineage pose a continuing threat to animal and human health. Since their emergence in 1997, H5 GsGd viruses spread across multiple continents and have become enzootic in poultry, causing considerable economic losses and regular zoonotic transmissions to humans with concerns about the potential for a new influenza virus pandemic. The hemagglutinin (HA) of H5 GsGd viruses diversified into several genetic and antigenic clades, posing serious challenges to pandemic preparedness and vaccine design. Antigenic drift is a well-studied phenomenon for seasonal human influenza viruses, but knowledge of the drivers and molecular basis of antigenic evolution avian influenza viruses is limited. Here, we identified the major molecular determinants in HA underlying the antigenic evolution and diversity of H5 viruses.

Method

Using synthetic HA genes representative of all H5 viruses, a three-dimensional antigenic map was generated based on cross-hemagglutination inhibition data, representing the antigenic evolution of H5 viruses from 1959 until 2022. Antigens were groupedd in 10 clusters using the k-means clustering algorithm and one representative prototype virus per cluster was selected. Site-directed mutagenesis and reverse genetics were used to generate mutant viruses, whose antigenic phenotype was assessed in hemagglutination inhibition assays against a selection of post-infection ferret antisera.

Result

Antigenic difference between prototype viruses from non-GsGd and early GsGd clusters and those from the other eight antigenic clusters were due to three to eleven substitutions in HA. These substitutions were in close proximity to the receptor binding site, similar to what has been observed for human seasonal influenza viruses.

Conclusion

The evolutionary patterns and molecular basis of antigenic change in H5 viruses are poorly understood, hampering the formulation of optimal vaccination strategies. We are currently using the knowledge generated here on substitutions that modulate H5 antigenic phenotype to refine the design of antigenically central vaccine antigens, aimed to confer broad-reactivity to the majority of viruses in the antigenic space.

Oral Abstract Session: Implementation: Burden of Disease for Influenza and Dual Epidemics

Chelsea Hansen - AOXI0207

Excess mortality from influenza and RSV during the COVID19 pandemic in the US: a natural experiment to clarify the etiology of respiratory deaths

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Background

The COVID-19 pandemic was marked by low circulation of endemic respiratory pathogens, providing a unique natural experiment to clarify the etiology of respiratory mortality in different age groups. Here, we compare US excess mortality estimates for influenza and RSV during 2019/2020 and 2020/2021, relative to excess mortality and seasonal baselines in the last 20 years.

Method

We used weekly age-specific US mortality counts for two underlying causes of death (respiratory diseases - excluding COVID19 codes, and pneumonia and influenza (P&I)) for 1999-2021. Weekly counts were extrapolated from monthly data for 2020-2021. We used linear regression models to attribute weekly age-specific mortality fluctuations to influenza and RSV viral activity.

Result

The 2019/2020 influenza season was characterized by a peak of influenza B followed by a peak of A/H1N1pdm09, and a sharp decline in March 2020. Influenza circulation remained low throughout 2020/2021 and was dominated by influenza B. RSV circulation also dropped sharply in March 2020 and rebounded in April 2021, an off-season increase. Underlying P&I and respiratory mortality were lower in 2020/2021 in all age groups compared to earlier seasons (Figure). Seasonal baseline mortality not accounted for by influenza or RSV was stable during the 2020/2021 season for those >5 years but decreased substantially in younger ages. In 2019/2020 we estimate 4999 (3681-6433) underlying P&I deaths associated with influenza, and 3508 (2857-4168) deaths associated with RSV, compared to historical annual averages of 5143 (4867-5419) for influenza and 3834 (3664-4005) for RSV. In 2020/2021 we estimate a 10-fold reduction in RSV mortality, and no excess influenza mortality in the youngest and oldest age groups. Similar trends were seen for underlying respiratory mortality.

Conclusion

Influenza and RSV excess mortality was typical of past seasons in 2019-2020 but greatly decreased during the 2020-2021 winter, likely due to non-pharmaceutical interventions. RSV activity rebounded in summer 2021, earlier than influenza; whether this rebound will lead to out-of-season excess mortality remains unclear given lags in data. Age-specific changes in baseline mortality during COVID-19 suggest differences in the viral and bacterial etiologies of respiratory deaths, which will be further explored. Underlying P&I mortality does not seem to capture many misattributed COVID-19 deaths and remains a reliable indicator of influenza and RSV mortality.

Oral Abstract Session: Implementation: Burden of Disease for Influenza and Dual Epidemics

Steph Wraith - AOXI0231

Presented by Aubree Gordon

Impact of heterotypic and heterosubtypic repeat influenza infection patterns in a pediatric cohort in Managua, Nicaragua

Steph Wraith[1], Angel Balmaseda[6], Fausto Andres Bustos Carrillo[3], Guillermina Kuan[5], John Kubale[2], Roger Lopez[4], Sergio Ojeda[4], Amy Schiller[2], Brenda Lopez[4], Nery Sanchez[4], Eva Harris[3], Aubree Gordon[2]

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Background

Influenza poses a significant public health burden each year, and the effects of hetero(sub)typic influenza interactions across seasons have not yet been explored thoroughly. Understanding these dynamics is critical to addressing future risk patterns for individuals and populations as well as improving influenza vaccine designs.

Method

Using data from a pediatric cohort study in Managua, Nicaragua, we explore and model the effects of repeated infections with distinct influenza virus types across multiple seasons. Children aged 0-14 were followed from 2011-2019 with yearly influenza captured by RT-PCR. The effect of influenza infection in a prior season (main exposure) on an infection with a different influenza virus type or subtype in the same or subsequent season (main outcome) was quantified using odds ratios and 95% confidence intervals estimated from logistic regression; models controlled for age and sex.

Result

Within same-season analyses, there was no significant evidence of protection from repeat hetero(sub)typic infection, with results generally centered around the null; however, there was a protective trend present in certain seasons. In cross-season analyses, we found that individuals infected with influenza virus when multiple types or subtypes were circulating were at significantly increased odds for symptomatic infection from other circulating types or subtypes during the subsequent season.

The increase in odds was particularly strong for hetero(sub)typic comparisons examining individuals who were initially infected with either influenza virus A or B in seasons where the two types co-circulated, and where the other influenza virus type circulated in the subsequent season. Children infected with H1N1pdm in 2018 exhibited heightened odds of infection with B/Victoria in 2019 (OR 3.57, CI 1.62-7.89); children infected with H3N2 in 2013 exhibited heightened odds of infection with H1N1pdm in 2015 (OR 2.61, CI 1.42, 4.78). The odds of infection remain elevated after age stratification and adjustment for healthcare-seeking behavior and pre-exposure antibody titer levels.

Conclusion

Overall, this study demonstrates that the odds of repeated hetero(sub)typic influenza virus infection increase in subsequent seasons following initial infection, and that the odds of repeated infection remain elevated irrespective of pediatric age or healthcare-seeking behavior. These findings highlight the importance of understanding patterns of exposure and infection, particularly among children who have a high burden of influenza. Our results further underscore how such patterns can drive both individual risk and the overall dynamics of influenza across multiple years of viral circulation.

Oral Abstract Session: Implementation: Burden of Disease for Influenza and Dual Epidemics

Ian McGovern - AOXI0245

Effects of Stacking Influenza Risk Factors on Odds of Influenza-Related Hospitalization

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Background

Patients with one or more influenza risk factors are a commonly targeted group for influenza vaccination and reimbursement. While several studies have evaluated factors that influence the risk of severe influenza outcomes, there is limited evidence on the potential additive impact of having multiple influenza risk factors and how this effect may vary by age. This study evaluated how an increasing number of influenza risk factors affected odds of having an influenza hospitalization in the overall population and among different age sub-groups.

Method

Patients 18 years of age or older in the US were evaluated retrospectively in five seasonal cohorts (2015-2016 through 2019-2020 seasons). Patient-level electronic medical records linked to pharmacy and medical claims were used to ascertain covariate and outcome information. Multivariable logistic regression models were fitted for the overall population and by age group to evaluate potential differences in the odds of an influenza-related inpatient hospitalization (ICD-10 codes J09*-J11*) based on number of influenza risk factors (as defined by the US CDC, excluding age). The logistic regression models adjusted for sex, race/ethnicity, geographic region, baseline healthcare resource use, vaccination status, presence of specific high-risk comorbidities, BMI, and smoking status. Odds ratios (OR) from each of the five individual seasons were summarized using fixed-effects meta-analysis.

Result

Season cohort sizes ranged in size from 887,260 to 3,628,168 individuals. There was minimal variation across seasons and the pooled estimates showed similar trends of the individual season estimates (Figure 1). There was a clear trend in increased odds of hospitalization with increasing number of risk factors compared to no CDC risk factors. The trend remained consistent across age groups, with the confidence intervals (CI) of individual age groups generally overlapping the overall population point estimate. For the overall population, when compared to patients with no risk factors, patients with one risk factor had a pooled OR of 1.8 (95%CI: 1.7 to 2.0) and patients with four risk factors had a pooled OR of 6.4 (95%CI: 5.8 to 7.0) for influenza hospitalization.

Conclusion

There is a clear trend towards increased odds of influenza hospitalization with increasing number of influenza risk factors. These results show that a simple measure like the number of influenza risk factors can be highly informative of a patient's potential for severe influenza outcomes.

Oral Abstract Session: Implementation: Burden of Disease for Influenza and Dual Epidemics

Sandra Chaves - AOXI0216

THE CONTRIBUTION OF INFLUENZA TO ISCHEMIC HEART DISEASE MORTALITY WORLDWIDE

Sandra Chaves[5], Joshua Nealon[7], Katrin Burkart[4], Daniel Modin[2], Tor Biering-Sørensen[2], Justin R. Ortiz[1], Victor Vilchis Tella[4], Lindsey Wallace[4], Gregory Roth[3], Cedric Mahe[6], Michael Brauer[4]

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Background

Ischemic heart disease (IHD) is the leading cause of global mortality, responsible for 16% of all deaths. Experimental and observational studies suggest that influenza virus infection causes cardiovascular changes that could contribute to IHD mortality. This study leverages extensive data on cause-specific mortality combined with influenza surveillance and other environmental data to estimate the burden of IHD attributable to influenza globally, with estimates spanning from 2010-2019.

Method

Our methodological approach builds upon tools developed at the Institute for Health Metrics and Evaluation for the Global Burden of Disease (GBD) project. We assessed the relationship between influenza activity and IHD mortality across 10 different countries in a non-linear meta-regression framework, integrating generalized additive models with shape constraints into the Meta-regression-Bayesian, Regularized, Trimmed (MR-BRT) tool. Then, we combined the derived relationship between influenza activity and IHD mortality with observed and modeled influenza activity data from 204 countries and territories to estimate the global burden of IHD mortality attributable to influenza by location, year, and age. We estimated the population-attributable fraction (PAF) of influenza upon IHD deaths, for each week and country, based on the relative risk (RR) associated with a given level of weekly influenza activity and multiplied PAFs by the burden of IHD deaths from the GBD study.

Result

Influenza activity was consistently associated with increased risk of both IHD and all-cause mortality across all countries analyzed. The relationship remained significant after adjustment. There was substantial heterogeneity in PAF estimates by country and region that could not be explained by population density, temperature, the proportion of positive influenza samples, or the socio demographic index. The PAF of influenza upon IHD mortality ranged from <1% to 10%, depending on location and year. Highest PAFs were observed in Southeast and South Asia, West Africa, and South and Middle America (Figure). The lowest global number of attributable deaths was observed in 2011 with 248,900 deaths and the highest in 2019 with 363,800 deaths. On average, we estimated that annually 300,000 IHD deaths can be attributed to influenza. Highest total numbers were observed in Southeast Asia, East Asia, and Oceania as well as South Asia.

Conclusion

This is the first study to estimate the global IHD mortality burden attributable to influenza. Our results suggest that by preventing influenza we could reduce up to 10% of IHD deaths in high-risk countries and regions.

Oral Abstract Session: Implementation: Burden of Disease for Influenza and Dual Epidemics

Eduardo Azziz-Baumgartner - AOXI0639

Incidence of respiratory virus illness and hospitalizations in a Panama and El Salvador birth cohort, 2014-2018

Eduardo Azziz-Baumgartner[1]

[1]CDC

Background

Respiratory viruses remain a key cause of early childhood illness, hospitalization, and death globally. The recent pandemic has rekindled interest in the control of respiratory viruses among pediatric populations. We estimate the burden of such viruses among children <2 years.

Method

Enrolled neonates were followed until two years of age. Weekly active symptom monitoring for the development acute respiratory illnesses (ARI) defined as cough, rhinorrhea, difficulty breathing, asthenia, anorexia, irritability, or vomiting was conducted. When the child had ARI and fever, nasopharyngeal swabbing was performed and samples were tested through singleplex RT-PCR. Incidence of respiratory viruses was calculated by dividing the number of laboratory-confirmed detections by the person-time accrued during weeks when that virus was detectable through national surveillance then corrected for under-ascertainment among untested children.

Result

During December 2014-November 2017, 1,567 enrolled neonates contributed 2,0186·9 person-years (py). Six in ten (64·4%) children developed ARI (total 2,493 episodes). Among children <2 years, incidence of respiratory syncytial virus (RSV) (21·0, 95%CI 19·3-22·8, per 100py) and rhinovirus -associated ARI (20·5, 95%CI 20·4-20·7) were similar and higher than parainfluenza 1-3 (14·2, 95%CI 12·2-16·1), human metapneumovirus (9·2, 95%CI 7·7-10·8), influenza (5·9, 95%CI 4·4-7·5), adenovirus (5·1, 95%CI 5·0-5·2), bocavirus (1·8, 95%CI 0·7-3·0), and seasonal coronavirus-associated ARI (1·6, 95%CI 0·6-2·7). Overall, few children (217, 13·8%) were fully vaccinated against influenza during the two-year follow-up (191 [21·9%] in Panama, and 26 [3·7%] in El Salvador). Children aged <3 months had the highest rates of RSV ARI (49·1, 95%CI 44·0-54·1 per 100py) followed by children aged 3-5 (25·1, 95%CI 20·1-30·0), 6-11 (17·6, 95%CI 13·2-21·9), and 12-23 months (11·9, 95%CI 10·8-12·9). One in ten children with RSV was referred to the hospital (2·5, 95%CI 2·1-2·8, per 100py).

Conclusion

hildren frequently developed viral ARI and a substantive proportion required hospital care. Despite the well documented effectiveness of standard dose influenza vaccines to protect young children against influenza illnesses, few cohort children were fully vaccinated against influenza (<15%). Such findings suggest the importance of exploring the value of new interventions and increasing uptake of existing prevention measures to mitigate burden of epidemic-prone respiratory viruses. Lastly, children were frequently ill with seasonal coronaviruses at an incidence similar to that estimated for SARS-CoV-2 during the COVID-19 pandemic.

Oral Abstract Session: Implementation: Disease Control Policy for Influenza

Brian Stamm - AOXI0007

Presented by Jonathan Temte

The Influence of Rapid Influenza Diagnostic Testing on Clinician Decision-making for Patients with Acute Respiratory Infection in Urgent Care

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Background

Urgent care facilities provide evaluation and treatment services for patients with acute respiratory infections (ARIs). Few studies have documented the potential benefits to clinical care and prescribing practices by utilizing rapid influenza diagnostic tests (RIDT) in these settings. We compared antiviral and antibiotic prescribing, imaging (x-ray), and laboratory ordering practices in the presence of positive and negative RIDT results with clinical encounters for which RIDT results were unavailable.

Method

We conducted a matched, case-control study comparing patients who received a RIDT when presenting with symptoms of ARI and control patients for which no RIDT was utilized at two urgent care facilities during two periods of elevated influenza circulation (February 7, 2019 - May 13, 2019, November 1, 2019 - March 4, 2020). Cases and controls were matched using one-to-one exact matching on participant sex, 5-year age bin, and week of healthcare encounter. McNemar's 2x2 tests were used to assess association between the likelihood of prescribing, imaging or laboratory ordering and use of RIDT for 1145 matched pairs.

Result

Use of an RIDT, regardless of the result, reduced the odds of antibiotic prescription (OR:0.52; 95% CI:0.43-0.63) and increased the odds of antiviral prescription (OR:3.07; 95% CI:2.25-4.26). Patients who tested positive for influenza infection by RIDT were more likely to be prescribed antivirals (OR:10.23; 95% CI:5.78-19.72), less likely to be prescribed antibiotics (OR:0.15; 95% CI:0.08-0.27), and less likely to have two laboratory tests ordered: rapid streptococcal screening (OR:0.40; 95% CI:0.24-0.67) and influenza A/B/RSV RT-PCR (OR:0.04; 95% CI:0-0.23) compared to matched controls with no RIDT. Patients who tested negative by RIDT were less likely to be prescribed antibiotics (OR:0.70; 95% CI:0.57-0.86), more likely to have imaging (OR:1.70; 95% CI:1.30-2.24), more likely to have a CBC (OR:2.48; 95% CI:1.62-3.90), and less likely to have rapid streptococcal screening (OR:0.68; 95% CI:0.51-0.90) and influenza A/B/RSV RT-PCR (OR:0.15; 95% CI:0.06-0.33) compared to matched controls with no RIDT.

Conclusion

In these urgent care settings, use of RIDTs drastically altered clinician diagnostic practices and patient treatment. This research suggests that implementation of RIDTs could provide clinicians information to improve their diagnostic and prescribing practice, benefit patients by reducing the burden of unnecessary testing, increase efficiency and reduce costs for urgent care centers, assist in mitigating the burden of influenza at the population level, and help confront the spread of antibiotic resistance.

Oral Abstract Session: Implementation: Disease Control Policy for Influenza

George Kassianos - AOXI0158

Influenza prevention during COVID19 pandemic (2020 to 2022): Data from 13 European countries and Israel

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Background

The COVID-19 pandemic has increased appreciation for vaccines among the public and health care practitioners (HCPs). However, the changes in influenza virus circulation during pandemic and prioritization of COVID-19 management by HCPs could potentially impact influenza vaccine coverage rates (VCR) in vulnerable populations. The objective of the RAISE forum is to Raise Awareness of Influenza Strategies in Europe.

Method

The third RAISE survey was conducted with the experts from 13 European countries (Bulgaria, Czech Republic, France, Germany, Greece, Lithuania, the Netherlands, Poland, Portugal, Romania, Slovakia, Spain, and the United Kingdom [UK]) and Israel. The survey assessed the VCR in 2020/21 and 2021/22 influenza seasons, the impact of COVID-19 on the local recommendations for influenza prevention, changes in vaccination strategies, vaccine access, and any challenges in flu campaign due to increased demand for COVID-19 vaccination. Results were presented descriptively by various countries.

Result

Overall, influenza VCR was increased in most of the Western European countries (Germany, Netherlands, Portugal, Spain, and the UK) during the COVID-19 pandemic. However, similar trend was not observed generally in Central and Eastern European countries. The COVID-19 pandemic impacted the local influenza recommendations in few countries. The co-administration of influenza and COVID-19 vaccines was recommended in most of the countries. Bulgaria, the Czech Republic, Israel, Romania, and Lithuania reported challenges in the influenza vaccination campaign. Influenza VCR for the 2021/22 season from few countries is awaited and will be presented at the congress.

Conclusion

The need to tackle with COVID-19 pandemic, combined with very low circulation of influenza viruses, the need for family doctors to do virtual consultations instead of in-person and the loss of chance to do opportunistic vaccination, challenged influenza protection in numerous European countries. Although the COVID-19 pandemic offered an opportunity to reinforce the benefit of vaccination, asymmetries on influenza VCR rates between Western and Central/Eastern European countries are observed due to difference in perception of adult vaccination and limited accessibility in few countries. Thus, achieving consistently high VCRs in the older adults and the other high-risk groups is a major public health challenge in Europe and calls for actions to address it.

Oral Abstract Session: Implementation: Disease Control Policy for Influenza

Wayne Ramkrishna - AOXI0399

Implementing seasonal influenza vaccination in the era of COVID-19, in South Africa (2011-2021)

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Background

Seasonal influenza causes substantial morbidity and mortality in South Africa resulting in an estimated 40,000 hospitalizations and 11,000 deaths annually. The South African Department of Health conducts annual national influenza vaccination campaigns since 2010, targeting people at increased risk for severe influenza and death (target groups). The influenza vaccination programme faced unique challenges during the COVID-19 period. The objective of this report is to describe the effect of COVID-19 on the national influenza vaccination programme.

Method

During 2011-2019, trivalent inactivated influenza vaccine (TIV) was used in the South African public health sector; from 2020, a combination of TIV and quadrivalent inactivated influenza vaccine (QIV) were used.

Influenza vaccines were procured by provinces and distributed to public health facilities country-wide. Vaccinations were recorded on tally sheets.

In 2020, during the start of the COVID-19 pandemic, target groups for government funded influenza vaccines were revised to include health care workers (HCWs) in addition to individuals with underlying medical conditions, elderly, people living with HIV/AIDS and pregnant women.

Result

Before COVID-19 on average 912 575 (range: 800 000-1 040 000) vaccines were procured annually. During COVID-19, 1 203 660 were procured and 1 008 311 utilised in 2020; 871 910 were procured and 612 848 utilised in 2021. Before COVID-19, vaccination campaigns were conducted from April to September. During COVID-19 vaccination campaigns were extended to October 2020 and December 2021. In 2021, staff were re-deployed from routine programmes, including influenza vaccinations, to manage COVID-19 patients and administer COVID-19 vaccines.

Conclusion

In 2020, uptake of influenza vaccines increased by 64 161 (6.8%) compared to 2019. This was mainly due to the Department of Health procuring and distributing additional vaccines to prevent influenza, protect HCWs and conserve medical resources for COVID-19 patients. HCWs, which was the newly added target group contributed to 17.6% (177 718) of the vaccinations.

In 2021, uptake of influenza vaccines declined by 395 463 (39,2%) compared to 2020; uptake was also lower than pre-COVID-19 levels. The main reasons for decline were the concurrent COVID-19 vaccinations which took precedence over influenza and because there was low influenza circulation in 2020, people thought there was no need to vaccinate.

Since influenza virus circulation was detected beyond the normal influenza season in 2020-2021, influenza vaccinations continued up to expiry date of vaccines i.e. 31 December 2021. In 2022, a policy change allowed concurrent administration of influenza and COVID-19 vaccines.

Oral Abstract Session: Implementation: Disease Control Policy for Influenza

Isa Ahmad - AOXI0386

Development of universal influenza vaccines: understanding the industry perspective

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Background

The successful development of future, 'universal' influenza vaccines will require active collaboration between academia, the pharmaceutical industry and public health agencies. As well as modelling target product profiles for future vaccines (see abstract AOXI0237), we sought to understand the industry perspective on the development of universal influenza vaccines: specifically, the barriers and drivers that shape commercial decisions on whether to invest in clinical development of promising new vaccine candidates.

Method

We engaged with a range of experts, from the pharmaceutical and life sciences industry as well as from public agencies. Experts were invited to address questions in a semi-structured interview, for example: 'How would pharmaceutical industry respond to promising new candidates for a broadly protective influenza vaccine?' In addition, we performed a literature search for preclinical studies of vaccines, published between 1996 and 2021. We then mapped how many of these candidates were taken forward to clinical trials, as published in clinicaltrials.gov and trialsearch.who.int.

Result

Despite its drawbacks, current, egg-based vaccine production has important commercial benefits for manufacturers who already heavily invested in this technology. However, it is likely that any future, broadly-protective vaccine would rely on newer, more flexible technologies such as mRNA or viral vectored vaccines. Thus, in order to justify the switch from current egg-based production, it is likely that the performance threshold that a future vaccine candidate needs to meet, to attract investment from major pharmaceutical companies, will be high. Progress towards meeting this threshold is most likely to be driven by academia, biotechnology companies, or other entities not currently invested in egg-based vaccine manufacture. In this context, our literature search found that only 24 published studies (25%) were present in published clinical trial protocols, illustrating a high rate of attrition from discovery to clinical trials.

Conclusion

Our findings suggest that incremental stages in the development of a future, broadly protective influenza vaccine may be unlikely to attract substantial support from major manufacturers of current vaccines, given their existing investment in egg-based production. However, the realisation of a truly disruptive new influenza vaccine may not be possible without the adoption and further improvement of such 'intermediate' vaccine products. Therefore, an important role for donors and public health agencies could be to examine whether appropriately designed push or pull support mechanisms will allow such 'intermediate' products to be sustained.

Oral Abstract Session: Implementation: Disease Control Policy for Influenza

Frederikke Lomholt - AOXI0586

Introduction of influenza vaccination in children aged 2-6 years in Denmark in times of COVID-19, 2021-2022.

Frederikke Lomholt[1], Katrine Finderup Nielsen[1], Ditte Rechter Zenas[2], Manon Chaine[2], Lisbet Krause Knudsen[1], Louise Hallundbæk[1], Palle Valentiner-Branth[1], Lasse Skafte Vestergaard[1]

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Background

Due to concerns about an influenza epidemic coinciding with an increase in COVID-19 cases during the winter season 2021-22, the national free-of-charge seasonal influenza vaccination programme in Denmark was expanded to include children aged 2-6 years. Children were offered a two-dose programme with a nasal spray vaccine. The target vaccination coverage was 85%. The aim of this study was to evaluate vaccination uptake in children aged 2-6 years in Denmark during the first year of the national vaccination programme and to identify determinants influencing vaccine uptake.

Method

We used national registries to identify all eligible children and to obtain individual information on influenza vaccination registrations, place of residence, date of birth, sex, who the child was living with, underlying medical conditions and immigration status. We calculated the national vaccination coverage and coverage by municipality as a percentage of eligible children vaccinated. We compared children receiving at least one influenza dose to unvaccinated children using a univariate logistic regression model for each potential determinant.

Result

Between October 1 2021 and May 25 2022 33.2% (108,060/325,020) of eligible children had received at least one vaccine dose and 32.0% (104,049/325,020) had completed the vaccination schedule. The achieved coverage at municipality level varied considerably ranging from 15.8% (9/57) to 45.1% (810/1,796) for at least on dose, Figure 1.

We found that children aged 4, 5 and 6 years were significantly less likely to be vaccinated than those aged 2 years (OR 0.91 (95% CI 0.88-0.92), 0.75 (0.74-0.77) and 0.60 (0.59-0.62) respectively) and children living with only one parent were also less likely to be vaccinated (OR 0.61 (0.59-0.62)). Further, first and second-generation immigrants were less likely to be vaccinated compared to children with Danish ethnicity (OR 0.84 (0.81-0.87) and 0.77 (0.75-0.78) respectively), while children with underlying medical conditions were more likely to be vaccinated than healthy children (OR 1.18 (1.16-1.21)).

Conclusion

After the first year of national childhood influenza vaccination, the coverage was far from the official target with marked geographical differences in vaccine coverage. We identified older age, living with only one parent and being an immigrant to be significantly associated with lower vaccination uptake. National initiatives are needed to increase the overall vaccine coverage in the coming season; however, our findings suggest that campaigns need to be adjusted to better address groups with low coverage.

Oral Abstract Session: Implementation: Modelling and Forecasting for Influenza

Matthew Biggerstaff - AOXI0009

Lessons learned for influenza forecasting from two-years of real-time forecasting for the COVID-19 pandemic

Matthew Biggerstaff[2], Michael A. Johansson[2], Estee Y. Cramer[3], Evan L. Ray[3], Roni Rosenfeld[1], Ryan J. Tibshirani[1], Velma K. Lopez[2], Pragati V. Prasad[2], Alexander Webber[2], Carrie Reed[2], Nicholas G. Reich[3]

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Background

Forecasts for the COVID-19 pandemic were produced on an unprecedented scale, and the U.S. Centers for Disease Control and Prevention (CDC) partnered with the University of Massachusetts Amherst and Carnegie Mellon University to create the COVID-19 Forecast Hub and public-facing tools to ensure that forecasts were solicited widely, stored openly, synthesized efficiently, communicated clearly, and evaluated honestly.

Method

For two years, starting in April 2020, the COVID-19 Forecast Hub has collected, disseminated, synthesized, and evaluated millions of real-time forecasts for reported COVID-19 cases, hospitalizations, and deaths at the national, state, and county (cases only) level from more than 90 different academic, industry, and independent research groups.

Result

from the Forecast Hub evaluations and experience from the operation of this system have led to several key findings:

• Collaborative, multi-team forecasting efforts and active collaboration and coordination between governmental public health agencies and these teams were critical for creating a forecasting system that provided useful information to partners; encouraged model development, evaluation, and comparison; and fostered a community with an open science ethos.

• An ensemble forecast that combined predictions from different teams was often one of the most accurate forecasts and provided a robust option for public communication by CDC and others.

• Forecasts, including the ensemble, did not reliably predict changes in trends.

• As expected, forecast skill degraded as models made forecasts further into the future.

• Reliability of forecasts for outcomes that occurred earlier in disease progression (e.g., report of a case) was often lower than for outcomes that occurred later (e.g., report of a death). This may be due to noise in the measurement of the former, and the availability of leading indicators for the latter.

• While individual forecasts showed high variability in skill across time, geography, and forecast horizons, a majority were better than a naïve baseline model on average.

• The lack of timely, granular, and regularly reported data limited forecast performance, but partnerships with data curation experts helped the forecasting community access critical data sources and address challenges together.

Conclusion

Short-term forecasts of influenza can help inform public health response and risk communication during influenza seasons. Ensuring that influenza forecasting efforts improve on and incorporate the lessons learned and best practices identified during the COVID-19 response will improve the utility of these forecasts in future influenza seasons.

Oral Abstract Session: Implementation: Modelling and Forecasting for Influenza

Yi Zhen Chew - AOXI0157

Presented by Hannah Clapham

Modelling informing policy in a highly vaccinated Singapore during the Delta and Omicron wave

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Background

Throughout the COVID-19 pandemic in Singapore, the government and academic institutions have collaborated to build and use transmission models to guide policy making. Here we present two examples of this, where transmission models were developed at the early stages of the Delta and Omicron COVID-19 waves that occurred in 2021 and 2022 respectively. During each wave, predictions in the early stages helped to guide policies by providing indications on its likely profile such as the timing of the peak, healthcare utilisation due to severe cases, and the total number of cases. They also enabled the assessment of the impact of various possible public health interventions and vaccination strategies.

Method

We used a deterministic age-structured SEIR model with vaccination, which was parameterised using data on severity, healthcare utilisation and vaccination from Singapore and by fitting to the number of reported cases.

Result

During the Delta wave in October 2021, we assessed the possible impact of a range of future disease control measures on its transmission. This work informed the implementation of disease control measures to ensure that the demand for intensive care beds stayed within manageable levels. It also showed that depending on how much transmission had occurred, there were variations in the remaining transmission potential in the population, which was important for the next steps after the wave. In addition, our models showed that vaccination of children would further reduce the transmission potential in the population, allowing for further lifting of measures.

With the many unknowns about Omicron, we modelled possible scenarios across a range of transmission, severity and vaccine efficacy parameters. These were used for planning healthcare resources for this wave in early January 2022. Three scenarios were finally adopted for policy planning - the best-case, worst-case, and an intermediate scenario.

As the Omicron wave unfolded, the actual COVID-19 case counts and the timing of the peak in cases were consistent with the modelled predictions. These fell between the best-case and intermediate scenarios, which reflect what we now understand of the Omicron variant. Marginally, however, the model over-projected ICU demand and under-projected deaths. Overall, our models were successful in predicting the key outcomes of the Omicron wave.

Conclusion

Mathematical modelling of transmission undertaken in close collaboration with policy makers can be of great support to decision-making on population-level control measures.

Oral Abstract Session: Implementation: Modelling and Forecasting for Influenza

Qiqi Yang - AOXI0237

Impact of broadly-protective vaccines on seasonal dynamics and variant escape of human influenza A

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Background

Human influenza A causes occasional potentially devastating pandemics and seasonal epidemics, resulting in substantial public health burden. Human influenza A viruses show rapid turnover of antigenic variants, due to continual immune selection and immune waning in the human population. Therefore, current variant-specific influenza vaccines need to be updated yearly. Furthermore, these existing vaccines have low efficacy and cannot target potential pandemic influenza strains. To tackle this challenge, there have been great efforts to develop a new generation of broadly-protective influenza vaccines. Three key characteristics of any such vaccine will be its efficacy, and breadth and duration of protection. Here we focus on the impact of cross-protection breadth on the seasonal dynamics of H3N2 and H1N1 subtypes, and the invasion of escape variants.

Method

To illustrate the interacting immunity landscape from vaccines and natural infections, we construct a 2-strain Susceptible-Infected-Recovered-Susceptible (SIRS) model. Assuming the deployment of a vaccine that primarily protects against H3, we focus on the impact of vaccine breadth on 1) seasonal strain dynamics of endemic infections; 2) immune escape at inter- and intra- clades. We model the vaccine as providing strong within-clade cross-protection of influenza A, but weaker cross-protection across clades.

Result

Simulations show that subtype dynamics would be strongly affected by the interplay of natural and vaccine-induced cross-immunity against H1. Notably, when increasing the strength of vaccine-induced cross-immunity against H1, the critical vaccination threshold for H3 subtype will increase. This might be due to a decrease of natural cross-immunity against H3, when H1 infections drop. Additionally, preliminary invasion analyses show that vaccine-induced cross-immunity against an invading strain has a significant impact on the success of invasion when the new variant evades natural immunity.

Conclusion

Our study underlines the importance of considering immuno-epidemiology in designing Target Product Profiles (TPPs) for future influenza vaccines, by translating individual-level immunological processes to population-level epidemiological processes. In future work, we will fit incidence data from the UK and the USA to model realistic seasonal epidemics.

Oral Abstract Session: Implementation: Modelling and Forecasting for Influenza

Hai Tuan Nguyen - AOXI0434

Using long short-term memory (LSTM) - a recurrent neural network model forecasting influenza activity with climate data for four geographic regions of Vietnam

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Background

The activity of influenza fluctuates year-round in Vietnam and represents substantial mortality and disease burden. Predicting the influence of climate factors on the influenza seasonality provided better understanding of influenza trends. The proven evidence is crucial to adjust important public health strategies to diminish the burden of disease.

Method

Influenza Like Illness (ILI) data in the period 2006-2020 collected from 17 sentinel surveillance sites in four regions of Vietnam are analyzed. The influenza surveillance data collected in period 2016-2020 from three hospitals in Northern Vietnam were analyzed for the period. The local aggregated monthly climate data including air temperature, humidity, rainfall, and sunshine hour collected from nearest hydro-meteorological stations to the sentinel site are examined association with influenza activity. The long short-term memory (LSTM) recurrent neural network is used to forecast the influenza trends in exploiting the effect of most significant climate factor.

Result

One in five ILI samples (20.05% or 10,246/51,088) tested positive for influenza virus. Influenza subtyping indicated 35.62% were A(H3N2), 12.66% were A(H1N1), 16.46% were A(H1N1)pdm09, 34.86% were influenza B virus, and 0.30% were positive for co-infection of two influenza viruses. The other 9 samples were tested positive for influenza A virus of unknown subtype. The A(H3N2) and B were dominant and year-round circulating influenza viruses. On a national scale, influenza A(H3N2) viruses were primarily detected in May and June, while influenza B viruses were detected in February and March. The overall positive rates fluctuated and diversified in the four regions of the country. In the North, influenza peaks were observed in February and again in July, while in the Centre it peaked in May and again in January. In the Highland, influenza peaked in July and again in November, while in South the peak was observed in June and remained highly circulated in the last four months of year (Fig. 1). Multivariate LSTM was capable of forecasting influenza activity trending in six months ahead (Fig. 2).

Conclusion

The activity of influenza regionally varied. The LSTM network can achieve state-of-the-art results on forecasting influenza activities captured through nationwide sentinel surveillance system. Our findings highlight influenza vaccine recommendations should be region-specific for upcoming season.

Oral Abstract Session: Implementation: Modelling and Forecasting for Influenza

Dong Wang - AOXI0577

Forecasting of influenza activity using multi-stream surveillance data in Hong Kong

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Background

Despite many efforts that have been paid to investigate influenza transmission in temperate regions, the influenza prediction and forecasting in the tropical regions are not straightforward for complex irregular transmission patterns with multi-peaks, which could be affected by seasonal factors.

Method

We developed a mechanistic predictive model based forecasting framework by considering different combinations of multi-stream surveillance data, including potential drivers of influenza, i.e., absolute humidity, temperature, and school holidays. We used a temporal cross-validation approach to evaluate the forecasting performance for different models for short-term and long-term forecasts. We also assessed the impact of COVID-19 public health and social measures (PHSMs) on influenza in Hong Kong by comparing the forecasted and observed peak magnitudes and attack rates during 2019-20 season.

Result

We forecasted two peaks of influenza activities in the 2019-20 influenza season, which were estimated to occur in Jan-Feb for the winter season and Jul-Aug for the summer season. We found that the model with absolute humidity and temperature had a better forecast performance. Following the strict COVID-19 PHSMs, we estimated a 14.01% (95%CrI 2.36%-23.38%) reduction in influenza for the first week, with a 66.91% (62.30%-70.49%) reduction for the first 5 weeks. The COVID-19 PHSMs helped to avoid the infections by 65.90% (95%CrI 56.14%-71.20%) for the winter season but entirely avoided the summer season in 2020.

Conclusion

The factors of multi-stream surveillance data were considered to model and forecast influenza activities for a subtropical city Hong Kong. Our forecasts suggest that the COVID-19 PHSMs would significantly suppress influenza transmission.

Oral Abstract Session: Implementation: Pandemic Preparedness and Response I

Rachael Pung - AOXI0106

Using high-resolution social contact networks to evaluate SARS-CoV-2 transmission and control in large-scale multi-day events

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Background

COVID-19 has created a need to understand how risks can be measured and mitigated at events held during a pandemic, particularly large-scale events over multiple days. Cruises are a microcosm of populations and represent an aggregation of different activities with multi-group passengers and crew. Their interactions offer insights to the potential dynamics of different individuals in other large-scale multi-day events and have implications for the control of SARS-CoV-2 or future respiratory disease pandemics.

Method

To examine how social contacts formed in different activities influence the ability to control SARS-CoV-2 outbreaks, we collected high-resolution contact data from passengers and crew on cruise ships and combined it with network transmission models. We simulated combinations of interventions including pre- and during event testing, mask wearing in different settings, vaccination and isolation of cases and compared the resulting outbreak sizes.

Result

Over 60% of contact episodes in passengers occurred in dining or sports areas where mask wearing would be limited. When applied individually, the number of secondary cases generated under each intervention exceeds one; the number of initial infected cases. However, we estimated a combination of rapid antigen testing at the start and halfway through the duration of sailing with at least 25% coverage of a vaccine conferring 50% protection against infection and 50% lowered infectiousness resulted in fewer onward transmission than the number of initial infectives. PCR tests have a higher sensitivity than rapid antigen tests at low viral load levels but tests were modelled to be conducted ahead of sailing to account for the turnaround time in reality. Thus, cases who develop symptoms days after the sailing may not be identified prior to the event, due to viral loads near the limits of detection, and large outbreaks could occur. Overall, we estimated mask wearing with passengers practising physical distancing could reduce transmission by about 54% after accounting for interactions in mask-off settings - implying that the overall intervention effectiveness of a mask mandate is substantially less than the assumed mask-on efficacy at the individual level.

Conclusion

Effectiveness of interventions needs to be assessed against the operational feasibility in the field and often results in a lowered effectiveness as compared to a controlled environment. We found that vaccination coverage and pre-event rapid antigen tests had a larger effect than mask mandates alone, indicating the importance of combined interventions to reduce event risk during pandemics. Our framework could be adapted for pathogens with different characteristics in future, whether new SARS-CoV-2 variants or a novel pandemic virus.

Kristin Tolksdorf - AOXI0221

Classification of different pandemic COVID-19-periods in Germany based on parameters from the Pandemic Influenza Severity Assessment Tool (PISA)

***Kristin Tolksdorf*[1], *Julia Schilling*[1], *Silke Buda*[1]**

[1]Robert Koch Institute

Background

To classify different periods and allow retrospective analyses of the several COVID-19 waves, we developed a distinct definition of pandemic phases based on a rational framework.

Method

We compiled a wide range of more than 20 potential parameters covering the aspects transmissibility, seriousness of disease and impact according to the Pandemic Influenza Severity Assessment (PISA) but also included testing and molecular data, vaccination coverage, non-pharmaceutical measures and school holidays. We explored data series looking for thresholds and trends indicating epidemiological changes such as the start or the end of COVID-19 waves (https://doi.org/10.25646/9787).

Result

As of March 2022, 8 distinct periods have been defined starting with the first period in week 5,2020, where mainly sporadic cases of younger age were observed and few regional outbreaks emerged. After this, the COVID-19-pandemic in Germany was characterized by 5 COVID-19-waves, of which the latest (Omicron wave) is still ongoing, and two low-incidence periods during summer 2020 and 2021, respectively.

Parameters, which showed an increase in transmission with high sensitivity (incidence, positivity rates from notification and sentinel data), were primarily used to determine the beginning of a period. For the end of a period, parameters describing individual disease severity (hospitalisation incidence from notification and sentinel) and impact on health care system (ICU prevalence) were more decisive. With the emergence of Variants of Concern (VOC), genomic data were included to separate intersecting waves dominated by different strains.

Certain parameters such as the number of PCR tests and the positivity rate could not be used as reliable markers since summer 2021 as testing strategies were modified. Moreover, completeness of some parameters based on notification data changes as underlying conditions (e.g. follow-up of cases, legal regulations, human resources) are adapted due to the transition phase. Thus, syndromic parameters and information on symptomatic and severe cases, using already existing and validated sentinel systems, are becoming more relevant.

Conclusion

In defining different COVID-19 periods and providing regular updates, we provided the essential groundwork for timely and national comparable analyses regarding epidemiological, mental and social aspects over the course of the pandemic and therefore enabled a better risk and severity assessment. Moreover, our work contributes to the preparedness of future respiratory disease pandemics and the management of public health measures and is a valuable basis for defining and assessing future endemic COVID-19 waves, especially with co-circulation of other respiratory viruses like Influenza and RSV.

Oral Abstract Session: Implementation: Pandemic Preparedness and Response I

Cecile Viboud - AOXI0518

The COVID-19 Scenario Modeling Hub: a multi-model effort to generate pandemic projections in the United States

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Background

The COVID-19 pandemic continues to present challenges for mitigation of public health burden. Pandemic planning often requires projections of epidemic trajectories over time scales of several months or more; accounting for uncertainties over such timescales is necessary to provide effective guidance. The COVID-19 Scenario Modeling Hub was initiated in December 2020 to generate multi-model projections of pandemic burden in the United States over 3 to 12 months.

Method

Thirteen rounds of projections were undertaken between January 2021 and May 2022 to address the impact of interventions and different epidemiological conditions on epidemic dynamics. In each round, weekly probabilistic projections of cases, hospitalizations and deaths were generated by 6-10 multiple modeling groups for a common set of 4 scenarios. Scenarios addressed vaccine administration and hesitancy, strength of nonpharmaceutical interventions, transmission potential and immune escape of SARS-CoV-2 variants, and waning immunity. Ensemble projections were obtained by aggregating individual model results using the Linear Opinion Pool approach. We evaluated projection accuracy given the match between scenario assumptions and reality.

Result

We will present estimates of the population impact of the 5-11 years old vaccination program and results from two emergency rounds of projections for the Omicron wave in winter 2021-2022. We will also discuss the potential impact of waning immunity and hypothetical new variants on epidemic dynamics in 2022-2023. After evaluating multiple past rounds, we find that the average accuracy of ensemble projections is greater than that of individual models. Further, projection accuracy can persist over several months (for instance during the Omicron period), although variant emergence and changes in human behavior remain key sources of uncertainty. Even when projection accuracy degrades, as seen for projections during the Delta variant wave, projections can retain some aspects of epidemic dynamics (including the timing and shape of outbreaks, or geographic heterogeneity in epidemic intensity) that can be useful for policy.

Conclusion

Repeated rounds and extensive interaction with policymakers have facilitated the development of scenario-based projections that are useful for mitigation of a large-scale respiratory virus pandemic. Maintaining collaborative modeling hubs in peace time is essential to ensure an effective and coordinated modeling response to future respiratory disease outbreaks.

Oral Abstract Session: Implementation: Pandemic Preparedness and Response I

Jiajie Chen - AOXI0553

Presented by Yiyang Guo

Comparison Between Online and Face-to-face Health Education Approach for School Students of Hong Kong During the COVID-19 Pandemic Era

Jiajie Chen[1], Yiyang Guo[1], Sze Sze Ning[1], Nicole Ngai Yung Tsang[1], Kin Keung Mok[1], Hau Chi So[1], Dennis Kai Ming Ip[1]

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Background

School settings bring potential risks of infectious disease (ID) transmission to young children. Although non-pharmaceutical interventions (NPIs) have proven to be effective in reducing IDs, it is harder for young children to learn and master the correct hygiene practices. Since the COVID-19 epidemic, health education programmes (HEPs) have been more vital for enhancing public health awareness. Yet HEP in a traditional face-to-face (F2F) format may not be sustainable when social restrictions are applied. With the growing acceptance of online learning, we examined and compared the effectiveness of a HEP in improving children students' knowledge related to COVID-19 prevention and NPI usage via either a F2F format or an online learning mode (OLM).

Method

The HEP was developed based on the Reach Effectiveness Adoption Implementation Maintenance (RE-AIM) framework for P1-3 primary school students in Hong Kong. The programme was delivered to invited schools during COVID-19 pandemic in 2021 when schools were affected by intermittent class suspension. A simple worksheet containing 27 questions on aspects including symptoms of common IDs, hand hygiene techniques and correct cough etiquette was used for evaluation before and after the HEP. Results of the pretest and posttest were calculated to assess the knowledge improvement. The effectiveness of the two delivery models, in terms of mean total scores and the proportion of students attaining full marks, was compared by the absolute difference and the chi-squared test. The 95% confidence interval (CI) of mean results was obtained by bootstrap (1,000 samples). Feedback was gathered from teachers to assess the acceptability of the HEP.

Result

The HEP recruited 2911 P1-3 children students in 9 local primary schools, 76% of students attended the HEP by OLM and 24% attended by F2F format. 2598 pre-tests (OLM:1988, F2F:610) and 2506 post-tests (OLM:1906, F2F:600) were completed. An overall significant knowledge improvement was achieved by the HEP. The mean total score of the OLM improved by 5.06% ($P<0.05$) compared to F2F increased by 12.29% ($P<0.05$). For students attaining full marks, OLM increased by 12.95% ($P<0.01$), while F2F raised by 17.56% ($P<0.01$). Over 90% of teachers agreed that the HEP helped to enhance hygiene concepts and 89% of teachers generally preferred OLM for HEP during the pandemic era.

Conclusion

Our results suggest that HEP could improve knowledge related to COVID-19 or other ID prevention for school students regardless of OLM or F2F formats. Despite the F2F format getting a higher amount of knowledge improvement, the significant increment achieved by OLM highlighted its use as an alternative HEP approach when F2F is difficult to implement.

Oral Abstract Session: Implementation: Pandemic Preparedness and Response I

Dennis Ip - AOXI0569

Presented by Hau Chi So

Daily Antigen Rapid Testing Surveillance (DARTS) System for COVID-19 - a large-scale ad-hoc participatory community surveillance initiative using self-performed lateral flow rapid antigen tests in Hong Kong

Dennis Ip[1], Nicole Ngai Yung Tsang[1], Chun Wai Leung[1], Ka Yan Ng[1], Hau Chi So[1], Benjamin J. Cowling[1], Michael Y Ni[1], Gabriel M Leung[1]

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Background

The daily number of PCR-confirmed cases (case count) represent the most fundamental data stream routinely reported to reflect the clinical and public health burden of COVID-19. However, it may serve poorly as a surveillance data for a representative picture of the overall disease activity and temporal trajectory in the community, due to its bias towards symptomatic cases or people having an identifiable risk exposure, and the high dependency on changing testing policy and service accessibility. During the fifth wave of the Omicron pandemic in Hong Kong, the manpower for sample collection and laboratory PCR testing was both overwhelmed by a sudden upsurge of cases, leading to both delayed and underdiagnosis, which jeopardized downstream control measures. We reported here an attempt to build a large-scale ad-hoc community surveillance initiative, Daily Antigen Rapid Testing Surveillance(DARTS) System, using self-performed rapid antigen tests(RAT) to monitor the real-time situation of the COVID pandemic.

Method

We recruited >10000 individuals over the territory, with a pre-defined age-stratified quota, and statistically weighted to provide a representative cohort. Participants were divided into 7 sub-cohorts to achieve a rolling schedule with 1400+ individuals on a daily basis. Participant performed the RAT regularly irrespective of symptom or exposure history, with a self-sampled throat-and-nasal swab, as instructed by simple infographic video. RAT result and photo were reported on the same day of testing through an online platform. Daily point prevalence was disseminated on a real-time dashboard to inform the public regarding the epidemic trajectory(https://covid19.sph.hku.hk/dashboard).

Result

Since its launch on Mar 3, 2022, the system had helped to track the rapidly changing trajectory of the fifth wave of pandemic, and reflected the gradually subsiding of daily point prevalence from an initial high value of 10.8%(7.4-15.3) on Mar 7 to a very low figure of 0.6%(0.2-1.3) in early Apr 2022, and maintained thereafter with a baseline non-zero prevalence(0.08-0.32%) on most days since then, signifying the persistence of residual activity in the community without achieving complete elimination.

Conclusion

Our DARTS system has demonstrated the feasibility of building a participatory surveillance system using self-performed RAT, and highlighted its utility as an ad-hoc surveillance to timely reflect the epidemic trajectory. Regular non-symptom-and-risk-based testing approach helps to give a more representative disease activity with all severity spectrum, including subclinical cases who still carried an implication of disease transmission. The use of RAT helps to avoid the constraint of manpower and testing capacity, and has been quickly adopted by the government for case definition.

Oral Abstract Session: Implementation: Pandemic Preparedness and Response II

Ryoko Takahashi - AOXI0017

Current efforts to prepare for the future respiratory pandemics: understanding public health and social measures

Ryoko Takahashi[1], Ramona Ludolph[1], Tim Nguyen[1], Sylvie Briand[1]

[1]WHO

Background

For the next pandemic of respiratory infectious diseases, understanding how public health and social measures (PHSM) protect and/or limit the health and well-being of individuals, communities and societies based on the current and past pandemics is critical. PHSM are non-pharmaceutical actions that individuals, communities, and governments take to reduce person-to-person contact and/or make them safer. PHSM subsequently reduce the pressure on the health care system to allow for the continuation of essential services and development and dissemination of treatments and vaccines.

Method

WHO has launched a global initiative in 2021 to build robust data and research evidence to better understand the effectiveness of PHSM and improve precision in future PHSM decisions and policies. The initiative convened a global technical consultation with 101 experts from over 30 countries to identify critical actions for systematic and comparable evidence generation on PHSM.

Result

The consultation meeting concluded with priority activities for the WHO initiative including PHSM research agenda, global evidence reviews and harmonious data collection and research methods with ethical and legal considerations. To lay the foundation for consistent and standardized approach to the development of these research and decision tools, the initiative focuses on the development of a harmonized taxonomy of interventions as well as a system-based, multifaceted conceptual framework. The conceptual framework serves as the foundational work to ensure that the effectiveness of PHSM is considered in light of associated trade-offs, contextual factors and mitigation measures to address intervention burden. In parallel, the initiative works to establish a robust evidence base on PHSM through a series of global evidence reviews on the effectiveness and impact of PHSM, contexts and determinants of PHSM implementation as well as social policies and mitigation measures.

Conclusion

The multi-year PHSM initiative aims to build on the existing knowledge from the current pandemic as well as previous influenza pandemics and endemics, and works to strengthen PHSM research and decision-making to enable agile, evidence-based PSHM implementation. The initiative gives due considerations to socioeconomic implications faced by individuals and societies and how those unintended negative consequences could be addressed towards systematic integration of PHSM into health emergency preparedness and response in the future.

Ioana Ghiga - AOXI0011

Enhancing Pandemic Preparedness - Understanding Global Population Needs for Pandemic Influenza Products

Ioana Ghiga[1], Tim Nguyen[2]

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Background

Planning for the deployment of vaccines, antivirals and other pandemic influenza response products enables stakeholders to act efficiently and ensures timely delivery of these life-saving interventions to populations in need. Many pandemic products are in short supply in the early stages of their availability (as production yields increase over time) and therefore an efficient planning needs to optimize the allocation of such scarce products to reach the desired public health goals as fast as possible. Fundamental to this effort is an understanding of the global needs, in order to correctly calibrate allocation and prioritisation approaches based on realistic and evidence-informed assumptions on supply and demand.

Method

A forward-looking analysis based on foresight and scenario analyses is employed. This consists of 1) horizon scanning allowing for identification of early detection of weak signals as indicators of potential change, 2) identification of key factors that inform scenario development and shape public health goals, 3) workshop with subject matter experts to discuss scenarios and public health goals and 4) numerical estimation of needs for each public health goal.

Result

The research identifies a plurality of drivers that impact the supply and demand of medical countermeasures. Key considerations are represented by the target populations for various medical interventions, which in turn are affected by the severity of a pandemic as well as the efficiency and effectiveness of the different medical products in different population groups. Availability and access to different medical countermeasures is another area that impacts global needs across geographies. This in turn is affected by production capacities and technologies. To a certain extent, effectiveness of public health and social measures also dictate demand for pandemic products. Throughout the unfolding of the pandemic the different uptake rates may further inform supply and demands which would differ for different pandemic waves. Numerical ranges obtained in accordance with each public health goal, provide a greater understanding of needs and gaps that should be addressed by the global community.

Conclusion

This work allows articulation of appropriate allocation and deployment strategies and operational components, including country planning for developing national plans for vaccine deployment.

Oral Abstract Session: Implementation: Pandemic Preparedness and Response II

Peter Daly - AOXI0084

Enhancing Influenza Surveillance through Cloud Computing Platforms

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Background

The Public Health Laboratory Interoperability Project (PHLIP) uses HL7 messaging to send influenza and other respiratory virus test results from state and local public health labs to the Centers for Disease Control and Prevention (CDC). Prior to the COVID-19 pandemic, from January 27, 2015 to January 31, 2020, the PHLIP system received approximately 1,200,000 HL7 messages. Between January 31, 2020 and March 15, 2022, the system received over 23,000,000 HL7 messages. The increase in messages was due to the COVID-19 pandemic and the addition of SARS-CoV-2 test results to PHLIP. Initially, analysis of data was done in SAS version 9.4 (Cary, N.C.) on staff computers, and the data were stored in Microsoft Access Databases on shared drives. We describe here a proposal to transition data processing to cloud computing in response to the time burden resulting from the increased volume of messages to be able to provide timely situational updates.

Method

PHLIP data processing is transitioning to cloud computing using a multi-step process. The first step was moving where the processed data were stored. To manage the increased message volume, the program transitioned from Microsoft Access databases to an on-premises SQL server. Once that move was made, the data processing was able to run via CDC's SAS/Grid Computing cluster. The final step in fully transitioning to cloud computing is the ongoing development of processing programs utilizing Microsoft Azure Databricks computing clusters.

Result

The switch from Microsoft Access to an on-premises SQL server and processing data on a SAS/Grid Computing cluster improved processing time, from over 10 hours on staff computers to under 5 hours on the cluster. This decrease in time allowed for data processing to shift from occurring twice a week to daily. Since the processing was done on a computing cluster, staff computers could be used for other analysis projects while PHLIP data were being processed. While the changes substantially improved the current workflow, SAS/Grid is not without limitations. Since the platform is shared within CDC, scaling up computing resources in response to increased data volume may not be feasible. Ongoing development of a new processing program using Microsoft Azure Databricks seeks to take advantage of its scalability. Initial testing of the Azure program revealed that processing time could be reduced even further to 2 hours.

Conclusion

While development and optimization of cloud-based data processing programs is ongoing, the transition to cloud computing platforms will allow PHLIP to be more agile in the events where the number of HL7 messages received substantially increases and situational updates might be required multiple times a day.

Oral Abstract Session: Implementation: Pandemic Preparedness and Response II

Rehana Jauhangeer - AOXI0107

Building a COVID Biobank in response to the Pandemic by repurposing residual primary samples

Rehana Jauhangeer[1], Neil Woodford[1], Sarah Alexander[1], Jaya Shrivastava[1], Bob Tarbuck[1], Pinar Erder[1]

[1]UKHSA

Background

Aim:

The worldwide outbreak of COVID-19 infections caused by the SARS-CoV-2 virus led to the development and establishment of a COVID biobank by UK Health Security Agency (UKHSA). The COVID-19 biobank provides a vital resource for the curation and distribution of samples received by our regional laboratory networks across England throughout the ongoing pandemic. When fully operational, the biobank will provide key specimens for use in research, evaluations and assay development which may inform health protection and intervention strategies for both national and international Public Health Laboratories, the UK's national health service (NHS), industry and the wider scientific community.

Method

aterials and Method:

Team: The Biobank team is currently composed of scientists and a Senior Project Manager and is located within the UKHSA Culture Collections Department. Strong steer is provided via UKHSA Scientific Advisory Group (SAG) composed of scientists, clinicians, epidemiologists, statisticians and experts in governance, IT, Quality, Health and Safety plus site services.

Samples: Residual positive and negative samples such as nose and throats swabs, extracted RNA, faeces, CSF, urine, sputum, nasopharyngeal specimens including post-mortem swabs have been stored at -80C, whereas serum samples have been stored at -20C.

A bespoke inventory system and customer interface is currently being developed for the Biobank.

Result

The number of samples have increased month on month as the pandemic progressed. In June 2021, the number of serum samples were 22, 751 and at April 2022 reached 148, 820 serum samples. The COVID-19 biobank stores approximately 300, 000 SARS-CoV-2 primary samples and 20, 000 RNA samples to date. The final acquisition numbers are still under discussion with the depositors and this number may vary.

Conclusion

Discussion and Conclusion:

A COVID-19 biobank containing residual primary diagnostic samples and associated metadata is currently being established by the UKHSA. It is envisaged that the biobank, when fully operational will provide clinical materials to scientists to support a variety of needs by public health organisations and industry. There is an ongoing need to educate depositors to distinguish between biobanked samples; which can be made available to others; from samples that simply require safe storage. Significant resources have been invested to ensure the COVID-19 Biobank is both compliant with regulatory and ethical requirements and has the infrastructure in place to store and supply biological materials.

Oral Abstract Session: Implementation: Pandemic Preparedness and Response II

Patrick Yang - AOXI0252

Development of an RNA strand-specific hybridization assay to differentiate replicating versus non-replicating influenza A virus

Patrick Yang[1], Natosha Zanders[1], Svetlana Shcherbik[1], John Barnes[1], David Wentworth[1], C. Todd Davis[1]

[1]CDC

Background

Replication of influenza A virus (IAV) from negative-sense viral RNA (vRNA) requires the generation of positive-sense RNA (+RNA). Most molecular diagnostic assays, such as conventional real-time RT-PCR, detect total RNA in a sample without differentiating vRNA from +RNA. These assays are not designed to distinguish IAV infection versus exposure of an individual to an environment enriched with IAVs, but wherein no viral replication occurs.

Method

Strand-specific hybridization (SSH) assay for differentiation and detection of vRNA (exposure) and +RNA (infection) was updated from previous designed procedures (Yang et al., Appl Microbiol Biotechnol, 2016; Yang et al., J. Clin Microbiology, 2020).

Result

The SSH assay exhibited a linearity of 7 logs with a lower limit of detection of 6x102 copies of molecules per reaction. No signal was detected in samples with a high load of non-target templates or influenza B virus, demonstrating assay specificity. IAV +RNA was detected 2-4 hours post-infection of MDCK cells, whereas synthesis of cold-adapted IAV +RNA was significantly impaired at 37ï‚°C and equivalent timepoints. The assay was used to test IAV rRT-PCR positive nasopharyngeal specimens collected from individuals exposed to IAV at swine exhibitions or while working at live bird markets (LBM). IAV +RNA was detected in all but one specimen collected from persons exposed to swine; the +RNA-negative sample was collected following 3 days of oseltamivir treatment. In swabs from asymptomatic LBM workers in Bangladesh, vRNA, but not +RNA, was detected. The SSH assay was able to detect and distinguish vRNA and +RNA in samples collected from infected, symptomatic individuals versus individuals who were only exposed to IAV in the environment, but had no active viral replication, or after several days of antiviral treatment.

Conclusion

Data generated with this technique, especially when coupled with clinical data and assessment of seroconversion, will facilitate differentiation of actual IAV infection with replicating virus versus individuals exposed to high levels of environmental contamination, but without virus infection.

Oral Abstract Session: Implementation: Pandemic Preparedness and Response II

Karen Hennessey - AOXI0276

Presented by Isabel Bergeri / WHO

A framework for seroepidemiologic investigations in pandemics: insights from an evaluation of WHO's COVID-19 Unity Studies initiative

***Karen Hennessey*[1], *Carsten Mantel*[1], *Lorenzo Pezzoli*[2]**

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Background

The WHO Unity Studies initiative supports countries, especially low- and middle-income countries (LMICs), to conduct seroepidemiologic studies for rapidly informing national and global public health responses to a pandemic. Early in the COVID-19 pandemic, 10 generic study protocols were developed which standardized epidemiologic and laboratory methods. WHO provided technical support, serological assays, funding, and training to implement these studies. We aim to inform planning and readiness for future pandemics by evaluating 1/the usefulness of study findings in guiding national and global COVID-19 response strategies, 2/capacity built from engagement with the initiative, and 3/management and support to conduct Unity studies.

Method

The three most used protocols (66% of the 339 studies tracked by the WHO Unity team), were the focus of this evaluation: first few cases; household transmission; population sero-survey. All 158 principal investigators (PIs) with contact information were surveyed with an online questionnaire. Interviews were conducted with 14 PIs (randomly selected by WHO region and country income level), 14 WHO Unity focal points at country, regional and global levels, 12 WHO global-level stakeholders, and 8 external partners. Interviews were coded in MAXQDA™, synthesized into findings, and cross-verified by a second evaluator. PI survey and interview responses were similar and are combined unless specified.

Result

Among 69 (44%) survey respondents, 61 (88%) were from LMICs. Nearly all survey respondents (95%) gave positive feedback on WHO technical support, 76% reported study results were useful for contributing to COVID-19 understanding, 51% for guiding public health and social measures, and 43% for guiding vaccination policy. Untimeliness was reported as the main barrier to usefulness, with ethical clearance, receipt of serological assays, and delays in sharing findings as major causes for overall delays. Unclear communication of findings was considered another barrier to usefulness. There was strong agreement that the initiative created equitable research opportunities, connected researchers and expertise across countries and facilitated study implementation. Around 90% agreed the initiative should continue in the future.

Conclusion

The Unity Studies initiative created a highly valued community of practice and contributed to research equity and enhanced surveillance, especially in LMIC. To better inform future pandemic responses, WHO should establish emergency-mode procedures for Unity studies to facilitate timely implementation and continue to build national capacity to enable rapid study implementation and communication of findings in a format friendly to decision-makers.

Oral Abstract Session: Implementation: Pandemic Preparedness and Response II

Marissa Malchione - AOXI0455

Exploring determinants of response-ready influenza vaccination programs in five middle-income countries

Marissa Malchione[3], Kate Hopkins[3], Theresa Sommers[3], Joel Tinkler[3], Cassidy Howell[3], Meredith Dockery[3], Nick Boehman[3], Alison Mack[3], Benjamin McCormick[4], Angus Thomson[1], Marie Mazur[2], Holly Seale[5], Stacey Knobler[3]

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Background

Influenza is both a pandemic threat and a persistent burden, but many countries do not meet World Health Assembly-recommended influenza vaccine coverage rates (VCRs). Policy and programmatic factors that improve and sustain influenza VCRs have been studied in high-income countries (HICs), but not in middle-income countries (MICs). With support from the Ready2Respond collaboration, Sabin is identifying and evaluating such factors in five diverse MICs (Albania, Bolivia, Brazil, Thailand and South Africa) and examining how influenza-specific evidence, investment and infrastructure contributed to their COVID-19 response. These results can guide the design and investment to improve influenza vaccination programs. This work will be completed in Q2 2022.

Method

Detailed case studies will identify specific factors driving positive trends in influenza VCRs and explore their interactions. For each country, we will conduct 1) a narrative literature review of published scientific and policy literature, 2) targeted semi-structured surveys of individuals and organizations critical to influenza vaccine access, and 3) key informant interviews (KIIs) to probe specific elements of program design and implementation. We will also investigate how VCR-associated factors-such as access to high-risk populations, data collection practices, and communications strategies-contributed to each country's COVID-19 response. Data analysis will employ a Framework methodology comprising the following stages: (i) familiarization, (ii) identification of themes, (iii) indexing, (iv) charting, and (v) mapping.

Result

Key determinants for increasing and sustaining influenza VCRs in each MIC studied will be categorized as follows: political (prioritization, accountability, partnerships), structural (access, data collection, management), communication & education (advocacy, messaging), economics, and socio-behavioral (trust, attitudes, perceptions). Determinants will also be analyzed to assess the effects of influenza-specific evidence, investment, and infrastructure on each country's response to COVID-19 and conversely examine how COVID-19 vaccination programmatic elements could increase influenza VCRs.

Conclusion

Sustained investment in influenza vaccination promises to enhance overall pandemic preparedness and response. Evidence demonstrating the benefits of specific investments in influenza vaccination programs in select MICs can guide similar countries' decisions on public health funding and prioritization.

Oral Abstract Session: Implementation: Public Health Genomics for Influenza

Emily Bendall - AOXI0038

Within-host evolution of influenza A virus during acute infection

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Background

While the global evolutionary dynamics of influenza viruses are dominated by positive selection of antigenic variants, there is little evidence for positive selection of antigenic variants within deeply sequenced within-host populations. This discrepancy could be attributable to technical difficulties with rare variant detection, insufficient longitudinal sampling, or differences in evolutionary dynamics across scales. We examined variant detections in specimens collected longitudinally during influenza A virus (IAV) infection.

Method

We sequenced samples from individuals enrolled in FluTES, a case ascertained household study of influenza. Infected individuals were sampled daily for up to 14 days. All samples with a qPCR cycle threshold ≤30 had two technical replicates sequenced using the Illumina platform. Intrahost single nucleotide variants (iSNV) were called at a 0.5% frequency threshold against a within sample consensus using iVar. We inferred loci under positive selection in two different ways - parallel evolution between individuals and allele trajectories within individuals. In the latter analysis, we estimated selection coefficients using a Wright-Fisher Approximate Bayesian Computation (WFABC) model and determined if iSNV reached consensus.

Result

The variant calling pipeline was validated against a prior dataset and had a specificity of 0.999 for variants ≥0.5%. We sequenced 233 IAV positive nasal swabs (185 H3N2 and 48 H1N1) from 92 individuals, with a median of 2 samples per individual (range=1-8). The median number of iSNV per sample was 14 (IQR=16.0-70.5), and 81% of iSNV had a frequency below 2%. As evidence for parallel evolution, we found 269 iSNV shared in ≥3 individuals, which is more than expected by chance based on a permutation test. Of these, only one iSNV was found in an antigenic site (HA 219, synonymous). In 13 serially sampled individuals, there were 22 iSNV that increased to consensus level over the course of the infection. Of these, one nonsynonymous iSNV was in an antigenic site (K176T). Using a WFABC model, we estimated a within-host effective population size of 332 (H3N2) and 316 (H1N1), and identified a positive selection coefficient for 36 iSNV. One of these was in an antigenic site (HA 197, synonymous).

Conclusion

We found that most within-host variants were either selectively neutral or removed by purifying selection. However, we found evidence for positive selection within the rare variant fraction that is detectable with extremely high depth of coverage, a low variant frequency threshold, and serial sampling. Nearly all sites inferred to be under positive selection laid outside of HA antigenic regions.

Oral Abstract Session: Implementation: Public Health Genomics for Influenza

Amanda Perofsky - AOXI0267

Impact of antigenic drift on influenza A/H3N2 vaccine effectiveness in the United States

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Background

Influenza A/H3N2 viruses have higher rates of antigenic evolution than other seasonal viruses, leading to frequent turnover in globally dominant A/H3N2 clades. Rapid strain replacement within and between influenza seasons has led to frequent antigenic mismatches between the A/H3N2 vaccine strain and naturally circulating viruses. Hemagglutination inhibition (HI) assays are the gold standard for measuring antigenic distance between strains. HI assays are typically used to predict vaccine effectiveness (VE) based on HI titers between circulating viruses and vaccine strains, but titers are available for a small subset of viruses. To overcome this limitation, we estimate antigenic drift based on hemagglutinin (HA) and neuraminidase (NA) genetic sequences of A/H3N2 viruses and use these variables to predict VE in the United States.

Method

We obtained A/H3N2 VE estimates across 22 influenza seasons (1997 - 2019) from 32 studies with test-negative case-control designs. We compiled an A/H3N2 dataset enriched for North American sequences and estimated genetic distances between each season's recommended vaccine strain and circulating strains in the US (H3=3249, N2=3033). Distance metrics were based on substitutions at epitope sites in HA1 (N = 129), substitutions at sites adjacent to the receptor-binding site (N = 7), and substitutions at epitope sites in the NA head domain (N = 53). To measure the effects of vaccine history and prior immunity on VE, we measured genetic distances between vaccine strains across successive seasons and between currently circulating strains and the prior season's vaccine. We used weighted linear regression and lasso regression to explore predictive models of VE.

Result

Antigenic distance based on a broad set of HA epitope sites was the strongest predictor of A/H3N2 VE (adjusted $R2 = 0.66$, $P < 0.001$) while NA antigenic drift did not significantly correlate with VE ($R2 = 0.03$, $P = 0.15$). The lowest VE estimates occurred during seasons with both high HA epitope distance from the current vaccine and egg-adapted mutations in the vaccine strain. Seasons with increased HA epitope change did not necessarily coincide with the CDC's definition of vaccine antigenic mismatch, which requires a >4-fold difference in HI titers. Multivariable models of VE retained HA epitope distance between the current and prior season's vaccine, in addition to HA epitope distance between circulating strains and the current vaccine and egg-adapted mutations.

Conclusion

HA epitope distance and egg-adapted mutations are the strongest predictors of A/H3N2 VE. Future VE analyses will compare the predictive accuracy of HI titers to sequence-based measures of drift and test model predictions for the 2021-2022 season.

Oral Abstract Session: Implementation: Public Health Genomics for Influenza

Simon De Jong - AOXI0470

Mobility and competition shape seasonal influenza epidemics in the United States

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Background

Seasonal influenza viruses cause substantial morbidity and mortality worldwide. Knowing how ecological, evolutionary and epidemiological factors interplay to shape this burden is crucial to its mitigation. The United States (US) offers an interesting setting to explore these factors due to its large geographical expanse, complex mobility structures, and sustained epidemiological and genomic surveillance efforts. However, much remains unknown about the nature of influenza virus circulation in the US, in part due to previous studies' reliance on syndromic surveillance data.

Method

We performed a large-scale analysis of >25,000 whole-genome influenza viruses, collected through routine surveillance in the US from 2014 until 2020. We clustered these viruses into groups that likely resulted from a common introduction into the US. Fitting the sampling dates of these clusters to epidemic trajectories in each state, we visualized how these clusters likely spread across the US. To estimate the migration dynamics and the mobility processes that are likely to underpin influenza spread, we fitted the cluster trajectories to an epidemiological model.

Result

The subtype-specific size of influenza epidemics varied substantially across seasons but was highly consistent among states within each season. The size of a subtype's epidemic correlated with its onset week, with later onsets being associated with smaller size. Individual seasons exhibited a large degree of within-subtype genetic structure, with the frequent cocirculation of many genetically distinct clusters. The success of these clusters was associated with a first-mover advantage, where competition dictated that the first clusters to arrive in a state were more successful. At the country level, this dynamic of competition was coupled to mobility networks, ensuring that clusters that originated from states that were more connected and saw earlier epidemic onset exhibited a larger degree of spatial spread. Modelling results suggested that long-distance travel had a larger effect on epidemic dynamics than commuting.

Conclusion

Influenza epidemics in the US are characterized by a large degree of genetic structure, with many distinct clusters of viruses co-circulating. The success of a cluster is largely determined by onset timing and connectivity, suggesting that states that are the first to see substantial epidemic activity and are more connected via travel have an outsized effect on epidemic genetic makeup. These findings have the potential to aid forecasting and surveillance efforts.

Oral Abstract Session: Implementation: Public Health Genomics for Influenza

Matthew Scotch - AOXI0463

Resurgence of H3N2 Influenza A virus (IAV) on a university campus in Arizona, USA during the COVID-19 pandemic

Temitope Faleye[1], Abriana Smith[1], Nicole Kaiser[1], Amir Elyaderani[1], Peter Skidmore[1], Sarah Finnerty[1], Helen Sandrolini[1], Rolf Halden[1], Arvind Varsani[1], Matthew Scotch[1]

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Background

During the SARS-CoV-2 pandemic, the number of global cases of human influenza viruses dropped 99% between the 2019/2020 and 2020/2021 seasons. In the United States, the availability of multiple effective SARS-CoV-2 vaccines, host immunity, and chemotherapeutic agents resulted in a return to pre-pandemic behavioral patterns during the beginning of the 2021/2022 influenza virus season. Here we describe the resurgence of H3N2 Influenza A virus (IAV) in a large university community (> 54K on-campus students) in Tempe, Arizona, USA during the 2021/2022 season.

Method

During two time periods, 29th November to 31st December 2021 (peak 1) and 3rd March to 13th May 2022 (peak 2), we received 40 and 52 nasopharyngeal swab samples, respectively, that tested positive for influenza A virus via a rapid antigen kit at the student health clinic. We subjected all samples to our IAV molecular characterization pipeline which includes RNA extraction, RT-PCR amplification of complete genome followed by nested M, HA and NA segments amplification and Sanger sequencing. We generated sequences to perform subtype and clade assignment, detection of vaccine escape mutations in HA, and susceptibility of NA to chemotherapeutic agents.

Result

Of the peak 1 and 2 samples, 55% (22/40) and 42% (16 of the 38 tested till date), respectively, were positive for IAV via our M assay. Of these, 41% (9/22) and 62.5% (10/16) were positive for H3 and confirmed by Sanger sequencing. None of these samples were positive for H1. Nextstrain analysis of the sequences suggested that they belong to H3N2 lineage 3C.2a1b.2a.2 and that there was more than one introduction into the campus community. Analysis of the translated H3 sequences identified amino acid substitutions Y159N, T160I, L164Q, G186D, and D190N which have been shown to result in an immune escape phenotype relative to the immune response elicited by the 2021/2022 H3 vaccine strain. An additional variant from a sample later collected in April 2022 had a T187M substitution in the antigenic region bordering the HA receptor-binding-domain. N2 amplification and sequencing showed lack of the H274Y substitution associated with resistance to Oseltamivir.

Conclusion

Our data shows the resurgence of H3N2 influenza A virus with immune escape markers in a large university community in Arizona, USA. Continued sequencing and genomic epidemiology of A/H3N2, A/H1N1, and influenza B viruses is essential for surveillance during and after the COVID-19 pandemic.

Oral Abstract Session: Implementation: Public Health Genomics for Influenza

Sidan Yao - AOXI0429

Presented by Lin Wang

Learning Protein Sequences to Predict Antigenic Variation of Human Influenza Viruses

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Background

Influenza vaccination is the primary strategy to prevent and control seasonal influenza epidemics. To keep pace with the antigenic drift of influenza, antigens included in influenza vaccines have to be updated regularly. Currently, the characterization of antigenic changes in human influenza viruses is based heavily on wet laboratory experiments using immunological assays such as the hemagglutination inhibition (HI) test. This needs intensive laboratory work and translation of HI measurements into antigenic distances. Fortunately, next-generation sequencing (NGS) can generate large-volume genetic sequences, providing useful data resources that may support wet-lab experiments with data-driven methods for antigenic analysis. However, using genetic sequences to predict antigenic changes remains challenging.

Method

We developed a two-stage deep learning framework to predict the antigenic distance between pairwise influenza viruses using their hemagglutinin (HA) protein sequences. In the first stage, we trained a sequence processing model built with BiLSTM using large-volume HA sequences retrieved from GISAID, which encodes HA sequences from the same type of influenza virus into a high-dimensional continuous space. In the second stage, we trained a residual neural network (ResNet) to predict the antigenic distance between pairwise influenza viruses from the same subtype/lineage using their HA sequence-based embedding distance.

Result

Our framework achieves high-level accuracy in predicting antigenic distances demonstrated by all four influenza subtypes/lineages, including A/H3N2, A/H1N1, B/Victoria, and B/Yamagata. Our prediction of "between-virus" antigenic distances outstrips the prediction of HI measurements using state-of-the-art computational methods. In addition, using only genetic sequence data as input, our framework is able to predict the emergence of new antigenic clusters and the distance between new strains and existing strains.

Conclusion

Our deep learning framework provides an effective solution to use genetic sequences to predict antigenic changes of human influenza viruses. Our framework has a great potential to alleviate the resource-intensive and time-consuming wet-lab efforts for antigenic drift analysis, and hence improve global influenza surveillance efforts.

Oral Abstract Session: Implementation: Surveillance for Influenza Including Joint Surveillance and Impact of COVID-19 on Other Respiratory Virus Diseases

Yaw Awuku-Larbi - AOXI0177

Impact of COVID-19 on influenza surveillance during 2020 in Ghana

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Background

Influenza activity in Ghana is usually monitored by sentinel surveillance sites in health facilities nationwide. Worldwide, COVID-19 resulted in about 5 million deaths at the end of 2021 and many health systems were overburdened affecting routine disease surveillance. We present here reports on influenza activity in 2020 during the height of the COVID-19 pandemic in Ghana.

Method

Despite the focus of the health service on COVID-19, 10% of respiratory samples submitted for COVID-19 testing that met the WHO case definition for influenza-like illness, were randomly selected for influenza testing at the Ghana National Influenza Centre (Ghana-NIC). These cross-sectional secondary analyses enabled the weekly detection of influenza virus infections.

Result

In 2020, the Ghana-NIC processed 2550 respiratory samples of which 77 (3.0%) tested positive for influenza. There was a 50% reduction in the number of samples received from the sentinel sites compared to the previous years (2017-2019). Two waves of influenza activity were identified; December 30, 2019, to March 8, 2020 (epidemiological weeks 1-10) and September 28 to December 28, 2020 (weeks 40-52) with 12 and 65 influenza positive samples respectively. There was no influenza activity between epidemiological weeks 10 and 40 which coincided with intense COVID-19 infections nationwide. Influenza AH3N2 subtype was dominant in the 1st wave (epidemiological weeks 1-10) with influenza B Victoria lineage more frequent in the 2nd wave (epidemiological weeks 40 - 52). Official vaccination for COVID-19 in Ghana begun in March 2021 and influenza vaccines are currently not part of routine immunization in Ghana.

Conclusion

The opening of the international airport from 1st September 2020, probably contributed to the increased influenza activity during the second influenza wave. It appears that the adherence to COVID-19 mitigation strategies such as wearing of face masks and handwashing hygiene also affected transmission of influenza viruses. Continuation of mitigation strategies at the community level with inclusion of influenza vaccination could help reduce seasonal influenza transmission.

Oral Abstract Session: Implementation: Surveillance for Influenza Including Joint Surveillance and Impact of COVID-19 on Other Respiratory Virus Diseases

Md Ariful Islam - AOXI0183

Utilization of influenza surveillance platform to monitor SARS-CoV-2: findings from hospital-based influenza sentinel surveillance during first two years of COVID-19 in Bangladesh

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Background

WHO recommends utilising the existing influenza sentinel surveillance system to monitor SARS-CoV-2. We explored and leveraged our hospital-based influenza sentinel surveillance to detect cases of COVID-19 in Bangladesh.

Method

From the existing influenza surveillance platform we analyzed data between March 2020-February 2022, from patients with severe acute respiratory infections (SARI, defined as a history of fever or measured fever ≥38.0°C with cough, onset within the past ten days, and requiring hospitalization) at 9 tertiary care hospitals across Bangladesh. Surveillance staff collected socio-demographic, clinical and laboratory data on a standardized surveillance case record form and obtained nasopharyngeal and oropharyngeal swabs; samples were sent weekly to the icddr,b virology laboratory to test for influenza viruses and SARS-CoV-2 by real-time reverse transcription-polymerase chain reaction (rRT-PCR). From November 2021, multiplex PCR assays for influenza and SARS-CoV-2 were used. We performed descriptive analyses to describe the SARI cases with influenza virus and SARS-CoV-2 and compared this with the national data of Bangladesh Government during the same time period. The national data includes all the symptomatic and asymptomatic cases tested for SARS-CoV-2 including samples tested for international travel.

Result

We included 6,871 SARI cases with a median of 55 cases (IQR: 34-85) reported weekly (6 work days). The median age of the SARI patients was 26 years (IQR: 1-54), and 63% were male. During this period, the laboratory tested between 10 and 180 specimens per week (median 55 specimens; IQR: 34-85). Among the 6,871 SARI cases, 1,124 (16.4%) were had a positive SARS-CoV-2 test, 596 (8.7%) tested positive for influenza virus, and 10 (0.1%) were coinfected. The first case of COVID-19 identified through this surveillance system was more than six weeks after the first COVID-19 case detected through national surveillance in Bangladesh. The positivity rate of SARS-CoV-2 (16.4%) from this sentinel surveillance was higher than the national average (7.0%, 1,942,680 /13,376,734). Overall, the SARS-CoV-2 positivity trend from this sentinel surveillance platform was aligned with COVID-19 national data in Bangladesh, with peaks during epi-week 22 to 35 in 2020, epi-week 15 to 30 in 2021 and since epi week 5 in 2022.

Conclusion

Our findings suggest that using existing influenza sentinel surveillance platforms may not be optimal (nor were they designed) for early SARS-CoV-2 detection in Bangladesh. However, the system may complement national surveillance for SARS-CoV-2 where COVID-19 testing facilities are limited, or when pandemic response systems shut down, especially during periods of high SARS-CoV-2 transmission.

Oral Abstract Session: Implementation: Surveillance for Influenza Including Joint Surveillance and Impact of COVID-19 on Other Respiratory Virus Diseases

Varsha Potdar - AOXI0254

Surveillance of Influenza and SARS CoV2 and Impact of COVID-19 on Other Respiratory Viral diseases in Pune western part of India

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Background

To understand the co-circulation of influenza and SARS CoV-2 viruses, vigilance is essential. The National Influenza Central at the National Institute of Virology Pune is continuously monitoring circulating influenza strains. With the use of a surveillance database, NIC performed cross-sectional research in order to compare and understand the flu and other respiratory virus activities during the two years of COVID-19 pandemic.

Method

During the two years of the pandemic; January 2020 to March 2022, systematic surveillance of SARI and ARI was conducted. Each week, the Center recruited 10-20 ARI and SARI (as per WHO Case Definition) patients across all age categories from sentinel hospitals. Participants were recruited by convenient sampling with due consent. A total of 4894 cases were enrolled, of whom 1301 (26.5 percent) were ARI and 3593 were SARI (73.5 percent) Clinical and epidemiological data for each recruited participant were recorded using a standardized case report form. The in-house combo test detected Influenza A, B, and SARS CoV-2 While PIV 1, 2, 3, 4, human metapneumovirus, RSV A/B, and adenovirus were detected using duplex real-time PCR. Next Generation Sequencing was used to sequence SARS CoV 2's genome. The HA gene sequencing was done for Influenza and G gene for RSV.

Result

4894 nasopharyngeal swabs were tested. The average age of 0-92 patients was 34.8 years, with 2885 males (58.9%) and 2009 females (41.1%). Participants aged 15 to 45 made up 31% of the total. We identified viruses in 1391 (28.4%) of the samples. SARSCoV-2 was the most frequent virus, with 683 cases (14%). RSV was found in 351 instances (7%) of which majority were pediatric. During the two years of the pandemic, only 3.8% of people tested positive for influenza, yet all three influenza viruses were present. Other respiratory virus percent positive was low (2%). It was discovered that A(H3N2) strains correspond to the 2a1b2a2 clade, A(H1N1)pdm09 strains to the 5A2 clade, and Type B Victoria strains to the A1A3 clade. The RSV A G gene sequencing identified the strains as ON1.1. The NGS analysis of SARS CoV 2 revealed the presence of clades L, O, and V early in the pandemic, and afterwards several Clade G lineages

Conclusion

Over the duration of the pandemic, influenza activity has decreased considerably, but an unanticipated surge in RSV positive has been reported in less than five years. Non-pharmaceutical treatments, including as mask usage, social isolation, and lockdowns, had a significant influence in reducing the spread of other respiratory viruses. Increased RSV activity in the pediatric population indicates the return of other non-SARS CoV-2 respiratory viruses.

Oral Abstract Session: Implementation: Surveillance for Influenza Including Joint Surveillance and Impact of COVID-19 on Other Respiratory Virus Diseases

Julia Rogers - AOXI0179

Results from a test-and-treat study for influenza and impact of the SARS-CoV-2 pandemic on influenza prevalence among residents of homeless shelters in King County, WA: a stepped-wedge cluster-randomized trial

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Background

Persons experiencing homelessness face greater risk for severe influenza virus infection. Overcrowding in shelters can facilitate virus spread. Data on the role of on-site testing and treatment for influenza within homeless shelters remains limited.

Method

We conducted a cluster-randomized stepped-wedge trial of point-of-care molecular influenza testing with antiviral treatment in shelters in Seattle, WA, USA. Shelter residents ≥3 months of age were eligible to participate if they had cough or ≥2 acute respiratory symptoms. At intervention shelters, influenza testing was conducted on-site for those with symptom onset within 48 hours, and if results were positive, individuals received baloxavir or oseltamivir treatment on-site. At control shelters, samples collected were not tested on-site and no treatment was offered. Asymptomatic and paucisymptomatic individuals were eligible for standard surveillance participation 1x/month pre-3/30/2020 and weekly henceforth. All samples were tested by RT-PCR for influenza at a University of Washington laboratory. We were able to generate full genome sequences (<10% missing data) from a subset of influenza-positive samples.

Result

Overall, 15 shelters participated in the study from 11/15/2019 - 3/31/2020 and from 11/2/2020 - 3/31/2021. Standard surveillance was conducted from 10/2/2019 through 5/31/2021. From a total of 11,009 episodes, influenza virus was detected in 59 (0.5%) specimens (54% from intervention shelters) using RT-PCR, including 41 flu B (69%), 2 flu A/H3N2 (3%), and 17 flu A/H1N1 (29%) subtypes. In the first year, 57 positive specimens were detected, and family shelters observed higher test positivity compared to adult-only shelters (11% vs. 2%; p<0.001). Of the 32 infections observed at intervention shelters, 21 (66%) were detected within 48 hours by on-site testing and treated with an antiviral, including 9 with baloxavir and 12 with oseltamivir; 38% of those treated were <5 years old. Of the 6 oseltamivir recipients with follow-up data, 5 (83%) were treatment adherent. The overall measure of agreement between on-site testing and RT-PCR was high (k=0.90). 86% (6 of 7) sequenced flu A/H1N1 and 81%

(13 of 16) of flu B/Victoria Lineage samples were most closely related to another sequence from the same shelter when analyzed with WA GISAID sequences collected during the same time period.

Conclusion

Shelter-based surveillance highlighted higher influenza detection in shelters serving families compared to adults only and strong evidence of intra-shelter transmission. Our findings suggest high feasibility of test-and-treat in shelters; additional studies to discern an intervention effect are needed when influenza circulation resurges.

Oral Abstract Session: Implementation: Surveillance for Influenza Including Joint Surveillance and Impact of COVID-19 on Other Respiratory Virus Diseases

Hanne-Dorthe Emborg - AOXI0227

Late influenza A(H3N2) season after relaxation of COVID-19 restrictions driven by the 7-14 years old

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Background

Since the COVID-19 restrictions in Denmark were implemented in March 2020 and abruptly ended the 2019-20 influenza season, influenza has been nearly absent with only sporadic cases detected. However, after two years of various degrees of COVID-19 restrictions all were lifted by the end of January 2022, which was followed by a surge in influenza cases. This study describes the late influenza season after the lift of COVID-19 restrictions and atypical age distribution.

Method

In the Danish Microbiology Database (MiBa), data on all patients swabbed at the GP or at the hospital and tested for influenza A and B viruses by PCR are registered in real-time with date and age on testing. A random subsample of the positive influenza tests is subtyped and genetically characterised at the National Influenza Center at Statens Serum Institut (SSI). Further, SSI has launched an influenza Dashboard that shows the weekly influenza incidence by age group in current and previous seasons.

Result

From mid-February 2022, upon the relaxation of almost all COVID-19 restrictions, the weekly number of influenza A cases doubled from 65 in week 6 to 3,389 in week 12. The circulating strain in the 2021/22 season was influenza A(H3N2) and mainly clade 3C.2a1b.2a.2. The increase in influenza A cases was first observed in the age group 7-14 years old and this age group also experienced the steepest increase in cases (Figure 1).

Conclusion

Following the relaxation of the COVID-19 restrictions, it was the age group 7-14 years of age that drove the increase in influenza A in Denmark during the late 2021-22 season starting February 2022. In previous influenza seasons 2016-17 and 2017-18, that were also dominated by influenza A(H3N2), the highest incidences were observed among people aged 75+ and 0-1 years old. This shift in age was unexpected and might be attributed to the lack of build-up immunity in the school age children after two years absence of seasonal influenza.

Oral Abstract Session: Implementation: Surveillance for Influenza Including Joint Surveillance and Impact of COVID-19 on Other Respiratory Virus Diseases

Lily E Cohen - AOXI0190

Increased severity of influenza-related hospitalizations in resource-limited settings: Results from the Global Influenza Hospital Surveillance Network (GIHSN)

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Background

Data on influenza disease burden remain scarce in middle and low-income regions. Here we used a comprehensive dataset from the Global Influenza Hospital and Surveillance Network (GIHSN) to assess geographic differences in the epidemiology and severity of influenza.

Method

Patient-level information was collected from active surveillance in 116 participating hospitals in five high-income countries (HICs), thirteen upper middle-income countries (UMICs), and four lower middle-income countries (LMICs). Respiratory specimens were obtained from patients who had influenza-like-illness presentation 7 days prior to hospital admission and tested by RT-PCR. The adjusted odds ratios (aORs) for intensive care unit (ICU) admission and in-hospital death were estimated with multivariate generalized linear models that included country income group, age, sex, number of comorbidities, influenza subtype and lineage, and season.

Result

From 2012-2019, we analyzed data for 73,121 hospitalized patients, of whom 15,660 (21.4%) were influenza-positive (72.4% A, 27.6% B). Influenza positivity varied between years (median of 10-20% in LMICs, 20% in UMICs and 10-40% in HICs). After adjustment for patient-level covariates, there was a 2-fold increased risk of ICU admission for patients in UMIC (aOR 2.31; 95% confidence interval (CI) 1.85-2.88, $p < 0.001$), and 5-fold increase in LMIC (aOR 5.35; 95% CI 3.98-7.17, $p < 0.001$), compared to HICs. The risk of in-hospital death in HICs and

UMICs were comparable (UMIC: aOR 1.14; 95% 0.87-1.50; p > 0.05), though substantially lower than that in LMIC (aOR 5.05; 95% 3.61-7.03; p < 0.001 relative to HIC). The presence of comorbidities was an independent predictor of severity, with two or more comorbidities significantly increasing the odds of ICU and death (aOR 5.71, 95% 4.40-7.43; p < 0.001 and aOR 3.12, 95% 2.16-4.56; p < 0.001, respectively). Age ≥65 years was also an independent predictor of death (aOR 2.65; 95% CI 2.05-3.42; p < 0.001) while female sex reduced the odds of ICU admission (aOR 0.80; 95% CI 0.69-0.93; p < 0.01) and in-hospital death (aOR 0.74; 95% CI 0.60-0.90; p < 0.01).

Conclusion

We find significant disparities in influenza severity among hospitalized patients, with increased risk of ICU admission and death among patients from lower and upper middle-income countries, relative to high-income countries. These findings could reflect an intrinsically more severe nature of the disease in poorer settings or differential access to care. These data demonstrate a burden of influenza in countries with limited resources, supporting global efforts to increase surveillance and vaccination programs.

Oral Abstract Session: Implementation: Surveillance for Influenza Including Joint Surveillance and Impact of COVID-19 on Other Respiratory Virus Diseases

Lilach M. Friedman - AOXI0533

Profiling the avian influenza antibody repertoires of wild migratory birds using influenza antigen microarrays

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Background

Migratory birds play a central role in the global spread of avian influenza viruses (AIV), causing costly outbreaks in wild birds and poultry farms along their migratory routes. Surveillance of H5N1 or H7N9 strains that may spread to mammals and are potential pandemic threats is important for public health preparedness.

Method

We developed an avian influenza antigen microarray spotted with recombinant hemagglutinin and neuraminidase proteins from 63 influenza strains belonging to 18 AIV subtypes, and used this assay to concurrently profile IgY and IgM antibodies of AIV infections in two wild migratory bird species sampled in Israel: (1) mallards (Anas platyrhynchos) - a natural reservoir and non-symptomatic carriers of AIV; and (2) steppe buzzard (Buteo buteo vulpinus) - less common carriers of AIVs with higher susceptibility to symptomatic infection.

Result

We demonstrated the subtype-specificity of our microarrays using samples from chickens vaccinated with an H9N2 vaccine. We compared the anti-AIV IgY and IgM repertoires in resident and migrant wild mallards sampled in Israel between 10/2018-02/2020 (n=94) and migrant buzzards sampled between 04/2017-04-2019 (n=58). A large diversity was observed in the antibody repertoires of different birds from the same species. Nevertheless, our results demonstrate important differences in the variety and magnitude of antibody responses to the different subtypes, both within and between the two species. In particular, migratory mallards had higher IgY levels to H9N2 viruses as compared with both resident mallards and migratory buzzards. Buzzards had higher antibody levels to H10N8 strains than mallards. Both species had high antibody levels to H5N1 strains. Significant differences in the immune history were observed between sex and age groups of the same species. IgM antibody repertoires were significantly altered in different capture years. Higher anti H7N9 IgM levels were detected in mallards trapped in 2019 as compared with those trapped in 2018. While this study was performed using serum samples, in chicken we found that dry blood spots that were stored frozen can also be used to profile the antibody repertoires of birds using the avian influenza microarrays with high correlations with serum profiles.

Conclusion

Antigen microarrays can be applied to profile the influenza immune history of wild birds by measuring IgY and IgM antibody responses. Identification of recent infections may be done by profiling IgM responses. Dry blood spots can be used instead of serum, allowing surveillance of AIV exposures in wild bird species from which serum

collection is difficult or impossible. More research is required to provide a more detailed interpretation of the AIV infection history in wild birds.

Oral Abstract Session: Implementation: Surveillance for Influenza Including Joint Surveillance and Impact of COVID-19 on Other Respiratory Virus Diseases

Erik Karlsson - AOXI0548

The echoes of COVID-19 on avian influenza prevalence and risk: stories from Cambodia

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Background

Cambodia is a Least Developed Country in Southeast Asia with a high dependence on agriculture and tourism. It is also a major hotspot of endemic and (re)emerging infectious disease. Poultry represent a major source of subsistence and economic stability for many households; however, many activities are practiced with minimal biosafety or biosecurity. Avian influenza virus (AIV) is endemic in the country with numerous subtypes circulating concurrently in live bird markets (LBMs). Previous studies have shown AIV introduction and prevalence is associated with social determinants such as festivals, and risk of zoonotic infection increases with poultry contact. While Cambodia had a strong response to COVID-19, it was critical to continue to monitor AIV, and to understand how public health measures impacted AIV prevalence and risk in tempore and for the future in Cambodia.

Method

To monitor AIV prevalence, active, longitudinal surveillance in key Cambodian LBMs was continued as conducted since 2011. AIV was detected using standard RT-PCR protocols and sequenced with multi-segment, amplicon-based Oxford Nanopore technology. Human zoonotic infections prompted One Health investigations. To understand impact of COVID-19 pandemic on food security and AIV risk factors, a cross-sectional, structured questionnaire was administered to 400 individuals in the tourism and agriculture sectors.

Result

Over the course of the COVID-19 pandemic, several novel subtypes of AIV were detected and continued to circulate in Cambodian LBMs (ex: A/H5N8, A/H7Nx). Two A/H9N2 human infections occurred, both in young children with high contact with backyard poultry. COVID-19 associated cancellation and alteration of festival periods significantly reduced AIV prevalence in LBMs, with AIV returning to high levels when gatherings were rescheduled or unrestricted. Travel restrictions and job loss as a result of the COVID-19 pandemic led to food insecurity in tourism professions, with many turning to backyard poultry farming to supplement subsistence and income. The majority of individuals who turned to farming had minimal training, increasing overall AIV risk.

Conclusion

It is clear that the COVID-19 pandemic had significant impact on AIV in Cambodia. The ability to continue monitoring AIV prevalence in LBMs allowed further understanding of social influences on AIV introduction, circulation, and risk. Reduction in human seasonal influenza but continued surveillance allowed detection of novel zoonotic infections. Economic hardship led to more individuals farming poultry, often with minimal training, potentially amplifying high-risk practices and interfaces in the future. All of these issues provide targets for future research, intervention, and policy.

Oral Abstract Session: Implementation: Surveillance for Influenza Including Joint Surveillance and Impact of COVID-19 on Other Respiratory Virus Diseases

Paola Cristina Resende - AOXI0633

Atypical seasonality of Influenza A(H3N2) in Brazil 2021/2022 and mismatch with the vaccine strain

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Background

Introduction of non-pharmaceutical interventions to control COVID-19 transmission in early 2020 coincided with a global decrease in active influenza virus circulation. These interventions significantly altered the circulation patterns of respiratory diseases worldwide and the pandemic event disrupted continued Influenza surveillance in many countries. In this study, we describe the introduction and spread of a new Influenza A (H3N2) strain in an atypical Influenza season in Brazil, the vaccine mismatch, and the epidemiological impact of this strain.

Method

Epidemiological data was recovered from the Brazilian Ministry of Health database and a representative number of Influenza A(H3N2) RT-PCR positive samples had the genome characterized and the phylogenetic relationship with other circulating viruses and the contemporary vaccine strain were performed.

Result

The usual influenza season in Brazil is mainly from April to July. But influenza activity has remained at historically low levels globally since March 2020, even when increased influenza testing was performed in some countries. In Brazil, Influenza strains were responsible for up to 1,551 Influenza like illness (ILI) cases and 3,737 Severe Acute Respiratory Infections (SARI), 668 of which were fatal revealed the atypical 2021/2022 outbreak from November 2021 to January 2022. The genetic evolution of 755 influenza H3N2 detected from all Brazilian regions: Northern (AC, AM and PA) = 78, Northeast (AL, BA, PB, PE, RN, SE and MA) = 61, South (SC, PR and RS) = 83, Southeast (SP, RJ, MG and ES) = 523, and Midwest (GO, MS and MT) = 10 from November 2021 to May 2022 comprised genetically similar viruses to the Clade 3C.2a1b.2a.2, related to the A/Darwin/6/2021-like recommended vaccine strain for the Southern Hemisphere in 2022. Maximum likelihood analysis revealed a highly supported monophyletic Brazilian cluster containing eight amino acidic signatures. Another two viruses were found outside this main cluster. The viruses circulating in Brazil were distinct from the WHO recommended influenza A(H3N2) vaccine virus components for 2021 Southern Hemisphere season (A/Hong Kong/45/2019-like Clade 3C.2a1b.1b). According to the WHO vaccine composition report ferret antisera raised against egg propagated A/Hong Kong/45/2019-like viruses (3C.2a1b.1b), representing the egg-based vaccine viruses for the 2021 southern hemisphere influenza seasons, recognized all viruses A/Darwin/6/2021-like poorly.

Conclusion

Maintenance of the influenza surveillance system in Brazil during the COVID-19 pandemic allowed the detection and response to recommend the intensification of non-pharmacological and Oseltamivir treatment to the atypical influenza A(H3N2) outbreak in late 2021.

Oral Abstract Session: Implementation: Vaccine Effectiveness for Influenza

Kayla Hanson - AOXI0066

Absolute and Relative Effectiveness of Cell Culture-Based Inactivated Influenza Vaccine against Medically-Attended, Laboratory-Confirmed Influenza in Northcentral Wisconsin, US, 2019-20

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Background

Mutations from egg-adapted vaccine viruses may contribute to reduced effectiveness of influenza vaccines, especially for A/H3N2. We assessed absolute vaccine effectiveness (aVE) of cell culture-based inactivated influenza vaccine (ccIIV4) (Flucelvax Quadrivalent, Seqirus Inc.), aVE of standard egg-based IIV (IIV4), and the relative VE (rVE) of ccIIV4 vs IIV4 during the 2019-20 influenza season.

Method

arshfield Clinic (MC) in Wisconsin, US conducted two test-negative influenza VE studies during 2019-20; a single-site study designed to estimate ccIIV4 VE and the CDC-funded US Influenza VE Network study. Data from MC participants aged ≥4 years in both studies were combined.

Patients were approached during outpatient primary and urgent care visits for acute respiratory illness (ARI) between 12/16/2019-3/13/2020. Recruitment ended in mid-March due to COVID-19. Patients with ARI were eligible if they had a cough with illness duration ≤7 days and had not taken an antiviral. Participants completed a brief survey and provided nasal and oropharyngeal swabs for influenza testing via RT-PCR. Additional data were extracted from the electronic health record.

aVE of ccIIV4 (vs no vaccination) and IIV4 (vs no vaccination), and rVE of ccIIV4 vs IIV4 were estimated from multivariable logistic regression models.

Result

There were 2,958 participants (mean age 33 years, 58% male, 89% non-Hispanic white). Forty-one percent tested positive for influenza; of those 52% were B/Victoria, 47% A/H1N1pdm09, and <1% A/H3N2. About half (47%) were vaccinated; of which, 75% received ccIIV4 and 13% received IIV4.

A/H1N1pdm09. aVE of ccIIV4 was 33% (95% CI 16%−46%) and aVE of IIV4 was 35% (95% CI 3%−58%). rVE was -4% (95% CI -61%−31%). VE estimates varied by age group, but CIs overlapped (Figure).

B/Victoria. aVE of ccIIV4 was 41% (95% CI 26%−53%) and aVE of IIV4 was 63% (95% CI 39%−79%). rVE was -59% (95% CI -176%−4%). VE estimates varied by age group, but CIs overlapped (Figure).

Conclusion

During 2019-20, a season characterized by co-circulation of A/H1N1pdm09 and B/Victoria viruses that were antigenically drifted compared to vaccine viruses, ccIIV4 was effective against medically-attended, laboratory-confirmed influenza A/H1N1pdm09 and B/Victoria. There was no difference in VE between ccIIV4 and IIV4, although some CIs were wide. Differences between ccIIV4 and IIV4 VE were not expected since egg-adapted changes have been rarely observed for A/H1N1pdm09 and B/Victoria.

Oral Abstract Session: Implementation: Vaccine Effectiveness for Influenza

Deshayne Fell - AOXI0236

EFFECTIVENESS OF INFLUENZA VACCINATION DURING PREGNANCY ON LABORATORY-CONFIRMED SEASONAL INFLUENZA AMONG INFANTS UNDER 6 MONTHS OF AGE IN ONTARIO, CANADA

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Background

Despite high-quality evidence from randomized clinical trials conducted in low- and middle- income countries showing efficacy of influenza vaccination during pregnancy against influenza infection among infants <6 months of age, assessments of effectiveness in settings with different influenza seasonality and across multiple seasons are limited.

Method

We conducted a test-negative study using population-based Ontario laboratory data to identify all influenza virus tests (in any clinical setting) among infants <6 months of age during 9 influenza seasons (2010-11 to 2018-19). These data were linked with health administrative data to ascertain information on maternal-infant dyads, including whether women had been vaccinated against influenza during pregnancy. Vaccine effectiveness (VE) was estimated from the adjusted odds ratio for vaccination, computed using logistic regression with adjustment for infant age at test, season of birth, prenatal care adequacy, neighbourhood income, and influenza season. Women who

received influenza vaccination less than 14 days prior to obstetric delivery or received the previous season's vaccine were treated as unvaccinated in the main analyses.

Result

Among 23,806 infants <6 months of age who were tested for influenza virus, 1,783 (7.5%) tested positive. Overall, 2,168 infants (9.1%) were born to women vaccinated against influenza during pregnancy-1,708 (7.2%) when women vaccinated less than 14 days before delivery or with the previous season's influenza vaccine were reclassified as unvaccinated. Across seasons, the adjusted effectiveness of influenza vaccination during pregnancy against laboratory-confirmed infant influenza infection prior to 6 months of age was 64% (95% confidence interval [CI]: 51% to 74%). In seasons where vaccines were well-matched to dominant circulating strains, VE was 71% (95% CI: 57% to 80%), while VE in poorly-matched seasons was 37% (95% CI: -10% to 64%). VE was similar when stratified by infant age at test, although it was not statistically significant in the oldest age group (<2 months: VE 65%, 95% CI: 48% to 76%; 2-4 months: VE 62%, 95% CI: 32% to 79%; 4-6 months: VE 77%, 95% CI: -70% to 97%). In a sensitivity analysis, VE was similar when women who received influenza vaccination less than 14 days prior to delivery and/or received the previous season's vaccine were treated as vaccinated. Results were robust in other subgroup and sensitivity analyses.

Conclusion

Since infants <6 months are at high risk for serious influenza-related illness, but not eligible for influenza vaccination, immunization during pregnancy is an effective strategy for protecting young infants during their first influenza season.

Oral Abstract Session: Implementation: Vaccine Effectiveness for Influenza

Esther Kissling - AOXI0336

Vaccine effectiveness against influenza A and A(H3N2): Results from the 2021-22 season European I-MOVE primary care multicentre study

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Background

In 2021-22, influenza A viruses and particularly influenza A(H3N2) dominated in Europe. The 2021-22 influenza A(H3N2) vaccine clade was 3C.2a1b.2a.1. The I-MOVE primary care network conducted a multicentre test-negative study in ten European study sites (nine countries) to measure vaccine effectiveness (VE) against influenza A and A(H3N2). During the COVID-19 pandemic, changes in healthcare-seeking guidance for respiratory infections in many countries led to operational changes in influenza primary care networks.

Method

Primary care practitioners swabbed patients presenting with acute respiratory infection or influenza-like illness, collecting also information on demographics, vaccination and clinical characteristics. Cases were RT-PCR positive for influenza viruses A (non-subtyped), A(H3N2) or A(H1N1)pdm09 and controls were negative for any influenza virus. Study sites with fewer than 10 influenza cases were excluded. We calculated VE using logistic regression, adjusting for study site, age, sex, onset time, and presence of chronic conditions. We estimated VE among all ages and among those aged 18-64 years.

Study sites selected all, or a sample selected independently of vaccination status, of influenza virus-positive specimens for genetic sequencing. Haemagglutinin (HA) sequences were used to determine clades.

Result

Between October 2021 and March 2022, we included 8645 patients from 7/10 study sites, of whom 630, 138 and 133 had influenza A(H3N2), A(H1N1)pdm09, and A non-subtyped, respectively.

Overall VE against influenza A was 35% (95%CI: 13-51) and 40% (95%CI: 14-58) among those aged 18-64 years.

Overall VE against influenza A(H3N2) was 31% (95%CI: 4-51) and 36% (95%CI: 3-58) among those aged 18-64 years.

Influenza positive cases among other age groups were too few to measure VE.

Sequencing information was available in six included study sites. Among 70/630 (11%) sequenced influenza A(H3N2) viruses, all belonged to the 3C.2a1b.2a.2 clade, with 36 harbouring D53G/S and other amino acid changes (subgroup ii) and 33 harbouring D53N, N96S and I192F amino acid changes (subgroup iii).

Conclusion

The 2021-22 VE against circulating influenza A and A(H3N2) was 31-40% overall and among those aged 18-64 years.

Our results suggest that at least one in three individuals vaccinated against influenza during the 2021-22 season were protected against presentation in primary care with laboratory-confirmed influenza. These results may be due to a mismatch between the circulating influenza A(H3N2) clade and the strain clade incorporated in the vaccine.

However, with changing healthcare systems and guidance in the COVID-19 pandemic context, in-depth validation of influenza VE studies is needed to rule out bias in estimates.

Oral Abstract Session: Implementation: Vaccine Effectiveness for Influenza

David Hodgson - AOXI0282

Using serological data to identify cryptical influenza infections and effect on vaccine responses

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Background

The seasonal influenza vaccination programme aims to reduce influenza burden. However, the magnitude of its effectiveness varies, both between seasons and also at the individual-level within-season. Heterogeneity in immune responses is driven partly by the ability of the host-specific antibody repertoire to neutralise novel infecting strains. We explore the mechanistic relationship between an individual's infection history with previous A(H3N2) strains and their observed antibody response with the aim of predicting individual antibody responses to vaccination.

Method

We combined serological data sampled at multiple time-points from cohort studies with a model that generated individual-level antibody landscapes by considering four main processes: prior infection history; antibody generation to infecting strains; the cross-reactivity antibody responses from infecting strains; and antigenic drift between strains. Using the probabilistically estimated infection history, we then explored how cryptical infections were associated with observed antibody responses to vaccination.

Result

We found that when we accounted for underlying serological dynamics, multiple individuals had evidence of prior infections that did not manifest as PCR-confirmed symptomatic infections or four-fold rises in titre. These individuals often exhibited different patterns of association between prior exposure and subsequent vaccine responses, which would not be detectable under traditional metrics alone.

Conclusion

Using this framework to estimate infection history from serological data, we are able to probabilistically determine infections which may be undetectable by traditional cutoff methods, such as a four-fold rise in titres. Improving estimates for individual-level infection history will help facilitate the understanding of the complex relationship between infectious history and antibody response due to vaccination.

Oral Abstract Session: Implementation: Vaccine Effectiveness for Influenza

Melissa Rolfes - AOXI0302

Influenza virus dynamics in uncomplicated infection: impact of age and vaccination

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Background

The impact of age and influenza vaccination on the viral dynamics of uncomplicated influenza virus infection is not well described; most studies evaluating the effect of vaccination on viral dynamics have been adult human challenge studies, and most data on children come from settings where vaccination has not been widespread. We examined patterns of influenza virus detection among individuals participating in a household transmission study over 3 consecutive influenza seasons.

Method

We conducted a case-ascertained household transmission study in Tennessee and Wisconsin during the 2017-2018 to 2019-2020 influenza seasons, recruiting index patients with laboratory-confirmed influenza and their household members. Enrolled participants completed baseline questionnaires and symptom diaries and obtained self-collected nasal swabs daily for 5 or 7 days. We verified seasonal influenza vaccination status using medical and immunization records. Influenza virus infections were detected using reverse transcription quantitative polymerase chain reaction (RT-qPCR). We examined the impact of age and current season vaccine on viral RNA detection over time, from symptom onset, using Kaplan-Meier survival analysis allowing for censoring and compared median time to end of detection using log-rank tests.

Result

Of 1,659 enrolled participants, 1,112 (67%) had influenza virus infection detected (24% A/H1, 30% A/H3, 13% B/Victoria, 5% B/Yamagata, 28% subtype/lineage unknown; 16% age <5, 43% 5-17, 28% 18-49, and 14% ≥50 years; 47% vaccinated). Mean cycle threshold (CT) values were lowest 1-2 days after symptom onset, suggesting peak viral loads during this time, and this timing of peak virus detection was similar by age and vaccination status. Compared with children aged <18 years, adults with A/H3 and B/Victoria infection were RT-qPCR positive for significantly shorter periods (Figure). Shedding duration was not significantly different in those who had and had not received the current season influenza vaccine.

Conclusion

From detailed data on influenza virus detection among people with uncomplicated infection, we saw longer shedding in children than adults, but similar shedding magnitude and dynamics in vaccinated and unvaccinated individuals. These data support the need for further analysis to understand whether seasonal influenza vaccines impact the natural history and transmission potential of uncomplicated influenza.

Oral Abstract Session: Implementation: Vaccine Effectiveness for COVID-19 and Implications for Influenza

Brendan Flannery - AOXI0239

Leveraging influenza vaccine effective networks for COVID-19 vaccine effectiveness studies

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Background

eginning in the 1990s, multiple US research networks were established to conduct observational studies of seasonal influenza vaccine effectiveness (VE) against influenza associated illness of varying severity. Estimation of influenza VE using test-negative designs assumed vaccination was not associated with risk of non-influenza acute respiratory illnesses. Validity of assumptions requires re-examination for estimation of both COVID-19 and influenza VE.

Method

We reviewed research methods of US influenza VE networks from 1996 through 2022 to assess implications of co-circulation of SARS-CoV-2 and influenza viruses. In influenza VE networks, persons eligible for vaccination presenting with acute respiratory symptoms were actively enrolled at participating healthcare facilities. Study staff administered questionnaires at enrollment to collect data on symptoms, date of illness onset, and patient characteristics. Data were extracted from medical records for underlying conditions and, in inpatient settings, for clinical markers of disease severity. Participant-reported influenza vaccination histories were used to complement immunization records. Respiratory specimens were systematically tested for influenza virus and, beginning in 2020, for both SARS-CoV-2 and influenza virus. Selected virus-positive specimens were antigenically or genetically characterized; genomic analyses of viral specimens expanded after 2014-2015. In May 2020, remote enrollment of symptomatic individuals seeking SARS-CoV-2 testing was added in ambulatory settings.

Result

Active screening for patients meeting predefined clinical criteria for enrollment has been part of US influenza VE networks since inception. Respiratory illness is assumed to be the reason for healthcare seeking. Systematic collection of respiratory specimens and testing regardless of vaccination status reduced potential biases of clinically driven testing. During the COVID-19 pandemic, symptomatic persons might have sought COVID-19 testing rather than care for illness, and healthcare seeking differed by COVID-19 vaccination status. COVID-19 and seasonal influenza vaccination were correlated, violating the assumption that vaccination is independent of risk of infection in the control group. Vaccinated and unvaccinated persons likely differ in exposures to respiratory virus infections given associations between adherence to non-pharmaceutical interventions and choosing to be vaccinated.

Conclusion

Estimation of both COVID-19 and influenza VE when viruses co-circulate requires validation of test-negative designs. Studies based on active enrollment provide patient information to test assumptions common to observational study designs.

Oral Abstract Session: Implementation: Vaccine Effectiveness for COVID-19 and Implications for Influenza

Chelsea Hansen - AOXI0208

Monitoring COVID-19 vaccine effectiveness against symptomatic infection with variants of concern through home-based testing in King County, Washington

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Background

The Greater Seattle Coronavirus Assessment Network Study has provided free, at-home testing for SARS-CoV-2 to residents of King County, WA, since March 2020. Eligible participants complete a questionnaire, including self-reported vaccination status, and receive a test kit delivered to their home. Samples are tested for SARS-CoV-2 and 26 other respiratory pathogens by RT-PCR.

Method

We used a case test-negative design to estimate COVID-19 vaccine effectiveness (VE) against symptomatic infection with variants of concern for fully vaccinated or boosted participants compared to participants who were not fully vaccinated, for all manufacturers combined, adjusting for age, sex, race/ethnicity, region, history of prior infection, week of data collection, and number of cases reported in the community at time of data collection. Variant detection for Delta and Omicron was based on molecular tests. We also used the screening method, a rapid approach which compares the proportion of cases vaccinated to the proportion of the source population vaccinated, to calculate weekly, unadjusted estimates over time using 9-week moving windows.

Result

Between January 2021 - March 2022, 738 test-positive cases (48% Omicron, 24% Delta) and 8121 test-negative controls ≥ 12 years of age enrolled. During June - July 2021, 80% of participants had been fully vaccinated within the past 6 months (62% Pfizer, 31% Moderna, 7% J&J). By December, 35% of participants had received a booster dose. Based on the test-negative design, we estimate VE for the primary vaccination series plus a booster to be 96.3 (82.5 - 99.8) against Delta and 50.1 (29.9 - 64.8) against Omicron. The primary vaccination series alone offered ~60% protection against Delta in the first 6 months after vaccination but did not provide significant protection against Omicron. Using the screening method (Figure) we estimate that between June-September 2021, VE hovered between 65-80%, but fell to 40-50% during October and November as more participants were >6 months past their primary vaccination series. Receiving an additional booster dose restored VE to >90% in November and early December, but this declined to ~50% when Omicron became the dominant variant in late December.

Conclusion

In this highly vaccinated population, VE was stronger against Delta than Omicron and declined with time since vaccination. Our complimentary approaches yielded similar results, but each had unique strengths. The test-

negative design provided robust, variant-specific estimates and allowed for adjustment for participant characteristics, while the screening method offered a rapid approach to monitor trends.

Oral Abstract Session: Implementation: Vaccine Effectiveness for COVID-19 and Implications for Influenza

Celine Gurry - AOXI0407

AFRO-MoVE: African network to monitor vaccine effectiveness for COVID-19 and other respiratory pathogens

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Background

As of 15 May 2022, some 14 months since the first Covid-19 vaccinations in Africa, over 9 specific products have been introduced, yet only 2 countries (Seychelles, Mauritius) have met the WHO set target of 70% of people fully vaccinated by June 2022. Regional coverage for full schedules ranges from 0% to 82% with a median of 19 % in the general population, and from 5% to 92% with a median of 27% in target populations across 38 of 47 countries represented.

The International Vaccine Access Center (IVAC) maps global COVID-19 vaccine effectiveness (CVE) studies to a total count of 272 studies in 38 countries as of 19 May 2022. Of these, three studies are from the African region. Strong evidence requires new information, and validation through replication, thus there is still a need to improve representation of populations for better understanding of vaccine effects in this unique context.

Method

The WHO Regional Office for Africa and partners launched the AFRO-Monitoring Vaccine Effectiveness (AFRO-MoVE) network in March 2021 to facilitate vaccine effectiveness (VE) studies in the region and to establish a platform for monitoring CVE and other respiratory pathogens including influenza and RSV . This project was enabled by the WHO Global Unity studies initiative that initiated and resourced activities for capacity building in the first 12 months, in alignment with other regions (EURO, PAHO), and negotiated global funding for sustainability.

WHO is providing technical assistance, advocacy and coordination with Member States, partners and implementing research institutions. Best practices from influenza vaccine programs were adapted to this network and contexts. AFRO-MoVE has adapted two generic protocols developed by EURO, to measure COVID-19 VE in the region: a prospective cohort study among health workers and a test-negative design nested in existing SARI surveillance. To strengthen CVE capacities and discuss methods, AFRO-MoVE hosts regular network meetings and workshops.

Result

AFRO-MoVE includes partners from 17 AFRO countries and 30 organisations. Twenty-two CVE studies have been mapped in AFRO across 13 countries, and four have published estimates (South Africa, Zambia).

Three countries have adapted the HW protocol to local context, and four the SARI protocol . Nine technical CVE meetings have been organised with participation of 18 countries, and a central database and data management support have been operationalised.

Conclusion

AFRO-MoVE provides a platform to pool data across sites to estimate regional CVE for under-powered objectives. Lessons and experience from Covid-19 will serve to strengthen influenza VE monitoring as seasonal vaccination is introduced in more countries, and in preparedness for future pandemics.

Oral Abstract Session: Implementation: Vaccine Effectiveness for COVID-19 and Implications for Influenza

Nancy Hiu Lan Leung - AOXI0516

Homologous and heterologous boosting with third-dose BNT162b2 and CoronaVac: a randomized trial (Cobovax study)

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Background

All vaccines against COVID-19 approved until now have originally been developed as either a single or homologous two-dose regimen. Two-dose inactivated COVID-19 vaccines have shown inferior immunogenicity compared to two-dose mRNA vaccines, but there are few randomised trials comparing the advantages of heterologous third doses for inactivated and mRNA vaccines.

Method

We conducted a randomized open label trial in adults aged ≥18 years old who had previously received two doses of inactivated vaccine CoronaVac (CC) or mRNA vaccine BNT162b2 (BB). Participants were randomly assigned in 1:1 ratio to receive a third dose of CoronaVac (C) or BNT162b2 (B), i.e. into one of four study arms CC-C, CC-B, BB-C and BB-B. Blood samples were collected at enrolment and after 28 days. The primary immunogenicity outcome was geometric mean titer (GMT) of SARS-CoV-2 serum neutralizing antibodies against vaccine strain (ancestral virus) 28 days after study intervention, measured by plaque reduction neutralisation test (PRNT). We also assessed neutralizing antibodies against Omicron variants, cellular immune responses at days 7 and 28, adverse reactions and COVID-19 infection following vaccination.

Result

We administered vaccines to 451 participants, evenly spread across the four study arms. In 219 participants who had previously received two doses of CoronaVac, neutralizing antibody level against the ancestral virus was increased significantly more by third-dose BNT162b2 (PRNT50 GMT=905) than CoronaVac (GMT=109). Similarly, in 232 participants who had previously received two doses of BNT162b2, neutralizing antibodies was increased significantly more by third-dose BNT162b2 (GMT=816) than CoronaVac (GMT=92). Data on T cell responses, adverse reactions and COVID-19 infections are being analysed.

Conclusion

Heterologous vaccination regime may provide immunologic advantages over homologous vaccination, and may be considered for vaccination against other respiratory virus infections such as influenza.

Theresa Kowalski-Dobson - AOXI0635

Symptom severity and long-COVID in vaccinated and unvaccinated individuals

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Background

After the acute phase of SARS-CoV-2 infection, persistent symptoms, or 'long-COVID', have been reported but little is known about the effect of vaccination on developing long-COVID. To better understand the role of vaccination in long-COVID we analyzed self-reported symptom data from participants enrolled in the Immunity Associated with SARS-CoV-2 (IASO), a prospective longitudinal study.

Method

Participants completed weekly symptom surveys and SARS-CoV-2 testing through the study. Surveys to assess long-COVID symptoms were sent monthly post-infection until all symptoms resolved.

Result

A total of 3,360 people participated in the study contributing 3,421.61 person-years. In total 884 infections were detected, 750 acute infections and 134 serologically confirmed infections. The study has collected 9,827 respiratory samples and 173,336 weekly surveys (completion rate 99.0%). To date, 26.3% (n=884) of participants have a documented infection.

During the first week of infection, unvaccinated individuals were significantly more likely to report lower respiratory symptoms [difficulty breathing (29.2% vs 8.4%, p<0.001), chest pressure (29.2% vs 8.6%, p<0.001)] and loss of taste (46.8% vs 33.0% p<0.001,) and smell (53.3% vs 41.8% p<0.001) compared to those with breakthrough infections. 607 long-COVID surveys on symptoms at 30 days post-infection were analyzed (310 unvaccinated and 297 vaccinated cases). The same proportion (38.4%) of unvaccinated cases and vaccinated cases reported symptoms at 30 days post infection. However, unvaccinated individuals were more likely to report difficulty breathing than vaccinated individuals (9.2% vs 3.4%, p=0.005). No difference was seen in fatigue, cognitive dysfunction, or loss of smell or taste (p>0.05). At 90 days post-infection 3.9% of unvaccinated cases were still experiencing symptoms compared to 1.7% of vaccinated cases (p=0.10). In addition, on average unvaccinated cases had 2.7 symptoms (SD: 3.3) compared to 1.3 symptoms (SD:1.9) in vaccinated cases.

Conclusion

During the first week of illness unvaccinated cases are more likely to report respiratory symptoms and loss of taste/smell than vaccinated cases. No differences were seen in the proportion of individuals with symptoms at 30 days. Vaccinated individuals were half as likely to report the presence of symptoms 90 days after infection and reported fewer symptoms. Data collection and analysis are ongoing. Rates of long-COVID in our prospective study are significantly lower than those from electronic database studies, indicating a potential bias in those studies. A better understanding of the impact of long-COVID prevalence and symptoms in breakthrough infections may inform vaccination policy.

Oral Abstract Session: Translational: COVID-19 - Antivirals and therapeutics

Rohan Narayan - AOXI0155

A natural broad-spectrum inhibitor of enveloped virus entry, restricts SARS-CoV-2 and Influenza A Virus in preclinical animal models

Rohan Narayan[5], Mansi Sharma[5], Rajesh Yadav[4], Abhijith Biji[5], Oyahida Khatun[5], Raju Rajmani[6], Pallavi Raj Sharma[1], Sharumathi Jeyasankar[1], Priya Rani[3], C. Durga Rao[2], Vijaya Satchidanandanam[2], Rachit Agarwal[1], Shashank Tripathi[5]

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Background

The ongoing COVID-19 pandemic has highlighted an urgent need for new antivirals. Human viruses primarily use receptor-mediated endocytosis or viral cellular membrane fusion, or both, during viral entry. The cellular factors involved in these processes are attractive targets for developing broad-spectrum viral entry inhibitors and are less likely to be affected by viral resistance. Picolinic acid (PA) (PubChem, CID 1018) is a naturally occurring metabolite produced during the catabolism of Tryptophan via the Kynurenine pathway. Recently, PA was shown to interfere with endocytic maturation, which we hypothesized could provide broad-spectrum antiviral activity.

Method

The broad-spectrum antiviral effect of multiple PA concentrations was tested in vitro using different cell lines by pre-treating cells with different concentrations of PA prior to infection. A panel of wild-type or reporter-based viruses were tested. Time of addition (ToA) experiments, Transmission electron microscopy (TEM), confocal microscopy, and lipid mixing assays were performed to understand the drug's mechanism of action. Animal experiments were done in Syrian golden hamsters for SARS-CoV-2 and BALB/c mice for IAV. In both cases, 20 mg/kg PA was administered via oral or intraperitoneal routes, using both prophylactic and therapeutic regimens.

Result

In our initial antiviral assays, we observed broad-spectrum antiviral effects of PA against a panel of enveloped viruses, including Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) Hong Kong and four different variants of concern, Influenza A virus (IAV), Dengue and Zika viruses, Herpes simplex virus-2, and Human Parainfluenza virus. In all cases, maximum inhibition was observed when cells were pre-treated with 2 mM PA. Upon testing against non-enveloped viruses, PA did not inhibit Coxsackievirus B3, Rotavirus, or Adeno Virus-5, but marginally reduced Adeno Associated Virus-6 replication. ToA assay data revealed that PA inhibits enveloped virus entry, and not subsequent late events during virus life cycle, and this was supported by confocal microscopy. TEM imaging of PA-treated IAV particles revealed damage to the virus envelope. Lipid mixing assay using octadecyl rhodamine (R18) labeled IAV particles showed inhibition of virus-endosome fusion in the presence of 2 mM PA. Finally, in vivo studies using 20 mg/kg PA against SARS-CoV-2 in Syrian golden hamsters and IAV in BALB/c mice showed significant antiviral efficacy, especially as an oral prophylactic.

Conclusion

Our data establish PA as a broad-spectrum antiviral targeting enveloped virus entry, with promising preclinical efficacy against pandemic respiratory viruses such as SARS-CoV-2 and IAV.

Oral Abstract Session: Translational: COVID-19 - Antivirals and therapeutics

Keiko Baba - AOXI0367

Analysis for specificity of SARS-CoV-2 viral titer testing in Ph2a and Ph2b part of ensitrelvir clinical study

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Background

In Ph2a and Ph2b part clinical study, a novel SARS-CoV-2 3C-like protease inhibitor ensitrelvir showed rapid and profound declines in infectious viral titer and viral RNA compared to placebo. In these studies, viral titer was measured by virus-induced cytopathic effects (CPE) in VeroE6/TMPRSS2 cells.

To evaluate specificity of viral titer measured by CPE, we analyzed results in Ph2a and Ph2b part. Comparison between viral titer and viral RNA by SARS-CoV-2 variants is also under analysis.

Method

In Ph2a and Ph2b, participants infected with SARS-CoV-2 were randomized 1:1:1 to ensitrelvir fumaric acid 125 mg (375 mg on day 1, 125 mg on day 2-5), 250 mg (750 mg on day 1, 250 mg on day 2-5), or placebo. SARS-CoV-2 viral RNA was quantified by RT-PCR for E-gene or N-gene in Ph2a and Ph2b, respectively. To investigate the specificity of viral titer, viral titer of selected nasopharyngeal swab samples in Ph2a was measured by ViroSpot immunostaining using specific anti-SARS-CoV-2 N protein antibody. The correlation between viral titer and viral RNA in swab samples was also examined. Viral titer and viral RNA higher than lower limit of quantification were used for analysis.

Result

In selected samples, a significant correlation was observed between viral titer measured by CPE and immunostaining (Pearson r = 0.81), and comparable values were detected by both assays. Specificity of viral titer by CPE was also confirmed by a positive correlation between viral titer and viral RNA at baseline in Ph2a and Ph2b. In addition, viral RNA decreased on day 2 from the baseline in proportion to declines of viral titer in all groups, and Pearson analysis showed a positive correlation of declines in viral titer with viral RNA.

Viral RNA (copies/mL) were approximately 1000-10000-fold and 10000-100000-fold higher than viral titer (TCID50/mL) in Ph2a and Ph2b, respectively. Even though there is no big difference in viral RNA between Ph2a and Ph2b at baseline, median viral titer in Ph2b was significantly lower than that in Ph2a. The viral titer may represent epidemic SARS-CoV-2 variant in the study period, because Ph2a was conducted in delta epidemic period and Ph2b was in omicron period.

Conclusion

Pearson analysis showed that a positive correlation was observed between viral titer measured by CPE and immunostaining, or viral titer and viral RNA at baseline. These results suggest that SARS-CoV-2 viral titer could be specifically measured by CPE in ensitrelvir Ph2a and Ph2b studies and viral titer testing by CPE in VeroE6/TMPRSS2 cells is an appropriate method for SARS-CoV-2 viral titer measurement in clinical studies.

Oral Abstract Session: Translational: COVID-19 - Antivirals and therapeutics

Emi Takashita - AOXI0418

Efficacy of therapeutic monoclonal antibodies and antiviral drugs against SARS-CoV-2 variants

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Background

Several therapeutic monoclonal antibodies and antiviral drugs are approved for the treatment or prophylaxis of COVID-19, which is caused by SARS-CoV-2. The emergence and spread of SARS-CoV-2 variants may reduce the efficacy of these antiviral agents and pose an increased risk to global public health. In this study, we examined the efficacy of therapeutic monoclonal antibodies and antiviral drugs against SARS-CoV-2, including an early SARS-CoV-2 strain (A) and alpha (B.1.1.7), beta (B.1.351), gamma (P.1), delta (B.1.617.2), and omicron (BA.1, BA.1.1, and BA.2) variants.

Method

Neutralization activities of therapeutic monoclonal antibodies (etesevimab, bamlanivimab, imdevimab, casirivimab, tixagevimab, cilgavimab, and sotrovimab), individually and in combination, against SARS-CoV-2 were determined by using a focus reduction neutralization test (FRNT); the results (ng/mL) are expressed as the 50% focus reduction neutralization titer (FRNT50). Susceptibilities of SARS-CoV-2 to antiviral drugs, RNA-dependent RNA polymerase inhibitors (remdesivir and molnupiravir) and a protease inhibitor (nirmatrelvir), were determined by using a focus reduction assay (FRA); the results (μM) are expressed as the 50% inhibitory concentration (IC50).

Result

The etesevimab/bamlanivimab combination showed remarkably reduced neutralizing activity against gamma and lost neutralizing activity against beta and omicron/BA.1, BA.1.1, and BA.2. The imdevimab/casirivimab combination inhibited omicron/BA.2 but did not inhibit omicron/BA.1 or BA.1.1. However, the FRNT50 value of this combination was higher for omicron/BA.2 than for the early strain and the alpha, beta, gamma, and delta variants. The tixagevimab/cilgavimab combination retained its neutralizing activity against omicron/BA.2 but showed reduced neutralizing activity against omicron/BA.1 and BA.1.1. Sotrovimab had reduced neutralizing activity against omicron/BA.1, BA.1.1, and BA.2 than against the early strain and the alpha, beta, gamma, and delta variants. The FRNT50 value of this monoclonal antibody was higher for omicron/BA.2 than for omicron/BA.1 and BA.1.1.

All variants showed comparable IC50 values to that of the early strain for remdesivir, molnupiravir, and nirmatrelvir.

Conclusion

Our results suggest that all SARS-CoV-2 variants tested are susceptible to the antiviral drugs remdesivir, molnupiravir, and nirmatrelvir; however, the efficacy of therapeutic monoclonal antibodies against SARS-CoV-2 variants differ even among omicron subvariants. Therefore, identification of variants may be useful to select therapeutic monoclonal antibodies for COVID-19.

Oral Abstract Session: Translational: COVID-19 - Antivirals and therapeutics

Andrew Ustianowski - AOXI0494

Safety, pharmacokinetics and anti-drug antibodies following a second dose of AZD7442 (tixagevimab/cilgavimab): open-label sub study of the PROVENT Phase 3 trial for symptomatic COVID 19 prevention

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Background

AZD7442 is a combination of two extended-half-life SARS-CoV-2-neutralising monoclonal antibodies (tixagevimab/cilgavimab) that bind to distinct epitopes on the SARS-CoV-2 spike protein. In the PROVENT study, a single 300-mg intramuscular (IM) dose of AZD7442 demonstrated 77% efficacy for prevention of symptomatic COVID-19 vs placebo at primary analysis (median follow-up [FU] 83 days), with 83% efficacy at 6-months' median FU, and was well-tolerated. We report safety, pharmacokinetics (PK) and anti-drug antibodies (ADA) following a second dose of AZD7442 in the PROVENT sub-study.

Method

The PROVENT sub-study is a Phase 3, multi-centre, open-label study (NCT04625725) that included participants from the PROVENT study who may benefit from ongoing protection against SARS-CoV-2. Participants received a second 300 mg IM AZD7442 dose ~12 months after the first dose. The primary endpoint was safety and tolerability of AZD7442. Secondary endpoints included PK and ADA response. Data are reported from an interim analysis (data cut-off February 25, 2022), planned for when ≥50 participants had received AZD7442 12 ± 2 months after the first dose in PROVENT and completed the sub-study Day 29 visit. PK and ADA are presented from the sub-study Day 29 visit.

Result

At interim analysis, 305 participants were enrolled and received their second AZD7442 dose (median FU 17.0 days) 12 ± 2 months after the first dose. Overall, 44/305 (14.4%) participants had ≥1 adverse event (AE); all were of mild or moderate intensity. One (0.3%) participant had a serious AE of moderate migraine. Two (0.7%) participants had AEs of special interest of injection-site reactions (pain) and there were no deaths. In the PROVENT study, geometric mean ± SD serum AZD7442 concentration (sum of tixagevimab + cilgavimab) 28 days after the first dose was 23.3 ± 1.9 µg/mL. At interim analysis in the PROVENT sub-study (n=53 participants with available samples), geometric mean ± SD serum AZD7442 concentration was 1.4 ± 2.2 µg/mL prior to second dose and 26.4 ± 1.5 µg/mL 28 days after the second dose. At interim analysis, 49 ADA-evaluable participants had available AZD7442 ADA Day 29 results. Treatment-emergent AZD7442 ADA were observed in 5 (10.2%) participants; the median of maximum ADA titre was low (80; min, max: 80, 640). No AEs were reported by participants with

treatment-emergent AZD7442 ADA and AZD7442 serum concentrations were similar between participants with and without AZD7442 ADA.

Conclusion

A second dose of 300-mg IM AZD7442 ~12 months after the first dose was well tolerated, with similar safety and PK to that seen following the first dose.

Oral Abstract Session: Translational: COVID-19 - Antivirals and therapeutics

Mirella Salvatore - AOXI0643

RISK FACTORS FOR PERSISTENT COVID INFECTION IN THE IMMUNOCOMPROMISED HOSTS

***Mirella Salvatore*[1], *Jing-Mei Hsu*[1], *Ayana Morales*[1], *Rosemary Soave*[1]**

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Background

COVID-19 caused by the Severe Acute Respiratory Syndrome Coronavirus-2 (SARSCoV2) is a devastating cause of severe pneumonia and multiorgan failure with a high mortality. Vaccination and therapeutic strategies are constantly hindered by the rapid emergence of viral variants that are highly transmissible and less sensitive to neutralization by antibodies. Some immunocompromised patients develop chronic infection with persistent viral shedding and are thus contagious for prolonged periods. There are increasing numbers of reported cases of chronic SARS-CoV-2 infection in immunocompromised hosts in which the prolonged viral replication has been identified as one of the key factors contributing to the development of SARS-CoV2 variants.

The emergence of antibody and drug-resistant variants during treatment makes clearing SARS-CoV2 infection from these patients a therapeutic challenge. Moreover, prolonged shedding of these resistant SARS-CoV2 variants facilitates spread to the population, resulting in surges of infection and disease. A definite lack of knowledge still exists on the risk factors for persistent infection and on the best management strategies to control viral replication in patients with chronic SARS-CoV-2 infection.

Method

Immunocompromised patients (hematologic malignancies, stem cell transplant recipients) with a positive COVID-19 test from 3/2020 to 5/2022 were included. Clinical data including demographics, comorbidities, laboratory tests, treatments dates of SARS COV-2 nasal swab positivity were collected longitudinally. Characteristics of patients with persistent viral infection (defined persistent nasal swab positivity lasting longer than 60 days from onset of infection) were compared to those who cleared the virus. This study was approved by the Weill Cornell IRB.

Result

In our preliminary review, 15 of 51 immunocompromised patients with COVID-19 met the criteria for having prolonged infection. Both groups had similar demographics (mean age 60.1 vs 62.1 years), gender composition (53,5 vs 52.7 % female).

B cell lymphomas (40%) and AML (26.6%) were the most common type of underlying malignancy in patients with prolonged infection. Seven of these patients received Remdesivir (up to 3 cycles) or monoclonal antibodies (3 patients) without any effect on viral clearance. SARS-CoV2 RNA sequencing is currently ongoing. Four of the patients with prolonged infection developed organizing pneumonia.

Conclusion

Antiviral therapies alone seem to be inefficient to drive viral clearance. Full results of this study can be used to optimize covid-19 treatment and public health prevention measures in the immunocompromised host.

Oral Abstract Session: Translational: COVID-19 - Vaccines (focus: vaccines in use, late stage development and pan-coronavirus vaccines)

Sho Miyamoto - AOXI0166

Determinants of humoral immune responses against antigenically distinct SARS-CoV-2 variants in COVID-19 vaccine breakthrough infection.

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Background

The immune profile against SARS-CoV-2 has diversified due to combinations of exposure to vaccines and infection by several variants, including the Omicron with numerous spike mutations. To properly assess the humoral immunity against circulating SARS-CoV-2 variants in individuals with various immune histories, we investigated the key drivers of magnitude and cross-neutralizing potency of humoral immune responses in COVID-19 vaccine breakthrough infection.

Method

The neutralization susceptibility of the variants, including the Omicron BA.1 and BA.2, and their ancestor was comparably assessed using a panel of serum derived from individuals with mRNA vaccine who suffered breakthrough infections by the non-Omicron variants. The impact of viral load in the respiratory specimens at diagnosis and time-interval from the second vaccination on the neutralization titers were assessed.

Result

obust cross-neutralization against the Omicron BA.1 and BA.2 were induced in vaccinees that experienced breakthrough infections. The increase of neutralization potency from the acute to convalescent phase against ancestral to omicron variants positively correlated with viral load in the respiratory specimen at diagnosis. In addition, the vaccination-infection intervals positively correlated with the cross-neutralizing potency against beta, BA.1, and BA.2 variants, distinguished by antigenic cartography.

Conclusion

These findings suggested that viral replication in upper respiratory tracts determines the magnitude of humoral immune responses, and the time interval between vaccination and infection expands the breadth of humoral immune responses against SARS-CoV-2 infection. Furthermore, two heterogeneous factors determined at the time of infection are independently involved in regulating the humoral immune response to SARS-CoV-2 in their convalescent phase, illuminating the driving forces behind the diversification of population immunity to SARS-CoV-2.

Oral Abstract Session: Translational: COVID-19 - Vaccines (focus: vaccines in use, late stage development and pan-coronavirus vaccines)

Irina Isakova-Sivak - AOXI0238

Development of a bivalent vaccine against SARS-CoV-2 and influenza using a live attenuated influenza vaccine platform

Irina Isakova-Sivak[1], Ekaterina Stepanova[1], Daria Mezhenskaya[1], Victoria Matyushenko[1], Sergei Niskanen[2], Elena Vinogradova[2], Alexander Dmitriev[1], Larisa Rudenko[1]

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Background

COVID-19 pandemic emerged in 2020 and has caused an unprecedented burden to all countries in the world. SARS-CoV-2 continues to circulate and antigenically evolve which enables multiple reinfections. In case of seasonal co-circulation of influenza viruses and SARS-CoV-2, a bivalent vaccine against both pathogens can help control these infections.

Method

We used live attenuated influenza vaccine (LAIV) viruses to generate recombinant influenza viruses expressing either structural antigenic fragment or T-cell epitopes of SARS-CoV-2. Chimeric influenza viruses were rescued by standard reverse genetics procedures. Western-blot analysis was used to confirm the expression of structural SARS-CoV-2 antigen. Stimulation of PBMCs of COVID-19 patients with recombinant LAIV-SARS-CoV-2 viruses was used to confirm correct processing of inserted T-cell epitopes. Immunogenicity of the vaccine prototypes was assessed in BALB/c and HLA-A2 mice, as well as in Syrian hamsters. Protective effect of the vaccines against both SARS-CoV-2 and influenza infections was evaluated in Syrian hamsters

Result

We generated a panel of recombinant LAIV viruses encoding immunogenic fragments of SARS-CoV-2. All rescued viruses retained their main phenotypic properties of LAIV viruses. Structural fragment of SARS-CoV-2 S protein was inserted into HA molecule of influenza virus and its expression was confirmed by WB with specific anti-HA and anti-Spike antibodies. Immunization of animals with LAIV encoding structural SARS-CoV-2 antigen induced robust anti-influenza and anti-Spike antibody responses. We also inserted cassettes encoding a number of conserved human T-cell epitopes of SARS-CoV-2 into NA or NS1 genes of LAIV viruses via the P2A self-cleavage site. Intranasal immunization of transgenic HLA-A2 mice with these recombinant viruses didn't result in significant SARS-CoV-2-specific T-cell responses, due to the immunodominance of NP366 influenza T-cell epitope. However, side-by-side stimulation of PBMCs of COVID-19 convalescents with recombinant viruses and LAIV vector demonstrated activation of memory T cells in samples stimulated with LAIV/SARS-CoV-2, but not LAIV alone. Hamsters immunized with LAIV/SARS-CoV-2 prototypes were protected against challenge with influenza virus and a high dose of SARS-CoV-2, which was confirmed by reduced weight loss, milder clinical symptoms and lower virus pulmonary titers, compared to LAIV- and mock-immunized animals.

Conclusion

LAIV is a promising platform for the development of a bivalent vaccine against influenza and SARS-CoV-2. The developed LAIV/SARS-CoV-2 vaccine prototypes warrant further assessment in preclinical and phase I clinical trials.

Funding. This study was funded by RSCF grant 21-75-30003.

Oral Abstract Session: Translational: COVID-19 - Vaccines (focus: vaccines in use, late stage development and pan-coronavirus vaccines)

George Carnell - AOXI0377

The receptor binding domain as a target for variant proof and pan-sarbecovirus vaccines

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Background

etacoronaviruses continue to represent one of the greatest threats for viral zoonotic spill over from existing animal reservoirs. Research has expanded rapidly in the detection and characterisation of new Coronaviruses in animal reservoirs. To mitigate this threat, pan-sarbecovirus vaccines are needed to provide immunity to existing circulating viruses in humans, and cross-reactive immunity to those at greatest risk of future spill-overs. Here we describe two structurally-informed computationally designed receptor binding domain vaccine candidates. The first (T2_8) is SARS-CoV-2 specific, and a candidate for a 'variant proof' booster vaccine. The second (T2_17) is capable of eliciting neutralising antibodies to diverse betacoronaviruses, as well as circulating SARS-CoV-2 variants of concern (VOC).

Method

A range of candidates were generated from an array of phylogenetically informed antigen structures displaying broad neutralising epitopes. T2_8 contains a masked epitope, redirecting the immune response to a neutralising epitope. T2_17 is a hybrid RBD containing epitopes from SARS-CoV-1 and SARS-CoV-2. Candidates were screened in BALB/c mice using DNA, mRNA and MVA platforms. Antisera were evaluated for neutralising antibodies (nAb) against a panel of lentiviral pseudotypes bearing the spike protein from diverse sarbecoviruses and SARS-CoV-2 VOCs. Binding antibodies against SARS-CoV-1 and SARS-CoV-2 RBDs were assessed by ELISA with mouse antisera. T2_17 was further evaluated in guinea pigs and rabbits, and tested in prime-boost challenge experiments using K18-huACE2 mice primed with licensed SARS-CoV-2 vaccines (Comirnaty or Vaxzevria).

Result

The T2_8 construct elicited strong variant proof antibodies in a DNA/MVA prime-boost, with antibodies both binding and neutralising VOCs significantly stronger than controls. T2_17 elicited antibodies that bound to and neutralised both SARS-CoV-1 and SARS-CoV-2 pseudoviruses, as well as those bearing the WIV16 or RaTG13 spikes. T2_17 elicited nAbs included coverage to VOCs alpha, beta, gamma, delta and omicron. Initial studies revealed a weak nAb response in mice, but strong nAb response in guinea pigs and rabbits, or mice primed with Comirnaty/Vaxzevria, where nAb response was boosted significantly. Crucially T2_17 boosted mice were fully protected from challenge by delta SARS-CoV-2.

Conclusion

The RBD is a viable target for booster vaccination against SARS-CoV-2, and as part of wider efforts to vaccinate against a variety of circulating or zoonotic potential viruses. Hybrid structurally modified RBDs containing cross-reactive neutralising or binding antibody epitopes are an important component of optimised antigens in the development of a pan-coronavirus vaccine.

Oral Abstract Session: Translational: COVID-19 - Vaccines (focus: vaccines in use, late stage development and pan-coronavirus vaccines)

Jordan Clark - AOXI0634

Non-neutralizing antibodies provide protection against lethal challenge with SARS-CoV-2 in murine and hamster infection models

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Background

Several studies have highlighted that COVID-19 vaccination elicits a higher number of non-neutralizing antibodies compared to SARS-CoV-2 infection, and that vaccinated individuals still exhibit protection from disease following infection with SARS-CoV-2 variants. These data suggest that other factors aside from neutralization are important for the control and clearance of infection. In order to explore this further, we utilized a previously described panel of 42 monoclonal antibodies (mAbs) derived from plasmablasts of three subjects vaccinated with the BNT162b2 vaccine.

Method

The protection conferred by the mAbs was investigated using in a lethal mouse challenge model and the effector functions of the mAbs were characterized using antibody dependent cellular cytotoxicity (ADCC) and antibody dependent cellular phagocytosis (ADCP) reporter assays. A subset of protective mAbs were cloned into IgG1 expression vectors harboring the LALA (L234A-L235A) and LALAPG (L234A-L235A, P329G) mutations which abolish effector cell binding and were utilized in a in vivo protection study.

Result

We identified 12 non-neutralizing antibodies that conferred protection from lethal SARS-CoV-2 challenge in a mouse model. Further experiments utilizing a lower challenge dose revealed that these mAbs were able to reduce lung viral titers. Reporter assays demonstrated that the majority of the mAbs exhibited ADCC and ADCP activity, with the highest activity elicited by neutralizing mAbs. When mutagenized to abrogate effector binding, three non-neutralizing mAbs lost protection, however three antibodies retained protection despite the presence of the LALA and LALAPG mutations.

Conclusion

This study characterized a panel non-neutralizing antibodies elicited by vaccination with the BNT162b2 vaccine. We found that several mAbs provided protection against lethal challenge with SARS-CoV-2 and were able to reduce lung viral titers, indicating that despite a lack of neutralizing ability, these mAbs are still capable of controlling infection. This protection is likely mediated via interactions with effector cells, thereby clearing infected cells via ADCC and ADCP in order to limit infection. By abrogating effector binding, we confirmed that three of the most protective non-neutralizing antibodies exert their protection via effector functions. Curiously, antibodies PVI.V5-4, PVI.V6-3 and PVI.V6-12 retained protection even in the absence of effector binding. As these antibodies are

incapable of neutralizing SARS-CoV-2, and no longer elicit effector cell functions, the mechanism through which they promote survival is unclear. This study highlights the protective capabilities of non-neutralizing antibodies, and hints at a mechanism of protection which is independent of neutralization and effector binding.

Oral Abstract Session: Translational: COVID-19 - Vaccines (focus: vaccines in use, late stage development and pan-coronavirus vaccines)

Tomer Hertz - AOXI0620

Correlates of protection for the BNT162b2 vaccine three and four booster doses during an Omicron outbreak in a healthcare workers multi-center prospective study

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Background

For the past two and a half years, the SARS-CoV-2 virus has driven a worldwide pandemic with mRNA vaccines having an enormous impact on its course. However, variants of concern (VOCs) and waning immunity continue to pose a serious problem. The main goal of this study was to identify novel binding antibody correlates of protection (COPs) against symptomatic SARS-CoV-2 infection in vaccinated healthy individuals comparing those who were vaccinated with three doses to those who received four.

Method

We conducted a multicenter prospective cohort study designed to assess the association between different serological profiles, neutralization assays and risk for SARS-CoV-2 infection, comparing those vaccinated with three doses of Pfizer-BioNTech vaccine to those who received a fourth dose. We enrolled 614 healthy adult health care workers, 221 received a 4th dose and 393 received 3 doses.

Result

During the first 90 days of followup, 245 (39.9%) were infected with SARS-CoV-2 of whom 71 (32.1%) received 3 doses, and 174 (44.3%) received 4 doses. Day 30 IgG and IgA serum antibody levels following a 4th dose elicited a significant rise in antibody binding and neutralizing titers to multiple VOCs including omicron, and reduced the risk of symptomatic infection by 31.9% [95% CI 7%-50%]. We then assessed various baseline binding antibody markers as COPs. We identified several IgA and IgG baseline markers that were associated with protection. Combinations of IgG and IgA markers performed better than single markers. The strongest marker was IgG Abs to RBD Mutants & IgA Abs to VOCs (boosted HR=20.2, p=0.007; unboosted HR=4.5, p=0.017, respectively). We also found that baseline binding antibody markers were associated with significant differences in neutralizing antibody titers. These differences were not found at 30 days following the booster dose. Despite the significant rise in antibody levels, the number of infections in the low-baseline group (n=16) was higher than in the high-baseline group (n=7, 43% vs. 20%, p=0.051).

Conclusion

Our study demonstrated that combinations of IgA and IgG antibody levels to SARS-CoV-2 VOCs are associated with protection from symptomatic infection in healthy adults, that received booster doses of the Pfizer vaccine. Our study identified a subpopulation of healthy adult individuals with low-baseline levels of antibodies to SARS-CoV-2 vwhich are at increased risk for SARS-CoV-2 infection, despite receiving three or four doses of the Pfizer-BioNTech vaccine. These findings warrant further study into this group to assess if they are also at higher risk for severe disease or to possibly spread the infection more readily than others.

Jeremy Jones - AOXI0077

Fitness, transmission, and mechanism of baloxavir resistance of influenza A viruses with PA E23X substitutions

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Background

loxavir marboxil (BXM) and its active metabolite baloxavir acid (BXA) are potent inhibitors of the influenza virus polymerase acidic (PA) protein. Clinically associated, treatment-emergent substitutions causing reduced BXM/BXA susceptibility most commonly occur at PA I38T and E23X. While I38T is well described, little data exists for E23X polymorphisms. Here we addressed the impact of E23X on virus fitness, transmission potential, and structural consequences for PA-BXA binding interactions.

Method

We generated recombinant A/California/04/2009(H1N1)pdm09 and A/Texas/71/2017(H3N2) influenza viruses carrying either single (E23G, E23K, E23R) or dual (E23G/K/R+I38T) PA substitutions. BXA susceptibility was measured by plaque reduction in MDCK cells. Polymerase activity was assessed in the minireplicon assay. Replication kinetics and plaque diameters were determined in MDCK cells. Direct or airborne routes of transmission (≤ 1x10^5 TCID50/animal) were studied in ferrets. Affinity of PA-BXA interactions was measured in thermostability assays, while molecular docking examined structural outcomes of each PA substitution.

Result

Single substitution E23G/K/R viruses displayed ≥ 2, 13, and 19-fold BXA EC50 increases vs. wild-type (WT), respectively. Combining E23G/K/R+I38T further increased BXA EC50s ≥ 138, 200, 928-fold vs. WT, respectively. Replication in MDCK cells, polymerase activity, and plaque diameters showed E23K viruses had greater in vitro fitness defects than E23G/R, with E23K+I38T being most impaired. All single E23G/K/R and dual E23G/K/R+I38T viruses transmitted to direct and airborne ferret contacts, though E23K+I38T virus failed to achieve 100% airborne transmission. Sequence analysis showed single or dual E23X genotypes were stable during both ferret transmission events and passaging in MDCK cells without BXA. Thermo-stable PA-BXA interactions were weakened more by E23K than E23G, and further compromised by addition of I38T. Modeling predicted that E23G negatively impacted 1 of 2 potential PA-BXA binding points, while E23K disrupted both, possibly explaining the higher E23K EC50s.

Conclusion

While E23G/K/R substitutions may negatively affect in vitro fitness, they do not alter ferret transmissibility and remain genetically stability. The synergistic effect of dual E23G/K/R+I38T substitutions dramatically elevated EC50s more than the sum of the EC50s for each individual substitution. Decreased PA-BXA interactions partially explain reduced BXA susceptibility caused by E23G/K. Our data indicates that E23G/K/R or dual substitutions with I38T should be considered important BXM reduced-susceptibility markers with potential to emerge in circulation.

Oral Abstract Session: Translational: Influenza - Antivirals and therapeutics

Jonathan Temte - AOXI0145

Rapid Detection of Influenza Outbreaks in Long Term Care Facilities Reduces Emergency Room Visits and Hospitalization: Rapid Assessment of, and Prophylaxis for Influenza for Denizens of Long-Term Care Facilities (RAPID-LTCF)

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Background

Influenza is a significant respiratory pathogen for residents of long-term care facilities (LTCFs). Rapid influenza detection tests (RIDT) may enable early outbreak detection allowing a timely response. We assessed whether the use of RIDT for LTCF residents with acute respiratory infection is associated with increased antiviral use and decreased healthcare utilization.

Method

This was a non-blinded, pragmatic, randomized controlled trial utilizing LTCFs in Wisconsin. The participants were residents of 20 LTCFs matched by bed capacity and geographic location. Intervention: the intervention had two parts: (1) modified case identification criteria and (2) nursing-staff initiated collection of nasal swab specimen for on-site RIDT. Outcome measures: the primary outcome measures, expressed as events per 1000 resident-weeks, included antiviral treatment courses, antiviral prophylaxis courses, total emergency department (ED) visits, ED visits for respiratory illness, total hospitalization, hospitalization for respiratory illness, hospital length of stay, total deaths, and deaths due to respiratory illness over three influenza seasons. This trial was registered at clinicaltrials.gov using the Identifier: NCT02964871.

Result

Oseltamivir use for prophylaxis was higher at intervention LTCFs (2.6 vs 1.9 courses per 1000 person-weeks; rate ratio: 1.38; 95%CI: 1.24-1.54; p<0.001); rates of oseltamivir use for influenza treatment were not different. Rates of total ED visits (7.6 vs 9.8/1000 person-weeks; RR=0.78; 95%CI: 0.64-0.92; p=0.004), total hospitalizations (8.6 vs 11.0/1000 person-weeks; RR=0.79; 95%CI: 0.67-0.93; p=0.004), and hospital length of stay (35.6 days vs 55.5 days/1000 person-weeks; RR=0.64; 95%CI: 0.0.59-0.69; p<0.001) were lower at intervention as compared to control LTCFs. No significant differences were noted for respiratory-related ED visits or hospitalizations or in rates for all-cause or respiratory-associated mortality.

Conclusion

The use of low threshold criteria to trigger nursing staff-initiated testing for influenza with RIDT resulted in increased prophylactic use of oseltamivir. There were significant reductions in the rates of all-cause ED visits (22% decline), hospitalizations (21% decline), and hospital length of stay (36% decline) across three combined influenza seasons. No significant differences were noted in respiratory-associated and all-cause deaths between intervention and control sites. This feasible, low-cost and simple intervention may provide significant benefit and should be further tested in other settings.

Oral Abstract Session: Translational: Influenza - Antivirals and therapeutics

Ali Zhang - AOXI0536

Broadly-neutralizing antibodies that bind to the hemagglutinin stalk domain enhance the effectiveness of neuraminidase inhibitors via Fc-mediated effector functions

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Background

Influenza A viruses (IAV) typically cause 3-5 million serious illnesses and more than half a million deaths annually worldwide. Seasonal vaccination is the best way to prevent infection, but the protection provided is narrow and ineffective against pandemic strains due to antigenic variability in the immunodominant hemagglutinin (HA) head domain. Broadly-neutralizing antibodies (bNAbs) against the conserved HA stalk domain have provided great promise towards development of universal influenza vaccines and therapeutics. These bNAbs require Fc receptor binding and immune cell function to confer maximal protection. We show that pharmacological inhibition of neuraminidase (NA) enhances Fc-dependent effector functions elicited by bNAbs, and that combining neuraminidase inhibitors with bNAbs lead to superior protection against influenza morbidity and mortality in murine IAV challenge models.

Method

We used in vitro antibody-dependent cell cytotoxicity (ADCC) assays to determine the effects of oseltamivir, an NA inhibitor, on monoclonal bNAb-mediated ADCC of IAV infected cells. Furthermore, we used ADCC assays to determine how oseltamivir influences the ability of serum from healthy, influenza vaccinated donors to modulate ADCC of IAV infected cells. The bNAb titers of serum samples were quantified using ELISA and HAI assays. Next, we used murine influenza challenge models to determine if oseltamivir in combination with bNAbs was superior at protecting against influenza clinical signs compared to using either therapeutic alone.

Result

We demonstrate that oseltamivir causes a dose-dependent increase in both monoclonal and polyclonal bNAb-mediated ADCC of IAV infected cells in an Fc-Fc receptor dependent manner. Using murine challenge models, we also show that oseltamivir in combination with monoclonal bNAbs or high titers of polyclonal bNAbs is superior at protecting against and treating lethal IAV infection compared to using either therapy alone. This protection is also dependent on an intact Fc-Fc receptor interaction.

Conclusion

The mechanism by which bNAbs protect against IAV propagation is incompletely understood. Our findings may explain the variable efficacy of oseltamivir in patients and guide design of universal influenza vaccines and other bNAb-based anti-viral therapeutics.

Oral Abstract Session: Translational: Influenza - Antivirals and therapeutics

NIRMAL KUMAR - AOXI0581

1,3-Diphenylurea derivatives inhibit the cellular entry of influenza A virus and SARS-CoV-2

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Background

In the fight against influenza and ongoing COVID-19 pandemic, while vaccination remains a cornerstone in prophylaxis, antivirals constitute an essential element in the treatment of affected patients. However, most of the potential antivirals currently being tested are targeted against viral proteins, and therefore, concern about the development of drug-induced resistance due to the rapid emergence of mutant viral strains remains.

Method

A high-content confocal imaging-based screening was performed to identify antivirals for IAV and SARS-CoV-2 using indirect immunofluorescence. 1,3-Diphenylurea derivatives (DPUDs) were chemically synthesised. IAV and SARS-CoV-2 entry were assessed using various imaging and spectrophotometric assays. The in vivo therapeutic effect of DPUDs was evaluated in C57BL/6 mice infected with IAV-WSN. The in vitro chloride transport assay was performed with lucigenin-entrapped large unilamellar vesicles.

Result

Screening of a small molecule library identified a group of 1,3-diphenylurea derivatives (DPUDs) that blocked IAV and SARS-CoV-2 infection in tissue culture cells by >99%. Investigating the mechanism of action of DPUDs, we found that they targeted cellular endocytosis and endocytic maturation processes as evident by a significant reduction in EGF and transferrin uptake, and a block in intra-vesicular acidification. Furthermore, we found that DPUDs are capable of transporting chloride ions across the cell membrane, possibly interfering with the cellular endocytic machinery. Previous studies demonstrated that chloride transport is tightly linked to vesicular trafficking, and therefore, perturbation by chloride-transporting molecules can potentially disrupt ion homeostasis, resulting in endosomal maturation defects. We also observed inhibition of infection by the DPUDs against different IAV and SARS-CoV-2 strains. Finally, we tested the efficacy of the DPUDs against IAV infection in mice, and observed a significant recovery and reduction in lung viral titer compared to the control mice.

Conclusion

Our results demonstrate that the diphenylurea-derived compounds identified through our study efficiently block the entry of IAV and SARS-CoV-2, which could be developed as a new class of broad-spectrum antiviral agents and could be an important addition to the armamentarium in the combat against the prevailing COVID-19 pandemic.

Oral Abstract Session: Translational: Influenza - Antivirals and therapeutics

Madduri Srinivasarao - AOXI0649

Design of Ligand-Targeted Immunotherapy for the Treatment of Influenza Virus Infections

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Background

Nearly 10% of world population is affected by influenza virus infections. Based on the most recent report from Centers for Disease Control and Prevention, during 2019-2020 flu season, more than 35 million people were infected in the US alone, of which 380,000 cases have ended up in hospitalization. While flu vaccines are widely available, their effectiveness has been only 39% during the 2019-2020 season. The approved drugs such as Tamiflu and Xofluza are most effective if taken in the early stages of infection. Therefore, there is an unmet need for developing novel therapeutics especially at later stages of infection.

Method

Herein we report a targeted therapeutic strategy with a dual mechanism of action that elicits host immune response against the virus and virus-infected cells. Because neuraminidase appears both on the influenza viral envelope and the infected cell surface, we repurposed the neuraminidase inhibitor zanamivir as the targeting ligand. Our targeted drug is designed by conjugating zanamivir to haptens that bind to naturally occurring antibodies in humans. Once recruited, these anti-hapten antibodies bind and activate innate immune system against the virus and virus-infected cells. Even though we recently showed remarkable antiviral activity with a zanamivir-mono-hapten conjugate, more recently we investigated whether a conjugate that has more than one hapten would improve the therapeutic outcome.

Result

When tested in BALB/c mice supplemented with intravenous IgG (IVIG) and infected with influenza A virus (H1N1, A/Pueto Rice/8/1934), a zanamivir-dual hapten conjugate demonstrated superior antiviral activity than the mono-hapten conjugates at both early and late-stage infection. More importantly, the dual hapten conjugate showed better or comparable activity in late-stage infection in comparison to standard-of-care drugs Tamiflu and Xofluza at a single dose. In addition to showing better therapeutic efficacy, the dual hapten conjugate also showed significantly higher reduction of viral titer in the lungs of infected mice.

Conclusion

Eradivir's targeted immunotherapeutic containing zanamivir-dual hapten conjugate has the potential to treat both early and late-stage influenza infection. While antiviral efficacy studies in mice showed superior performance by the targeted drug compared to standard-of-care drugs, further testing in more clinically relevant ferret studies is currently underway.

Oral Abstract Session: Translational: Influenza - Broadly protective/universal influenza virus vaccines

James Allen - AOXI0075

Universal mRNA vaccines for influenza elicit broadly protective antibodies against co-circulating and future drifted H1N1 and H3N2 viral variants

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Background

The implementation of mRNA vaccines against COVID-19 has successfully validated the safety and efficacy of the platform, while at the same time revealing the potential for their applications against other infectious diseases. Seasonal influenza vaccines often induce strain specific antibody responses that offer limited protection against antigenically drifted co-circulating viruses, leading to reduced vaccine efficacy. The magnitude and strength of these antibody responses can be enhanced through the addition of adjuvants. However, only a few adjuvants are approved for clinical use in humans, and other novel adjuvants must be fully investigated for safety and efficacy before they can be included in seasonal vaccine formulations. Universal influenza vaccine antigens and mRNA delivery platforms can be combined to generate more effective and safe vaccines that elicit cross-reactive antibodies against antigenically drifted strains of influenza without the use of adjuvant.

Method

ice were vaccinated with 3mcg of H1 and H3 Computationally Optimized Broadly Reactive Antigen (COBRA) hemagglutinin (HA), or wild-type (WT) H1 and H3 influenza HA mRNA sequences encapsulated in lipid nanoparticles (LNPs) in a prime-boost regimen. Blood was collected from the animals 2 weeks after both the prime and boost, which was assessed for sero-protective HAI antibody titers against contemporary, co-circulating, and future drifted strains of H1N1 and H3N2 influenza. One month after the boost, all mice were challenged with a lethal dose of either A/California/04/2007 H1N1 or A/Kansas/14/2017 H3N2 virus to assess protective efficacy. Lungs were collected from infected animals on day 3 post infection to assess the presence of replicating virus.

Result

Mice vaccinated with H1 and H3 COBRA HA mRNA-LNPs possessed neutralizing antibody responses against more antigenically distinct contemporary, co-circulating, and future drifted H1N1 and H3N2 influenza strains than those vaccinated with WT H1 and H3 HA mRNA-LNP vaccines. COBRA mRNA-LNP vaccines also elicited antibody and cellular responses that were associated with protection against lethal H1N1 and H3N2 viral challenge in mice by preventing viral replication in the lungs of infected animals at day 3 post-infection.

Conclusion

The H1 and H3 COBRA mRNA-LNP vaccines showed improved efficacy over WT mRNA-LNP vaccines at preventing influenza illness, including severe disease in the mouse model against multiple subtypes of influenza viruses. This study highlights the potential benefits of combining cutting edge universal influenza antigen design technology with modern vaccine delivery platforms, and how they can be advantageous over traditional WT vaccine antigens at eliciting superior immune responses without the use of adjuvant.

Oral Abstract Session: Translational: Influenza - Broadly protective/universal influenza virus vaccines

Adrian Creanga - AOXI0080

Neutralization Landscape of influenza hemagglutinin stem to characterize antibody behavior and decompose antibody mixtures

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Background

Current influenza vaccines protect against influenza strains antigenically matched to those used for vaccine preparation, but vaccine efficacy becomes minimal against antigenically mismatched influenza variants circulating among humans or emerging viruses from the animal reservoir. Discovery of broadly neutralizing antibodies (bnAbs) targeting conserved epitopes on influenza hemagglutinin (HA) head and stem domains set the blueprint for universal influenza vaccines that can elicit broader protective immunity. However, due to the lack of standardized, high-throughput influenza neutralization assay, deep characterization of the breadth and potency of bnAbs was not performed until recently.

Method

We build a panel of representative replication-restricted reporter (R3) influenza viruses carrying a reporter gene to replace an essential viral gene. Replication of R3 viruses is restricted to cells expressing the missing viral gene, allowing virus propagation in a biosafety level 2 environment. Using these R3 influenza viruses, we developed a high-throughput influenza neutralization assay for in-depth profiling of influenza bnAbs. We applied multi-dimensional scaling to antibody-virus neutralization dataset and project them on two-dimensional space, yielding a new form of antigenic cartography called Neutralization Landscape (NL). The NL was used to decompose mixtures of bnAbs and characterize the neutralization profiles anti-HA stem antibodies within each mixture.

Result

We used the 50% inhibitory titers (IC50) of 27 bnAbs against a panel of 51 seasonal R3 influenza A viruses to define the NL that captures the full scope of antibody-virus interactions for those targeting the HA stem. Furthermore, using the NL analysis, we decomposed the collective neutralization of 14 antibody mixtures (either 2 or 3 bnAbs with the same or different specificities) to identify the composition and fine specificities of the stem antibodies within each mixture.

Conclusion

Our framework offers deeper understanding of the antibody-virus interactions and represents a new tool for in-depth analysis of polyclonal sera to study the antibody repertoire elicited by influenza vaccination or infection, which will provide insights towards developing a universal influenza vaccine strategy.

Oral Abstract Session: Translational: Influenza - Broadly protective/universal influenza virus vaccines

Jonah Sacha - AOXI0235

Cytomegalovirus vaccine vector-induced, unconventionally MHC-restricted effector memory T cells protect cynomolgus macaques from lethal aerosolized H5N1 challenge

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Background

A novel vaccine approach to influenza that overcomes the problem of viral sequence diversity and provides long-lived heterosubtypic protection is urgently needed to protect against both seasonal and pandemic influenza viruses. To circumvent the problem of HA and NA sequence variability, we hypothesized that lung-resident effector memory T cells induced by cytomegalovirus (CMV)-vectored vaccines expressing conserved internal influenza antigens would protect against lethal heterologous influenza challenge.

Method

We constructed two sets of cynomolgus macaque CMV (CyCMV) vaccines expressing 1918 influenza M, NP, and PB1 antigens (CyCMV/influenza) using bacterial artificial chromosome technology. One set utilized full length (FL)-CyCMV, while the other utilized double deleted (dd)-CyCMV devoid of all identified inhibitors of unconventional T cell priming, to create vaccine sets that prime either conventional MHC-Ia- (FL-CyCMV) or MHC-E- and MHC-II- (dd-CyCMV) restricted CD8+ T cells targeting influenza internal proteins. Two separate groups of six Mauritian cynomolgus macaques (MCM) each received two subcutaneous doses of each vector set three months apart. A third group of six MCM received no vaccine and served as the control group. Beginning sixteen weeks after final vaccination, macaques were challenged in blinded groups with 5.5 log10 PFU of aerosolized H5N1 (A/Vietnam/1203/2004), then monitored via telemetry, plethysmography, and bronchoalveolar lavage (BAL).

Result

Both FL- and dd-CyCMV/influenza vaccines induced lung-resident effector memory influenza-specific T cells. FL-CyCMV/influenza induced CD8+ T cells that were MHC-I-restricted, while dd-CyCMV/influenza induced CD8+ T cells that were either MHC-E or MHC-II restricted. Following challenge with aerosolized H5N1 influenza, all six unvaccinated MCM died by seven days post infection due to acute respiratory distress syndrome. While FL-CyCMV/influenza protected 2/5 MCM from death, this did not reach statistical significance as one MCM was lost during the vaccine phase. In contrast, dd-CyCMV/influenza protected 4/6 MCM from death following aerosolized H5N1 challenge ($p=0.04$). This T cell-based vaccine did not protect against viral acquisition as all MCM developed symptomatic infection, but viral titers in bronchoalveolar lavage fluid were 10-100-fold lower in the dd-CyCMV vaccine group following infection.

Conclusion

These data demonstrate that CMV-induced effector memory T cells targeting conserved internal influenza proteins can provide protection against highly pathogenic heterologous influenza challenge and establish proof-of-concept for CMV-induced effector memory T cell-based vaccines in the development of universal influenza vaccines.

Oral Abstract Session: Translational: Influenza - Broadly protective/universal influenza virus vaccines

Joanne Marie Del Rosario - AOXI0229

An Immune Optimised Influenza Vaccine Generates Broad Neutralizing Immune Responses to Human and Swine H1N1 Viruses and Protects Mice and Swine from Challenge

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Background

Influenza A virus (IAV) zoonotic transmission and constant evolution in multiple species heightens the risk of emerging novel strains at the human-animal interface. The cornerstone of influenza prevention and control remains strain-specific vaccination, however decreased vaccine effectiveness still occurs. To cover seasonal, zoonotic and pandemic threats, we developed a Digitally designed, Immune Optimised, Synthetic vaccine antigen (DIOSynVax) platform to induce broad subtype-specific H1N1 immunity, providing protection against divergent strains in mouse and pig challenge models.

Method

In murine studies, immunogens were injected subcutaneously 4 times in 2w intervals and terminal bleeds taken 10w post-first immunisation. For swine, prior to challenge, two doses of DNA were administered by PharmaJet® Tropis at 4w intervals. Controls received whole inactivated virus (WIV) representing swine and human H1N1 influenza vaccine strains. Pigs were challenged with A/swine/England/1353/2009 (sw/EN/09) (matched to the control WIV swine vaccine), 10w post-prime. Efficacy was measured as reduced viral RNA nasal shedding. Serum neutralising titers were monitored using pseudotype neutralisation (pMN), enzyme-linked lectin assay (ELLA) and hemagglutination inhibition (HAI).

Result

F1. Computational mapping of hemagglutinin (HA), neuraminidase (NA) and M2 from sw/EN/09, A/Victoria/2454/2019 (VIC/19) and our DIOS-H1N1 confirmed defined antigenic sites with conserved residues between sw/EN/09 and DIOS-H1N1 and sw/EN/09 and VIC/19. These translated into broad immune responses in all mice (n=6) vaccinated with DIOS(HA) against all H1N1 strains tested that are comparable or superior to the control vaccine strain A/Michigan/45/2015(H1) (MI/45/15) (*$p<0.05$). Values are calculated as fold dilution of sera that resulted in 50% neutralisation of virus via pMN.

F2. Markedly reduced viral shedding was detected in pigs by nasal swabs (expressed as mean log Relative equivalent units (REU) of viral RNA) post-challenge in the DIOS (n=5) and WIV1353 (homologous to the challenge strain) (n=5) groups. Serum neutralising antibodies against sw/EN/09 and other relevant H1N1 viruses were also detected in the DIOS group as monitored at specific times.

Conclusion

We demonstrate broad immunogenicity and efficacy of the candidate DIOSynVax-H1N1 vaccine against relevant IAV H1N1 strains in vitro and in vivo in mice and pigs. This strategy may lead to broadened protection and control within the same IAV subtype with implications for pandemic preparedness whilst protecting against current circulating human influenza. This approach is being translated for IAV/IBV with the ultimate aim of providing broadly reactive antigens for universal influenza vaccine platforms.

Oral Abstract Session: Translational: Influenza - Broadly protective/universal influenza virus vaccines

Daniel Perez - AOXI0412

Modified live attenuated influenza B virus vaccines and sex differences on humoral immune responses in the DBA/2J mouse model

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Background

Influenza B virus (FLUBV) is a major respiratory pathogen in humans. Seasonal influenza vaccines include either one or both FLUBV lineage strains, Victoria, and Yamagata. Vaccine mismatch frequently occurs, particularly in countries where vaccines contain only one of the lineages. We have previously described the safety and efficacy of modified live attenuated FLUBV vaccines based on either virus with rearranged genomes (FluB-RAM and FluB-RANS) or carrying a PB1 segment with a combination of temperature-sensitive mutations and a C-terminal HA tag (FluB-att).

Method

We compared the immunological responses in female and male DBA/2J mice vaccinated with either one of these vaccines and those with isogenic backgrounds encoding a chimeric HA segment carrying an N-terminal peptide encoding the IgA inducing peptide (IGIP).

Result

combinant viruses were genetically stable over multiple passages in eggs. In mice, the introduction of IGIP improved attenuation of the vaccine candidates, particularly for the FluB-RAM/IGIP compared with the non-IGIP counterpart. In a prime-boost regimen, mice were completely protected against lethal challenge with a homologous FLUBV strain. Recombinant viruses induced antibodies against HA considered of protective value. Compared to male mice and regardless of the vaccine used, female mice showed a clear trend towards enhanced humoral and cross-reactive IgG and IgA anti-HA responses and against NA and NP. The presence of IGIP resulted in an overall trend towards reduced anti-HA responses but enhanced anti-NA and anti-NP responses, particularly of the IgA isotype. Mucosal and serological responses two weeks after the challenge showed similar trends with clear differences observed based on sex, vaccine backbone, and whether the vaccine carried the IGIP modification.

Conclusion

Different FLUBV LAIV backbones and the incorporation of the chimeric IGIP-HA led to similar protective outcomes but qualitatively different humoral and mucosal response patterns in mice. We further investigated the effect of biological sex on antibody-mediated immune response stimulation. These observations confirmed that female mice are less susceptible to FLUBV than males. However, female mice can mount better and broader antibody responses against FLUBV than males. Further investigation is warranted to broaden our understanding of those factors driving the opposite sex-related susceptibilities towards FLUAV and FLUBV.

Oral Abstract Session: Translational: Influenza - Improved seasonal vaccines

Yingxia Wen - AOXI0046

Quadrivalent influenza self-amplifying mRNA bicistronic vaccines elicit potent HA and NA-specific antibody and cell-mediated immunity against seasonal influenza strains

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[1]Seqirus

Background

Seasonal influenza vaccination is an effective means of generating widespread immunity to limit the significant burden of annual influenza epidemics. The current generation of licensed influenza vaccines focus immunity on viral hemagglutinin (HA), while the other major viral coat glycoprotein neuraminidase (NA) is largely or entirely absent from some vaccine formulations despite evidence from both animal and human studies that anti-NA antibodies reduce influenza virus replication, shedding, transmission, and clinical disease. Next generation bicistronic self-amplifying mRNA (sa-mRNA) vaccines offer an opportunity to generate immunity to multiple viral proteins, and in this work we utilized multiple sa-mRNA bicistronic constructs expressing both HA and NA from four influenza strains to create a quadrivalent influenza vaccine.

Method

We immunized Balb/c mice with either monocistronic or bicistronic seasonal sa-mRNA HA, NA, or HA-NA vaccines in monovalent or a quadrivalent formulations. A current generation adjuvanted quadrivalent subunit vaccine group was included as a control. Humoral immune response to vaccination was evaluated by measuring anti-HA and anti-NA antibodies by ELISA, hemagglutination inhibition, NA inhibition, and virus microneutralization. Cell-mediated immune responses were measured by flow cytometric analysis for HA and NA-specific T cells.

Result

Sa-mRNA vaccines containing an NA component induced potent NA-inhibiting antibodies and CD4+ T cell responses in both monovalent and quadrivalent formulations. Increasing the antigen number by using bicistronic sa-mRNA vaccines and/or quadrivalent formulations resulted in reduced antibody titers and antigen-specific T cells compared with monocistronic vaccines and monovalent formulations respectively. However, the anti-HA microneutralization responses were comparable to those elicited by a current generation adjuvanted subunit quadrivalent influenza vaccine and the NA antibody was similar or superior. Additionally, the sa-mRNA vaccines induced HA-specific CD8+ T cell responses which were absent in the adjuvanted subunit vaccine group.

Conclusion

Next generation bicistronic sa-mRNA vaccines expressing HA and NA induce potent antibody and cell-mediated immune responses when formulated alone or as a quadrivalent seasonal influenza vaccine.

Oral Abstract Session: Translational: Influenza - Improved seasonal vaccines

Raffael Nachbagauer - AOXI0113

Interim Analysis of a Phase 1/2 Randomized Clinical Trial on the Safety, Reactogenicity, and Immunogenicity of a Quadrivalent, mRNA-based Seasonal Influenza Vaccine (mRNA-1010) in Healthy Adults

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Background

Influenza is associated with substantial disease burden worldwide, causing seasonal epidemics of variable severity. Vaccines for prevention of influenza using traditional technology are available but often provide limited efficacy; messenger RNA (mRNA) technology has the potential to address limitations of traditional platforms, including avoidance of mutations acquired by egg- or cell-culture, improved efficacy in older adults through the induction of strong cellular immune responses (as demonstrated by mRNA vaccines against severe acute respiratory syndrome coronavirus 2), and by potentially enabling future strain selection closer to the influenza season to decrease the chance of a vaccine mismatch. mRNA-1010 is an investigational mRNA-based quadrivalent seasonal influenza vaccine that encodes the hemagglutinin surface glycoproteins of strains recommended by the World Health Organization: A/H1N1, A/H3N2, B/Victoria, and B/Yamagata. Here, we present interim safety and immunogenicity findings of mRNA-1010 in healthy adults from a phase 1/2 clinical trial.

Method

This first-in-human, observer-blind study (NCT04956575) enrolled healthy adults (aged ≥18 years), stratified by age, who were randomly assigned to receive different dose levels of mRNA-1010. This interim analysis presents safety and humoral immunogenicity data in younger and older adults against vaccine-matched influenza A and B strains measured 28 days after vaccination.

Result

No study pause rules were met, and there were no reported serious adverse events (AEs), discontinuations due to AEs, or deaths among mRNA-1010 recipients related to the study vaccine. Solicited local and systemic adverse reactions were more frequent among younger compared with older adults and were dose-dependent. All mRNA-1010 dose levels elicited increases in hemagglutination inhibition antibodies from baseline across all age groups.

Conclusion

A single dose of the quadrivalent mRNA-1010 candidate vaccine was immunogenic against all tested influenza strains in younger and older adults and had an acceptable safety profile. These interim safety and immunogenicity data support continued development of mRNA-1010.

Oral Abstract Session: Translational: Influenza - Improved seasonal vaccines

Larisa Gubareva - AOXI0163

Monitoring antigenic drift in the neuraminidase of recent influenza A(H3N2) viruses

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Background

Antibodies targeting influenza neuraminidase (NA) have been shown to correlate with immune protection and reduced disease severity. As hemagglutinin (HA) and NA antigenic advancement is discordant, analysis of antigenic properties of NA may be used to improve vaccine compositions. NA antigenic analysis requires testing of viruses with antigenically distinct HA from those used to induce a humoral response.

Method

An optimized enzyme-linked lectin assay (ELLA) was employed to assess antigenic differences in NAs of A(H3N2) viruses. NAs used in this study had sequences of recent vaccine strains, candidate vaccine viruses (CVV), and representative viruses. PR8-based reassortant A(H1N2) viruses containing A(H3N2) NAs of interest were tested using ferret antisera raised against A(H3N2) viruses.

Result

Antiserum raised against A/Hong Kong/45/2019 virus (α-HK45/19) had reduced reactivity (4-fold) with NA of A/South Australia/34/2019 (SA34/19), which differed by S315R + E344K. Both α-HK45/19 and α-SA34/19 sera reacted poorly (6- to 10-fold reduction) with NAs that gained a putative glycosylation site (CHO+) at residue 463 (D463N + N465S). Notably, most viruses circulating between 2020 to 2022 shared D463N(CHO+) (e.g., vaccine viruses A/Cambodia/e0826360/2020 and A/Darwin/6/2021). Furthermore, NA amino acid sequences of viruses collected during the 2021-2022 season clustered into D346G and S329N(CHO+) subgroups. Reactivity of α-A/Cambodia/e0826360/2020 was reduced (3- to 6-fold) to NAs carrying D346G, R150H, or a combination of S329N(CHO+) + D113G. α-A/Darwin/6/2021 had a similar reactivity pattern, but also reacted poorly (6-fold) with NA of A/Darwin/9/2021, which has R150S. NAs of A/Darwin/9/2021 (egg-based CVV) and A/Darwin/11/2021 (cell culture-based CVV) are identical and share R150S. α-A/Darwin/9/2021, α-A/Darwin/11/2021, and α-A/Maryland/02/2021 (D346G) sera reacted well with all NAs tested, except HK45/19. Notably, α-A/Alaska/01/2021 (S329N(CHO+)) showed slightly reduced reactivity (~3-fold) with NAs carrying D346G, R150H or R150S. The effect of R150H was also noted with α-SA34/19, whose reactivity was reduced by 6- to 14-fold. Viruses carrying NAs with either R150H or R150S or the dual substitutions S329N(CHO+) + D113G have rarely been detected among recently circulating viruses.

Conclusion

In this study, several NA changes, including the acquisition of a putative glycosylation site at 463, were associated with NA antigenic advancement in recent A(H3N2) viruses. As CVVs may differ in NA amino acid sequences, NA antigenic analysis may benefit the vaccine selection process.

Oral Abstract Session: Translational: Influenza - Improved seasonal vaccines

Vivek Shinde - AOXI0294

Safety and immunogenicity of COVID Influenza Combination Vaccine

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Background

We developed a COVID-Influenza Combination (CIC) vaccine, comprising recombinant SARS-CoV-2 Spike (rS) and quadrivalent influenza hemagglutinin (HA) protein nanoparticles (qNIV), and Matrix-MTM adjuvant. rS/Matrix-M previously demonstrated efficacy against COVID-19 in Phase 3 trials, while qNIV/Matrix-M previously demonstrated induction of broadly cross-reactive antibodies. Here we report preliminary safety and immunogenicity results of a first-ever Phase 1/2 CIC dose-finding trial.

Method

Seropositive (COVID-19 vaccinated >=8 weeks prior) participants (N=642) aged 50-70 years were randomized equally, to receive two intramuscular doses, 56 days apart, to 1 of 14 different dose/formulations of CIC using a design of experiments approach (dose range: rS 2.5-22.5ug, HA 5-60ug; and 50ug Matrix-M), or to 1 of 2 reference formulations of either standalone rS with Matrix-M [2 doses] or qNIV with Matrix-M [1 dose only]. Pre- and post-vaccination (Days 0, 28, 56, 70, 84, 182) immunogenicity assessments including SARS-CoV-2 anti-S IgG and influenza HAI antibodies to vaccine-homologous strains. Reactogenicity was assessed 7 days following each dose, and safety outcomes assessed through Day 70. Multiple regression was used to create predictive models to assess antibody response surfaces and for dose optimization.

Result

All CIC formulations were well tolerated, with a reactogenicity and safety profile generally comparable to standalone rS or qNIV. Regression modelling of post-first dose responses revealed that both rS and HA antigens in a CIC formulation modestly interfered with each other, however, interference was overcome with dose adjustment across a range of rS/HA doses. Specifically, higher rS dose (>20ug), in a dose dependent fashion, overcame HA interference, closely matching standalone rS IgG reference responses (GMEU 16,818), whereas lower, intermediate HA dose overcame rS interference, closely matching standalone HA reference HAI responses for H3N2 (GMT 145), H1N1 (GMT 134), and B-Victoria (GMT 66); while modestly (at least 34%) lower than the reference B-Yamagata response (GMT 101).

Conclusion

IC formulations were well tolerated and immunogenic, with various dose combinations achieving response comparable to standalone vaccines

Oral Abstract Session: Translational: Influenza - Improved seasonal vaccines

Matthew Miller - AOXI0714

Leveraging mucosal immunity for next-generation influenza and COVID-19 vaccines

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Background

The propensity to administer vaccines parenterally harkens back to the days of variolation. While parenteral administration is convenient and can be highly effective against certain pathogens, especially those that cause systemic infections, it may not be the optimal for pathogens that infect mucosal tissues. Advancements in the understanding of unique features of the mucosal immune system, including tissue-resident B and T cells, local trained innate immunity, and IgA-mediated mechanisms of protection, have catalyzed immense interest in the development of mucosal vaccines for pathogens including influenza virus and SARS-CoV-2.

Method

We have analyzed and compared unique features of the mucosal immune response in individuals enrolled in a cluster-randomized control trial comparing efficacy of inactivated and live-attenuated seasonal influenza virus vaccines. Using this data, we have developed novel, next-generation active and passive mucosally-administered vaccines to protect against influenza virus and SARS-CoV-2 infections.

Result

We demonstrate that despite equivalent clinical efficacy, inactivated and live-attenuated influenza vaccines induce fundamentally different immune responses - suggesting that unique surrogates of protection should be established for mucosally-administered vaccines. We show that IgA specific for influenza and SARS-CoV-2 are capable of eliciting unique, Fc-dependent antiviral functions. Finally, we developed an aerosolized, viral-vectored, pan-SARS-CoV-2 vaccine and demonstrate that it is superior to the homologous parenterally-administered vaccine in terms of both immunogenicity and protection.

Conclusion

Our data suggest that mucosal administration of vaccines for respiratory viruses like influenza virus and SARS-CoV-2 may be more effective than parenterally-administered vaccines. When delivered mucosally, the vaccines stimulate immune responses that are distinct from those stimulated by parenteral immunization, underscoring the need to establish unique correlates of protection to aide in the evaluation of novel mucosal vaccines.

Oral Abstract Session: Translational: Influenza - Mechanisms of vaccine induced and broadly protective adaptive immunity

Maireid Brigid Bull - AOXI0404

Next generation T cell activating vaccination increases influenza virus mutation prevalence

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Background

The development of next-generation universal influenza vaccines continues to advance in order to improve protection against antigenically drifted and emergent strains. Vaccines utilising conserved T cell epitope regions can confer long lasting heterosubtypic immunity but the effects of increased T cell-mediated immune pressure on the viral genome requires further characterisation. Influenza viruses are adept at responding to selection pressure, and as universal vaccines may provide non-sterilising immunity, this could lead to unintended selection bottlenecks. Viral immune evasion by antigenic drift is well defined, however mutations within immunodominant T cell targeted epitopes have also been seen to arise during natural infection, leading to a potential impairment of immunity. As such it is important to determine if T cell immune pressure elicited by a next-generation vaccine may inadvertently drive mutational frequency and lead to a loss of T cell recognition.

Method

To assess T cell-mediated immune pressure on the influenza genome, an experimental next-generation universal vaccine Wyeth/IL-15/5Flu was used within a mouse challenge model. Wyeth/IL-15/5Flu utilises a vaccinia backbone, incorporating five H5N1 proteins and encodes IL-15 as a molecular adjuvant. Mice were H1N1 challenged and viral RNA was isolated from lung homogenate at varying timepoints and in T cell depleted conditions. Viral RNA was sequenced using Illumina NovaSeq in order to quantify influenza gene mutagenesis in the context of a next-generation T-cell activating vaccine.

Result

Vaccination with Wyeth/IL-15/5Flu was coincident with increased mutation incidence and frequency across the influenza genome. Mutations were not seen to be enriched within T cell epitope regions, but rather stochastically arose across influenza genes, including in those that were not included in the Wyeth/IL-15/5Flu vaccine. Depletion of CD4+ and CD8+ T cell subsets led to a reduced frequency of observed influenza mutations. High allele frequency mutations within conserved haemagglutinin stem regions as well as previously identified mammalian adaptive mutations in PB2 were seen to arise in vaccinated mice.

Conclusion

Our findings indicate that heterosubtypic universal influenza vaccination imposes a greater selection bottleneck on influenza viruses than current vaccines, showing how even partial immune responses can shape virus evolution. These results show that more is needed to fully characterise the effects of T cell activating vaccines and could help inform future vaccine design.

Oral Abstract Session: Translational: Influenza - Mechanisms of vaccine induced and broadly protective adaptive immunity

Nina Urke Ertesvåg - AOXI0432

Seasonal influenza vaccination expanded antibody breadth and induced cross-reactive antibodies to future A/H3N2 viruses

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Background

Influenza A/H3N2 undergoes considerable antigenic drift. Past A/H3N2-exposure introduce diversity in subsequent antibody responses. However, differences in healthy adults' and children's cross-reactive antibody responses after vaccination or infection have not been characterized in detail.

Method

We investigated the breadth, magnitude and durability of influenza-specific hemagglutinin-inhibition (HI) antibodies against 14 antigenically distinct prototype vaccine A/H3N2 viruses circulating from 1968 to 2018 after live-attenuated influenza vaccine in children (aged 3-17 years, n=42), and after inactivated influenza vaccine or infection in adults (aged 22-61 years, n=42). Adults had either one seasonal vaccination or repeated annual vaccination. Previous vaccination status was collected through questionnaires.

Result

We found cross-reactive HI antibody responses elicited against older and future strains in vaccinated children and vaccinated or infected adults. Broader and more durable A/H3N2-specific antibodies were observed in repeatedly annual vaccinated adults than in children and previously unvaccinated adults. Childhood H3-priming in adults increased the breadth and magnitude of back-boosted A/H3N2-specific antibodies.

Conclusion

Our findings suggest that early A/H3N2 exposure, vaccination with advanced drifted strains, and frequent seasonal vaccination could increase the pool of cross-reactive antibodies and thereby improve vaccine protection.

Oral Abstract Session: Translational: Influenza - Mechanisms of vaccine induced and broadly protective adaptive immunity

Jorgen de Jonge - AOXI0511

A universal influenza mRNA vaccine candidate boosts T-cell responses and reduces zoonotic influenza virus disease in ferrets

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Background

For the protection of humankind from continuously changing seasonal and occasional pandemic influenza virus outbreaks, universal influenza vaccines are essential. T-cell responses targeting conserved internal influenza proteins could contribute to protection from a broad range of influenza viruses.

Method

We assessed a new mRNA vaccine coding for NP, M1 and PB1 proteins of pH1N1 influenza virus for the induction of protective cellular immunity in ferrets. We followed a prime-boost strategy in which ferrets were primed by H1N1 influenza infection and boosted by mRNA vaccination, resembling the adult human situation. We additionally modelled naïve individuals by prime-boosting with mRNA vaccine only.

Result

mRNA vaccination successfully induced and boosted systemic CD4+ and CD8+ T-cell responses where especially the CD8+ T-cell response was increased even compared to a prime-boost with two heterosubtypic influenza virus infections. Importantly, the mRNA vaccine was able to boost existing T-cell responses in the upper and lower respiratory tract of H1N1-primed ferrets and was a potent inducer of T-cells residing in the bone marrow. Moreover, these ferrets showed enhanced protection against a severe pneumonia induced by intratracheal challenge with H7N9 influenza compared to only H1N1-primed ferrets.

Conclusion

mRNA vaccination is a promising strategy to increase protection against seasonal drifting and new pandemic influenza viruses through boosting of existing T-cell responses.

Oral Abstract Session: Translational: Influenza - Mechanisms of vaccine induced and broadly protective adaptive immunity

Robert Mettelman - AOXI0528

Defining cellular correlates of protection to influenza across human cohorts

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Background

Mounting evidence suggests that cell-mediated immunity (CMI), comprising antigen-specific CD4/CD8 T cells, and innate immunity mediate tolerance and resistance during influenza infection and confer protection after vaccination. However, while humoral (antibody) responses to influenza HA and NA are well-known mediators of protection against influenza, fewer studies have resolved the contribution of cellular responses independent from pre-existing antibody (ab) titers. Thus, the individual CMI and innate cell subsets that correlate with protection against influenza independently from or synergistically with humoral responses remain to be identified in humans as well as the relative, quantitative, contributions of cellular and humoral responses to protective anti-influenza responses.

Method

Here, we use high-dimension spectral flow cytometry to resolve CMI and innate cell populations from adult peripheral blood across two influenza infection/vaccination cohorts. Using logistic regression modeling, we analyzed these cell populations, accounting for anti-influenza ab titers and participant demographics, to define independent CMI and innate cell correlates of protection. Using a third adult cohort, we compared CMI and ab responses to two quadrivalent influenza vaccine platforms (inactivated and live attenuated) to evaluate if a given vaccine strategy elicited these protective populations.

Result

From these analyses, we identify individual cell populations and co-regulated immune cell modules that correlate with protection. Th17, cTfh, CD4, and CD8 T cells are among adaptive populations having the strongest correlations, while NK cells and DCs were the strongest innate signals. Preliminary AUROC modeling also identified baseline infection classifiers predicting infection susceptibility with up to 80% accuracy after cross-validation. In comparing vaccine preparations, we find split-inactivated vaccine FluZone stimulates broad innate and adaptive responses comprising neutrophils, NK, and CD4/CD8 memory cells, while LAIV FluMist induces activated monocytes and CD4 effectors. Both induce cTfh and activated CD4s. While FluZone promotes higher anti-influenza ab titers, select FluMist participants with high ab titers have larger changes in immune cell frequencies suggesting interplay between protective humoral and cellular responses.

Conclusion

Together, our data highlight the complexity and variability of baseline, post-infection, and post-vaccination CMI and innate responses. These studies also provide insight into vaccine design strategies to improve CMI, innate, and humoral immunity, which are of particular importance with emerging or antigenically novel influenza strains.

Oral Abstract Session: Translational: Influenza - Mechanisms of vaccine induced and broadly protective adaptive immunity

Annette Fox - AOXI0637

Influenza vaccine responses among young children first exposed to influenza antigens via infection versus vaccination

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Background

Evidence suggests that the influenza subtype first encountered in life imprints subsequent antibody responses, whereas the effect of first exposure via multi-subtype vaccines is unclear. We compared antibody responses induced by quadrivalent inactivated vaccine among children aged 6 to < 60 months who had prior influenza A infection only; prior vaccination only, or who were influenza A antigen naive.

Method

Children attending two tertiary Australian Paediatric Hospitals for vaccination from 2019 to 2021 were enrolled based on their influenza exposure history. Samples were collected 0, 7-21 and 28-65 days after vaccination. Serum HI antibody titres were measured against 12 A(H3N2), 7 A(H1N1) and 4 B viruses representing vaccine and circulating strains.

Result

19 children participated, 8 followed for 1 year and 11 for 2 years. All received 2 doses of vaccine 28 days apart: 2 (aged 6 months) were previously naive, 8 (aged 23-54 months) had prior vaccination, and 9 (aged 12-50 months) had prior infection. Geometric mean titres (GMTs, 95% CIs) against vaccine A(H3N2) strains were similar at d0, but were substantially higher post-vaccination in previously infected (2560, 958-6843) than in previously vaccinated (233, 60-901) children. Accordingly, average titre rise (with 95%CI) was 20 (11-36) and 2.7 (1.9-4.2) fold, respectively. Pre- and post-vaccination GMT's against vaccine A(H1N1) strains were lower among previously infected children, although titre rise was 7 (2-24) versus 6 (4-8) fold in previously infected and vaccinated children, respectively. After a second year of vaccination H1N1 titre rise was greater among previously infected children. Previously naïve children had low titre rises against both subtypes.

Conclusion

These pilot study results indicate that infection is substantially better than vaccination for priming vaccine induced antibody responses against A(H3N2), whereas vaccination appeared to be at least as good as infection for priming responses against A(H1N1) viruses.

Oral Abstract Session: Translational: Influenza & COVID-19 - Human challenge models

Fuminari Miura - AOXI0161

How human challenge studies reveal vaccine efficacy and mode of action

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Background

To assess the impact of a vaccination programme, we need to know both the efficacy and the mode of action of the vaccine. While influenza vaccines are often considered "leaky" vaccines, the actual mode of action is difficult to infer from field trials, as it requires measuring the heterogeneity of exposure to infection. Here, we propose an approach to determine both vaccine efficacy (VE) and the mode of action of vaccines from human challenge studies.

Method

In the proposed approach, we express the probability of infection for both vaccinated and unvaccinated individuals as a function of the challenge dose by incorporating the variability between individuals in susceptibility to infection. The proportional reduction in the infection risk between those groups is defined as the dose-dependent VE. We apply the method to observed frequencies of infection among vaccinated and unvaccinated volunteers at various challenge doses of influenza A and B viruses.

Result

An estimated VE that is constant irrespective of the dose indicates a pure "all-or-nothing" effect of the vaccine (Fig-1(A)). An estimated VE that declines monotonically with dose indicates a "leaky" effect (Fig-1(B)). The actual effect might be in between these extremes. We thus applied the mixture model that captures both effects. VE was estimated to be 0.48 [95%CI:0.41, 0.55] and constant irrespective of the dose, suggesting that influenza vaccines might induce the all-or-nothing effect (Fig-1(C)). Another model that allows for describing vaccinated individuals more flexibly was also examined, and this generalized model induced the best fit to the analyzed data. Interestingly, the estimated VE from the generalized model first decreased to 0.39 at the intermediate dose range and then increased asymptotically to 0.48 (Fig-1(D)).

Conclusion

Human challenge studies have an advantage over the standard vaccine field trials in assessing the effect of vaccines, as the heterogeneity in exposure to infection is known and controlled. This makes it possible to infer rather than assume the mode of action. With the proposed method, we can design challenge studies such that they are effective to infer both VE and the mode of action.

Oral Abstract Session: Translational: Influenza & COVID-19 - Human challenge models

Loukas Papargyris - AOXI0499

Local and systemic mediators associated with symptomatic and asymptomatic infection after human influenza A(H3N2) challenge

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Background

Influenza is a respiratory infection the severity of which varies considerably between individuals. Despite extensive research, morbidity and mortality remain high, with vaccines unable to confer cross-strain protection and antivirals showing limited efficacy. Pathogenesis and immune determinants of influenza severity in the human respiratory tract are under-studied.

Human infection challenge involves the deliberate inoculation with pathogens and has the unique capacity to investigate host responses before, during and after infection while controlling for factors such as virus strain, dose and exposure. Here we report the clinical findings and expression of local and systemic cytokines and chemokines associated with symptomatic and asymptomatic infection in volunteers challenged with influenza A(H3N2).

Method

Healthy adults aged 18-55 years with serum neutralising antibody titres less or equal to 1:20 were enrolled for inoculation with influenza A/Belgium/4217/2015 (H3N2) virus (SGS CPU, Belgium) intranasally, at a dose of 5x105 TCID50 in 0.5ml PBS. Following inoculation , participants were quarantined until day 10 and returned for assessment and sampling at days 14, 28 and 180. Symptoms were monitored using self-reported symptom diaries. Viral load was quantified in nasal lavage fluid by qPCR. Soluble mediators in plasma and nasal lining fluid were measured using MesoScale Discovery. Antibody responses were assessed by ELISA, haemagglutination inhibition, and microneutralisation assays. Transcriptomics analysis of the blood and nasal curettage samples using RNA sequencing was performed to investigate differential gene expression.

Result

Following inoculation of 28 individuals, 22 (78.5%) developed PCR-confirmed infection and 6 remained uninfected. Viral load correlated with symptoms in symptomatic participants. The pro-inflammatory mediator response, including IFN-γ and IP-10, was most marked in the nose and significantly greater in symptomatic than asymptomatic participants. In contrast, asymptomatic infection was associated with unique expression in the nose of other markers, indicating a possible protective role. Transcriptomics analysis revealed differences between symptomatic and asymptomatic infection in pathways associated with immune regulation. No differences were observed between the antibody responses of symptomatic and asymptomatic individuals.

Conclusion

Unique patterns of soluble mediator expression associated with symptomatic and asymptomatic influenza are detectable in the nose, highlighting potential protective pathways and targets for intervention.

Oral Abstract Session: Translational: Influenza & COVID-19 - Human challenge models

Alex Mann - AOXI0658

Influenza, COVID-19, RSV and HRV: translating efficacy testing of vaccine and drugs from human challenge models to the field

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Background

Respiratory infections such as flu, COVID-19, RSV, and HRV, while in many ways are similar in presentation, still present differences, virologically, clinically and immunologically. Human challenge studies using wild-type viruses replicate what is seen in the field in the same populations, and as such they have been used to aid vaccine and drug development for decades, and more recently have supported RSV vaccines obtain fast track status. How infections differ in their presentation, understanding the implications for vaccine and drug study design, and defining clinically relevant endpoints can be important in translating efficacy to the field.

Method

VIVO recruited over 500 subjects that received one of several intranasally administered wild-type viruses: Influenza, COVID-19, RSV (including older subjects 60 to 75yrs), or HRV (including subjects with mild intermittent asthma). Apart from the RSV older subjects, eligible subjects were pre-screened for low or no antibodies to the challenge virus. Once admitted to quarantine and confirmed eligible for inoculation, subjects were monitored 24/7 for safety, with frequent sampling for viral shedding and assessments of disease including subject self-reported symptom diary cards, temperature, and nasal discharge. Endpoints assessed included peak and AUC viral loads by PCR and culture, incidence of symptomatic disease, "ILI-like" criteria [e.g., using CDC, WHO, PHE definitions], febrile illness, symptom type and severity.

Result

Clinical endpoints are summarised and compared for subjects infected with each virus and in each population group. Fever of ≥37.8oC occurred in ~40% of infected subjects with COVID-19, ~20% with influenza, ~25% in subjects with asthma and HRV, while only ~5% in healthy subjects infected with HRV or RSV. Subjects exhibiting fever and "ILI-like" illness or more severe presentations of disease are described in detail, including incidence and virology, symptoms, temperature, blood cells, and immune marker time-course. The particular relevance of the more severe presentation seen within the challenge model and the associated efficacy endpoints and the translatability and impact on design is considered.

Conclusion

Within the respiratory infection challenge studies and using wild type viruses in different populations, a diverse presentation of disease is observed. Clinical profiles are similar to that seen in community infections with the more severe of which, being translatable, are important endpoints to include in vaccine and drug efficacy studies.

Oral Abstract Session: Translational: Influenza & COVID-19 - Human challenge models

Thomas Luke - AOXI0452

Efficacy and safety of SAB-176, a novel anti-Type A and B influenza immunotherapeutic: a Phase 2a, randomized, double blind trial in H1N1 challenged adults

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Background

SAB-176 is a novel anti-Type A and B influenza therapeutic comprised of human polyclonal anti-influenza antibodies produced in the DiversitabTM platform to prevent and treat influenza infections.

Method

We conducted a randomized, double-blind, phase 2, placebo-controlled trial of SAB-176 in healthy adults nasopharyngeally inoculated with A/California/2009/H1N1. A single 25 mg/kg dose of SAB-176 or placebo was infused 20-24 hours later. The primary efficacy outcome was nasopharyngeal quantitative reverse transcriptase polymerase chain reaction viral load area-under-the-curve (qRT-PCR VL-AUC) measurements from day 1 through 8 days after inoculation.

Result

62 healthy volunteers were randomized and inoculated with A/California/2009/H1N1. Thirty received SAB-176 and 30 received saline intravenously. 2 were inoculated but were not infused. One subject in the SAB-176 group did not complete quarantine and was (a priori) excluded from primary efficacy analysis. SAB-176 statistically reduced qRT-PCR VL-AUC compared to placebo with a mean (standard deviation) of 91.98 (166.03) or 273.05 (337.25) log10 copies*hour/ml, respectively (p=0.026, one sided). Total Symptom Score AUC, peak and peak daily total symptom score as measured by a 13-item symptom questionnaire showed a trend towards symptom reduction p=0.066, p=-0.065 and p=0.05, respectively. No serious adverse events occurred, and no adverse events led to study discontinuation. Peak hemagglutination inhibition assay (HAI) serum titers against A/California/2009/H1N1 and other Type A and B influenza strains reached a peak in SAB-176 participants soon after infusion.

Conclusion

SAB-176 was safe, well tolerated, and effective in reducing nasopharyngeal viral load after challenge with A/California/2009/H1N1 in healthy adults. A clinically relevant trend toward symptom reduction was seen. SAB-176 should be evaluated for the prevention or prompt treatment of influenza in patients at substantial risk of developing serious disease in future clinical trials.

Oral Abstract Session: Translational: Influenza & COVID-19 - Human challenge models

Nicolas Noulin - AOXI0659

The key facets that drive success or failure of an influenza challenge studies in vaccine and antiviral testing: learning from past challenge studies.

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Background

Challenge studies can be a powerful tool in the development of both therapeutic agents and vaccines. However, key decisions made in the study design process can have a profound impact on the outcome of the efficacy assessment. These include factors such as the selection criteria and screening of subjects as well as timing and frequency of the virological sampling and illness symptom data. Probably the single most important aspect is the choice of the primary efficacy endpoint and how precisely that is defined and calculated. While the principles of the endpoints are the same in challenge studies and field trials, subtle but vital differences in the endpoint definitions need to be made between the two clinical trial formats to account for the different context.

Method

We have conducted a comprehensive review of the influenza challenge studies conducted in recent years. We have compared the study design, disease definitions and endpoints across the different studies to assess and identify key success criteria in the study design and analysis of influenza human challenge study data. Our analysis incorporates both data that is in the public domain as well as a significant body of unpublished influenza challenge model data. Utilising an extensive database of individual subject data we have further explored a range of different disease parameters in greater depth. Using a meta database from a range of studies facilitated the re-analysis of data and the impact of different analysis endpoint without being restricted by subtle but important differences in endpoint definitions between the different clinical studies.

Result

Our analysis highlights some important study design aspects that should be carefully considered when designing influenza challenge studies. The data demonstrate the importance of a thorough understanding of the expected variation in response to the infection and how this affects the powering considerations for challenge studies. We present our findings on what are the most commonly used study endpoints and discuss the relative pros and cons of the different study design approaches used. In addition, we provide a detailed analysis of the impact of subtle changes in endpoint definitions and how changes can facilitate an improved product efficacy assessment.

Conclusion

This insightful assessment of previous challenge studies both highlights key learnings and success criteria as well as illuminating as yet poorly understood or explored aspects that could further enhance the utility of influenza challenge models.