Marie-Anne Rameix-Welti - ARNI0003

RSV viral factories are druggable multiphasic biocondensates

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Background

Formation of cytoplasmic inclusions or inclusion bodies (IBs) in infected cells is a well-documented feature in the life cycle of most Mononegavirales. However, the critical importance of these structures has only recently been understood. Using metabolic labeling, we previously demonstrated that RSV RNA synthesis (transcription and replication) takes place inside IBs. RSV IBs play the role of essential viral factories during the virus life cycle. More recently evidence from different Mononegavirales demonstrated that IBs are liquid membraneless organelles (MLO). MLOs play an important role intracellular organization in normal and infected cells. They are composed of a small number of viral proteins and form by liquid-liquid phase separation (LLPS).

Method

We created recombinant RSV expressing a fluorescent P or fluorescent M2-1 fusion proteins to probe the dynamics of IBs and their components in infected cells.

Result

We show that IBs display key properties of LLPS biocondensates: (1) spherical shape and ability to fuse and to split, (2) sensitivity to hypotonic shock or hydrophobic agents, and (3) internal mobility of IB components. Interestingly a sub-compartment, IB associated granule (IBAG), is created within the IB during transcription of viral mRNA. IBAGs concentrate newly synthetized viral mRNA together with the viral transcription factor M2-1. Analysis of the dynamics of these compartments revealed liquid like behavior indicating that IBs are multiphasic biocondensates. Recently, we demonstrated that the plant steroidal alkaloid cyclopamine and chemical analogs resulted in a rapid and specific loss of IB/IBAG organization within minutes. Furthermore, we showed that the compound induced a liquid-to-solid phase transition (hardening) of the IBs as evidenced by alteration of shape, abolition of fusion events, loss of sensitivity to hypotonic shock and loss of internal mobility of components. IB hardening was not observed when cells were infected with RSV expressing the M2-1 R151K resistance mutation.

Conclusion

RSV viral factories are LLPS biocondensates that can be targeted by small molecules. This is the first description of condensate targeting drugs resulting in a therapeutic effect in vivo. Our findings open the possibility that MLOs of other viruses or MLOs in other pathological processes like cancer can be targeted by small molecule therapeutics.

Respiratory Syncytial virus NS1 protein targets the transactivator binding domain of MED25

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Background

Human RSV is the most frequent cause of infantile bronchiolitis and pneumonia worldwide. It is a major unmet target for vaccines and anti-viral drugs. The pathology associated with RSV infection results from both viral replication and the host immune response mediated first by the production of type I interferons (IFN-I). However, upon infection by RSV, IFN levels remain surprisingly low. RSV codes for two non-structural proteins NS1 and NS2. Both NS1 and NS2 act as IFN antagonists, and many of their cytosolic targets have been identified. Recently it was shown that in infected cells, nuclear NS1 could be involved in transcription regulation of host genes linked to innate immune response, via interactions with chromatin and the Mediator complex.

Method

Yeast two-hybrid (Y2H) screen was used to identify cellular binding partners of RSV NS1 protein. The hits were then validated in cellula using Nano Luciferase assay and direct interaction was further shown by GST-pulldowns using recombinant proteins. NMR interaction experiments were performed to map the α3 helix interaction site on MED25 ACID by titrating the NS1 α3 helix peptide into 15N-labelled MED25 ACID.

Result

After identifying MED25 as an NS1 interactor in a Y2H screen, we demonstrated that NS1 interacts with the MED25 ACID domain in cells. We found that both the NS1 α,β-core domain and the α3 helix directly bind to MED25 ACID. By investigating this interaction by NMR, we found out that NS1 α3 preferentially targets the MED25 ACID H2 face. Moreover, we revealed that NS1 could compete with the transactivation domain (TAD) of ATF6α, involved in the innate immune response to viral infections.

Conclusion

Our results suggest that NS1 possesses a TAD domain and that this TAD contributes to displace those of other regulation factors from the Mediator complex. On this basis we propose that NS1 could act as a transcription suppressor. This would present a new mechanism to control the host response upon RSV infection by interfering with activation of innate immune response genes by cellular transcriptional activators. Given the central role of NS1 in antagonizing the innate immune response to RSV, and MED25 being targetable by allosteric small molecules, our data could open a new avenue for RSV drug design.

EDP-323, A NOVEL L-PROTEIN INHIBITOR, FOR THE TREATMENT OF RESPIRATORY SYNCYTIAL VIRUS

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Background

Respiratory syncytial virus (RSV) infection is associated with substantial morbidity and mortality. Aside from the prophylactic pediatric monoclonal antibody, Palivizumab, and ribavirin, there are no approved vaccines or therapeutics for the management of RSV infection. Herein, we detail the preclinical characteristics of EDP-323, a novel small molecule, non-nucleoside RSV L-protein inhibitor as a potential treatment for RSV infection.

Method

Inhibition of RSV L enzyme activity was measured in an enzyme-coupled luminescence assay. Antiviral activity against clinical isolates and laboratory strains of RSV-A and RSV-B were evaluated in HEp-2 cells or differentiated primary human airway epithelial cells cultured at an airway-liquid interface (pHAEC-ALI), utilizing cytopathic effect (CPE) and RT-qPCR as readouts. Viral load, time-of-addition, and drug resistance mapping were performed using RSV-A Long strain. Animal efficacy studies were performed in BALB/c mice infected with RSV A2 strain.

Result

EDP-323 inhibited RSV L RNA polymerase activity in vitro with an IC50 of 14 nM. In HEp-2 cells, EDP-323 inhibited virus-induced CPE of RSV-A and RSV-B strains and clinical isolates with EC50s of 44-360 pM. In the pHAEC-ALI, EDP-323 inhibited RSV-A Long with an EC90 of 160 pM. A CC50 of 18 μM (HEp-2 cells) was observed, which provides a selectivity index >30,000. EDP-323 EC50 and Emax did not significantly shift with a multiplicity of infection (MOI) from 0.1-10. Treatment delayed by 3-days post-infection had no impact on the potency of EDP-323 in the pHAEC-ALI system. Resistance profiling mapped to the capping domain of the L-protein and mechanism of action studies showed inhibition of both RSV transcription and replication. EDP 323 protected RSV-infected BALB/c mice from viral-induced changes in body and lung weights and reduced viral replication by up to 2.9 logs as quantified by RT-qPCR and plaque reduction assays. Treatment with EDP-323 was also associated with improved lung histopathology and dose-dependent reductions in pro-inflammatory cytokines such as TNF alpha, IL1 beta, and MCP-1.

Conclusion

EDP-323 is a potent inhibitor of RSV replication with picomolar antiviral activity against all RSV-A and RSV-B clades tested. EDP-323 effectively blocks RSV replication and the associated pathology in a rodent infection model. Taken together, these data support the further evaluation of EDP 323 as a potential oral therapy for RSV.

Advanced modeling of RSV infection in neonatal lambs

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Background

Respiratory syncytial virus (RSV) is a common cause of respiratory infection in infants worldwide. The neonatal lamb model of RSV infection has similarities to RSV infected infants in terms of lesions, immunological responses, lung structure and cellularity, and clinical signs. It has been used to test the efficacy of various types of anti-RSV therapeutic regimens with various routes of treatment. Recent studies have further expanded the model to develop the best RSV infection method, other respiratory viruses, and bacterial respiratory infections. We have tested various types of RSV delivery, including intratracheal injection, intranasal deposition, fiberoptic bronchoscope deposition, intranasal spritzers, and jet whisper nebulization. Recently, we tested RSV mesh nebulization and compared it with the jet whisper nebulization. Additionally, the effects of RSV infection on secondary bacterial infection with Streptococcus pneumoniae demonstrated enhanced RSV titer and lung lesions. We are evaluating induced bacterial pneumonia in lambs with two of the most common bacterial pathogens in the human's Klebsiella Pneumonia and Methicillin-resistant Staphylococcus aureus (MRSA).

Method

In the first project, we had two groups of lambs treated identically. Both groups were infected with 6 ml of RSV 1.27×10^7 IFFU/ml by nebulization. Jet whisper nebulizer was used for the first group, while mesh nebulizer was used for the second group. Lambs were monitored daily for clinical signs and humanely euthanized at day six postinfection. Gross and microscopic lung lesions with viral titer were assessed to evaluate the severity of RSV lower respiratory tract infection.

In the second project, we tested the possibility of developing bacterial pneumonia using two common bacterial pathogens in humans, K. pneumonia and MRSA. Two groups of lambs were infected intratracheally with 2 ml of 2×10^6 CFU with either K. pneumonia or MRSA. The third group was inoculated with PBS and served as the control non-infected. Lambs were monitored and humanely euthanized six days postinfection. Bacterial colonization and lung lesions were determined to demonstrate infection.

Result

A significant difference was not seen between nebulizers in terms of viral titers and lesions, although there were more pronounced clinical and respiratory signs as noted by increased lung sounds, wheezing, and respiratory effort in the group nebulized with mesh nebulizer. We have also determined that lambs infected with K. pneumoniae and MRSA develop moderate multifocal bronchopneumonia.

Conclusion

Mesh nebulization induces more severe RSV lower respiratory tract clinical signs in the neonatal lamb model of RSV infection of infants. K. pneumonia and MRSA induced moderate bronchopneumonia in lambs after intratracheal inoculation setting the baseline for such infection secondary to initial RSV infection.

Dorothea Morris - ARNI0048

INVESTIGATING THE ROLE OF HIF-1α IN HMPV AND RSV INFECTIONS

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Background

Hypoxia-inducible factors (HIF) are transcription factors that mediate oxygen utilization by regulating cellular metabolism and redox homeostasis. When oxygen is present, HIF-1α is produced and rapidly degraded following ubiquitination to allow for normal metabolic activity. Under hypoxic conditions, HIF-1α is stabilized and translocated to the nucleus where it forms a heterodimer with HIF-1β. This HIF complex then influences many biological activities through the hypoxia response element, primarily by upregulating glycolytic activity. Viruses can hijack host cell metabolism by activating HIF-1α, through hypoxia-independent mechanisms, to produce components vital for virion production. HIF-1α activation also controls the expression of genes involved in inflammation and airway remodeling, two important pathogenetic component of respiratory viruses-associated lung diseases. Human metapneumovirus (hMPV) and respiratory syncytial virus (RSV) are leading causes of bronchiolitis among infants and young children.

Method

Recent work in our lab has shown hMPV and RSV to induce changes to core metabolic pathways through a HIF-1α dependent process in primary epithelial cells. In vitro blockade of HIF-1α with PX-478, a specific HIF-1α inhibitor, significantly reduced the viral titer of either virus, demonstrating anti-viral therapeutic potential. Here, we compare the effects of blocking HIF-1α in an hMPV or RSV mouse model of infection.

Result

Administration of PX-478 to hMPV infected mice results in improvements to early disease outcomes including hMPV-induced bodyweight loss, bronchoconstriction, total protein expression, and LDH activity. Towards the end of disease progression, hMPV/PX-478 mice failed to regain bodyweight, resulting in a chronic like illness. These mice also showed significantly increased peak viral replication on day 5 that was not cleared from the lung on day 7 post-infection. Additionally, hMPV/PX-478 mice had a near abrogation of lymphocytes in the bronchoalveolar lavage fluid at any time point tested. RSV infected mice treated with PX-478 had similar outcomes to airway function, viral replication, and BAL cell activity. RSV/PX-478 differed from hMPV/PX-478 mice in that there was never a timepoint with improve bodyweight loss as compared to the RSV/PBS control mice.

Conclusion

The differing activity noted between in vitro and in vivo models highlights the complex and important compartmental contributions of HIF-1α activity to the outcome of disease during hMPV and RSV infections. The use of anti-HIF-1α therapeutics for viral respiratory infections should be carefully considered.

Safety and Immunogenicity of mRNA-1345, an mRNA-Based Vaccine Against RSV in Older Adults through 6 Month Follow-up

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Background

An mRNA-based RSV vaccine (mRNA-1345) encoding the membrane-anchored RSV prefusion stabilized F glycoprotein is currently under clinical investigation.

Method

This phase 1, randomized, observer-blind, placebo-controlled, dose-ranging study assessed the safety, reactogenicity, and immunogenicity of mRNA-1345 in several populations, including older adults aged 65-79 years (NCT04528719). Participants were randomized to receive 1 dose of mRNA-1345 (12.5, 25, 50, 100, or 200 µg) or placebo. Data presented represent follow-up period through 6 months (M6) after dose 1.

Result

Overall, 298 participants received study injections (mRNA-1345, n=239; placebo, n=59). mRNA-1345 was well-tolerated at all dose levels. The most frequently reported local solicited adverse reaction (SAR) was injection site pain (mostly grade 1) reported by 66.2% (n=153/231) of mRNA-1345 recipients and 12.7% (n=7/55) of placebo recipients (Figure 1). Overall, 60.2% (n=139/231) of mRNA-1345 recipients and 45.5% (n=25/55) of placebo recipients reported ≥1 systemic SAR; the most common systemic SARs in the mRNA-1345 cohorts were headache, fatigue, myalgia, and arthralgia. Unsolicited treatment-emergent adverse events (TEAEs) within 28 days of dose 1 were reported by 24.3% (n=58/239) of mRNA-1345 recipients and 20.3% (n=12/59) of placebo recipients; among these, 7 events (2.9%) were grade 3 or higher, reported in the 50, 100, and 200 µg mRNA-1345 dose arms. Unsolicited treatment-related TEAEs were reported by 7.1% (n=17/239) of mRNA-1345 recipients and 10.2% (n=6/59) of placebo recipients. No treatment-related unsolicited serious adverse events (SAEs), adverse events of special interest, or fatal TEAEs were reported. All participants had neutralizing antibodies against RSV at baseline (Figure 2). A single dose of mRNA-1345 boosted RSV-A and RSV-B neutralizing antibody geometric mean titers (GMTs) at all dose levels evaluated with minimal dose response observed. GMTs steadily declined between month 1 (M1) and M6 but remained substantially above baseline for all mRNA-1345 dose cohorts. Across dose levels, neutralizing antibody geometric mean fold rise (GMFRs) for RSV-A at M1 ranged from 9.9 to 16.6; and 3.1 to 5.8 by M6; GMFRs for RSV-B ranged from 5.3 to 12.6 at M1; and 2.9 to 5.5 by M6.

Conclusion

mRNA-1345 is well-tolerated and induces a functional immune response in older adults, with neutralizing antibody levels substantially greater than baseline maintained through to M6. These data support the continued development of mRNA-1345 as an RSV vaccine for older adults.

Michelle Dimitris - ARNI0056

Examining the relationship between respiratory syncytial virus, influenza, and rotavirus seasons' timing and severity, and infant age at viral seasons' peaks, with subsequent childhood asthma

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Background

Most infants are exposed to respiratory syncytial virus (RSV) in the first year of life. Studies have observed an association between severe RSV infection and asthma, yet it is unclear if this relationship is causal or due to underlying factors that increase susceptibility to both conditions. One study partially mitigated the influence of these factors by examining the relationship between infant age at winter virus peak and subsequent asthma. We extended this approach by examining infant age at RSV, influenza, and rotavirus peaks, as well as proxies for the timing and severity of RSV, influenza, and rotavirus seasons, and their relationships with subsequent incidence of childhood asthma.

Method

We analyzed province-wide administrative data for 1,437,731 infants born in Ontario, Canada from 2002-2013. We ascertained RSV, influenza, and rotavirus hospitalizations by 1 year and asthma by 5 years of age using inpatient/outpatient ICD-9/10 codes. We used regression models to investigate: (1) infant age in the calendar week with highest incidence of hospitalization for each virus and subsequent asthma (unit of analysis infant); (2) incidence of RSV-, influenza-, and rotavirus-related hospitalizations by 1 year and asthma by 5 years (unit of analysis calendar week of birth).

Result

We observed highest likelihood of subsequent asthma at infant ages of approximately 13-, 11-, and 16-weeks during RSV, influenza, and rotavirus peaks, respectively. We observed apparent seasonal variation in childhood asthma by infant week of birth. The relationship between RSV seasonal variation and asthma appeared small in magnitude, while an unexpected relationship between rotavirus seasonal variation and asthma emerged (Figure).

Conclusion

We find limited evidence in support of a causal relationship between RSV and asthma, and suggest further investigation of other mechanisms, including underlying seasonal characteristics.

A large-scale multi-national prospective observational cohort study documenting the summer 2021 RSV epidemic in the United Kingdom and Ireland

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Background

An up-to-date understanding of the burden of RSV bronchiolitis and viral lower respiratory tract infection (LRTI) is required, as long-acting monoclonal antibodies are likely to be introduced soon. However, these are unlikely to be fully effective in preventing disease, and may be susceptible to viral genetic variability. Therefore information is also needed on factors leading to the seeding and transmission of RSV, to ascertain whether other non-pharmaceutical interventions might be effective.

Method

The BronchStart Project was set up April 2021 to study an aseasonal summer RSV epidemic in the United Kingdom (UK) and Ireland. Clinicians and research nurses in 61 Emergency and Paediatric Departments collected information on clinical presentation, demographics (including a history of prematurity, socioeconomic status, number of siblings, day care attendance, and travel history), management and outcomes for 0-23 month olds with bronchiolitis, LRTI, or a first presentation of viral induced wheeze.

Result

A total of 16,983 patients were recruited to BronchStart; cases peaked in September 2021. Of this cohort 10,554 attended Emergency Departments and were discharged, and 6,429 hospitalised. Of those admitted low flow oxygen was administered to 2,550 (39.7%); 1,778 (27.6%) received nasogastric fluids, and 715 (11.1%) intravenous fluids. 428 children (6.6%) were cared for in a high dependency unit and 137 (2.1%) in an intensive care unit; 90 (1.4%) received invasive mechanical ventilation. Patients with a history of prematurity constituted 11% of attendances; prematurity was a risk factor for admission (risk ratio=1.7, 95% CI 1.6-1.9, p < 0.001). Those in the most deprived quintile were over-represented in attendances (28.5% vs 16.7%) and admissions (27.5 % vs 18.3%) when compared to the least deprived. Data on number of siblings was available for 83%, on attendance at day care for 63%, and on travel history for 52%. A minority of children (23%) attended day care, but a majority (68%) had 1 or more siblings. By month, a travel history was associated with between 5.6 to 14.6 % of attendances, peaking August 2021. Most travel was within the UK (75%), followed by Europe (17%); 8% had travelled outside Europe. Stored samples will undergo whole genome sequencing to elucidate the role of travel in seeding RSV infection.

Conclusion

In addition to the substantial hospitalisation burden of bronchiolitis/LRTI, we found that for every child admitted a further 1.6 attended an Emergency Department. Ongoing large scale studies, incorporating genomic data, are likely to be required to understand the impact of long-acting monoclonal antibodies on the burden of RSV disease and transmission dynamics.

A Phase 3 Pivotal Study of Efficacy and Safety of Oral Ziresovir in RSV-Infected Hospitalized Infants

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Background

Respiratory syncytial virus (RSV) infection is a cause of substantial morbidity and mortality in infants, elderly, and adults with immune-compromised conditions. Currently there is no known effective anti-RSV therapy available. Ziresovir is a potent, selective, and orally bioavailable RSV F protein inhibitor. Positive signs of efficacy of ziresovir was observed in a phase 2 proof-of-concept study in hospitalized RSV-infected infants.

Method

We conducted a randomized, double-blind, placebo-controlled phase 3 pivotal study with ziresovir in hospitalized RSV infants aged at 1 to 24 months age. Patients were assigned in a 2:1 ratio to receive either 2-4 mg/kg ziresovir or placebo every 12 hours for 5 days. Bronchiolitis sign & symptom (S&S) score, viral load, and safety were evaluated daily during drug treatment period and were followed up through day 14.

Result

A total of 311 patients underwent randomization, and 200 patients received ziresovir and 102 received placebo. The mean S&S score change from baseline at Day 3 post treatment was -3.4 (95% CI: -3.7, -3.1) with ziresovir versus -2.7 (95% CI: -3.1, -2.2) with placebo, with a mean difference of -0.8 (95% CI: -1.3, -0.3; P = 0.002), indicating an additional 30% reduction in S&S score for ziresovir compared to placebo. Viral load was lower with ziresovir than with placebo at Day 5 of treatment, with a mean difference of −0.64 log copies/ml (95% CI: -1.093, -0.189; P = 0.006), or 4.4-fold decrease in viral load relative to placebo. In the patients at age ≤ 6 months, the mean S&S change from baseline at Day 3 was -3.5 with ziresovir versus -2.2 with placebo, with a mean difference of -1.2 (95% CI: -1.9, -0.6; P value < 0.001), or an additional 55% reduction in S&S score compared to placebo. Ziresovir exhibited an excellent safety profile in the study with a comparable adverse event rate of ziresovir (61.0%) versus placebo (52.9%).

Conclusion

In infants with RSV infection, ziresovir significantly reduced both bronchiolitis S&S score at Day 3 and viral load at Day 5. Ziresovir is well tolerated with good safety profile in the infant patients. To our knowledge, this was the first successful pivotal phase 3 study for a direct anti-RSV drug in hospitalized infant patients.

Sheerien Manzoor - ARNI0084

Differential responses of paediatric airway epithelium from wheezers and non-wheezers to RSV and/or aeroallergens.

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Background

Asthma affects 300 million children worldwide. Early age viral infections and allergen sensitizations are associated with subsequent asthma development. We aimed to understand the consequences of respiratory syncytial virus (RSV) infection and/or house-dust mite (HDM) exposure on cytopathogenesis, virus replication, and innate immune responses in airway epithelium from young children.

Method

Well-differentiated primary nasal epithelial cell (WD-PNEC) cultures were derived from children (aged 1-6 years) identified as (n=5 each):

1. Healthy

2. Mild wheezers

3. Severe wheezers

WD-PNECs were infected with RSV following HDM or mock stimulation.

Result

HDM pre-treatment attenuated RSV growth kinetics, probably through a partial cleavage of IGF1R and NCL RSV receptors/entry factors, regardless of wheeze phenotype. Post-infection ciliated cell loss was greatest from severe wheezer WD-PNECs. HDM pre-treatment did not alter innate immune responses to infection. Expression of IL-29 and interferon-stimulated genes (ISGs), including IFI6, ISG15, DUOXA2, and DUOX2, were significantly increased following infection, as were IL-29, CEACAM1, TRAIL, CX3CL1, GM-CSF, CXCL8, and CXCL16 protein secretions. Interestingly, IRF9, IFI6, and ISG15 gene expressions were significantly lower in mild compared to severe wheezers or healthy individuals. Furthermore, CEACAM1 and IL-33 secretions were highest, and GM-CSF was lowest, in WD-PNECs from severe wheezers. CXCL16 secretions were higher in wheezers compared to healthy children, irrespective of wheeze severity. Surprisingly, genes associated with asthma and airway remodelling, including TSLP, HMGB1, and KRT5, were reduced following RSV infection in all cohorts.

Conclusion

However, although differential gene/protein expression was observed in children with no, mild or severe wheeze, the functional consequences of these responses on the progression of wheeze pathogenesis remain to be determined.

Megan Barnes - **ARNI0087**

Severity-Associated Transcriptional Signatures in the Respiratory Mucosa during Human Paediatric RSV Infection

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Background

Understanding of the mechanisms that determine RSV disease severity in infants is increasing, but the responses to RSV in the respiratory mucosa need additional study. We have previously shown that viral load is not increased in babies with severe disease: indeed, it may be reduced, indicating that reducing viral load in those with advanced disease may not be therapeutically effective. We and others have shown that reduced interferon signalling may occur in severe disease, indicating possible alternative therapeutic approaches.

Method

Nasopharyngeal aspirates (NPAs) were obtained from 51 babies under the age of two, hospitalised for RSV bronchiolitis during a single season (Oct 2016 to March 2017). Transcriptomic profiling was performed on RNA from NPA using NanoString, to study 785 transcripts covering innate and adaptive immune responses. Participants were categorised by severity based on their need for intensive care (PICU) (severe, n=31) or whether they were kept in pediatric wards (moderate, n=20).

Result

Gene expression analysis revealed 19 statistically differentially expressed genes (DEGs) (adjusted P value<0.05) between the moderate and severe RSV groups (Figure 1). Pathway analysis indicated reduced T cell function during severe RSV, including chemotaxis, antigen processing, and cytotoxicity. Lower expression of several guanylate-binding proteins (GBPs), including a significant (P=0.0264) decrease in GBP1 expression, could also suggest a dysfunction in innate antiviral immunity in severe RSV. The most novel finding was of a significant (P=1.51x10-10) increase in expression of IL-1 family epithelial alarmin IL36A in severe RSV. Measuring protein levels of interferons and inflammatory cytokines showed that most were reduced in severe RSV, but IL-36α levels were consistently high.

Conclusion

The transcriptional landscape in the airway during RSV infection distinguishes disease severity groups. These data indicate reduced T cell signalling but enhanced epithelial dysregulation in more severe RSV. Given the role of IL-36α in neutrophil chemotaxis and activation, we hypothesise that it is an effector in driving neutrophilic inflammation in RSV-induced bronchiolitis, enhancing the clinical severity of the infection.

Jim Boonyaratanakornkit - ARNI0096

Cross-Protective Antibodies Against Common Endemic Respiratory Viruses

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Background

Respiratory syncytial virus (RSV), human metapneumovirus (HMPV), and human parainfluenza virus types one (HPIV1) and three (HPIV3) are a major cause of death, morbidity, and health care costs worldwide, and they can exact a significant toll on immunocompromised patients, the elderly, and those with underlying lung disease. There is an unmet need for safe and effective medications to prevent many of the viruses responsible for common respiratory viral infections in vulnerable patients. While a protective monoclonal antibody exists for RSV, clinical use is limited to high-risk infants. Here, we present the discovery, in vitro characterization, and in vivo efficacy testing of two cross-neutralizing monoclonal antibodies, with one targeting both HPIV3 and HPIV1 and the other targeting both RSV and HMPV.

Method

We leveraged a "bait and switch" strategy whereby antibodies produced by B cells binding to one virus (bait) were screened for the ability to neutralize a different virus (switch). For this, fluorescent-conjugated tetramers of viral prefusion proteins were used to isolate B cells capable of binding to HPIV3. Individual B cells were then stimulated, and culture supernatants were screened for their ability to neutralize HPIV1. The heavy and light chain sequences of candidate cross-neutralizing B cells were cloned and produced as monoclonal antibodies. Binding kinetics were determined using biolayer interferometry; neutralization potency was measured using live virus in a plaque reduction neutralization assay; epitope analysis was performed using cryo-electron microscopy; and in vivo protection was tested in a hamster challenge model.

Result

We isolated the 3x1 antibody which is capable of targeting multiple parainfluenza viruses. Using a related approach, we also isolated the MxR antibody which shares features with other previously reported monoclonal antibodies capable of neutralizing both RSV and HMPV. We obtained structures using cryo-electron microscopy of these antibodies in complex with their antigens, providing a structural basis for binding and neutralization. 3x1 binds to a novel antigenic site called site X on HPIV3 preF, whereas MxR binds to antigenic site III on RSV preF. Prophylactic administration of 3x1 and MxR suppressed viral replication in the lungs of hamsters challenged with HPIV3 and RSV, respectively. Prophylactic co-administration of 3x1 and MxR also protected hamsters from co-infection with HPIV3 and RSV.

Conclusion

Together, a cocktail of 3x1 and MxR could have clinical utility in providing broad protection against four of the respiratory viruses that cause significant morbidity and mortality in at-risk individuals.

Hospitalization rates and outcomes for RSV-associated hospitalizations in adults aged 18 years and older in the United States during two respiratory seasons, October 2018 - April 2020

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Background

Respiratory syncytial virus (RSV) is a major cause of hospitalizations in older adults and typically circulates in the fall and winter in the United States. Population-based data about RSV-related hospitalizations in adults are limited.

Method

RSV-NET is a population-based active surveillance system that collects clinical information on RSV-associated hospitalizations in acute care hospitals across 75 counties in 12 states in the United States. An RSV-NET case is a resident of a defined catchment area who tests positive for RSV through a standard of care test ordered by a healthcare professional within 14 days prior to or during hospitalization. Surveillance data were analyzed from October through April for the 2018-19, and 2019-20 seasons. Hospitalization rates (presented per 100,000 population) were calculated using U.S. census catchment denominators. Rates were adjusted for test sensitivity, and to adjust for under-ascertainment of RSV cases due to testing practices, the number of RSV-positive patients identified through RSV-NET was multiplied by 1/proportion of patients tested for RSV in a convenience sample of patients with discharge diagnosis codes for any acute respiratory illness at RSV-NET sites. Data on ICU admission and in-hospital death were abstracted from the medical chart for all RSV patients.

Result

2,536 and 3,195 laboratory-confirmed RSV-associated adult hospitalizations were identified in 2018-19 and 2019-20, respectively (Figure 1). Across both seasons, 785 (13.7%), 746 (13.0%), 2518 (43.9%) and 1682 (29.4%) of hospitalizations were among those aged 18-49, 50-59, 60-79, and ≥80 years, respectively. Adjusted rates for those aged ≥18 years were 42 (95% CI 30-73) in 2018-19 and 53 (95% CI 37-92) in 2019-20 per 100,000 population. Rates increased with age, from 8 - 11 per 100,000 in those aged 18 - 49 to 237 - 277 per 100,000 in those aged ≥80 years. (Figure 2). Across both seasons, 19.7% of RSV patients were admitted to the ICU and 3.8% died. Mortality increased with increasing age, ranging from 0.9 to 5.4% in those aged 18-49 years and ≥80 years, respectively.

Conclusion

The burden of RSV hospitalizations is high in older adults, indicating the need for targeted interventions such as vaccination in older adults. RSV can cause severe disease in adults of all ages, with one in five hospitalized adults needing ICU care. Hospitalization rates and mortality increased with increasing age.

Elucidating the role of the Pep27 fragment in RSV fusion protein function and stability

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Background

The respiratory syncytial virus (RSV) fusion protein (F) is responsible for facilitating viral-cell and cell-cell membrane fusion as well as viral entry. These critical roles make F an excellent therapeutic target. To be fusogenically active, RSV F must be cleaved by a host cell protease into two disulfide linked subunits. Unlike many type I fusion proteins, RSV F has two cleavage sites, both containing a furin recognition motif (R-X-R/K-R), separated by 27 amino acids. Cleavage at both sites releases a 27 amino acid fragment, termed Pep27. Previous work has provided conflicting results on when the two cleavage events occur, and the fate of Pep27 is unclear. Recent studies have suggested that Pep27 is present on the cell surface; however, a better understanding of RSV F proteolytic processing is needed.

Method

We have used a combination of techniques to explore the proteolytic processing of RSV F and the potential function of Pep27 in RSV A F and RSV B F. Mutagenesis permitted the deletion or alteration of specific residues to identify importance. Metabolic label experiments determined cleavage product formation and protein expression. Pulse chase experiments indicated protein cleavage state and stability over time, while surface biotinylation assays detected targeted proteins on the cell surface. To observe and quantify cell-cell fusion, syncytia assays and reporter gene assays were used.

Result

We demonstrate that both cleavage sites within F are viable targets for proteolytic processing, and that mutating either site to alter the furin recognition motif blocks cell-cell fusion activity, consistent with early studies. Examination of F cleavage kinetics in both infected and transfected systems over time determined that cleavage of both sites occurs within the same time frame. To identify the role of Pep27 in F processing and expression, we deleted the fragment, but preserved the cleavage sites. We found that the deletion of Pep27 does not block F surface expression or cell-cell fusion. Within Pep27, there are two N-linked glycosylation sites conserved among both RSV A2 and RSV B9320 lab strains. Randomization of Pep27, while conserving the two N-liked glycosylation sites, did not significantly change the surface expression of pre- or post-fusion F, stability of F over time, or cell-cell fusion. However, preliminary data indicates that the mutation of either Pep27 glycosylation site reduces cell-cell fusion and alters surface expression of Pep27 compared to WT.

Conclusion

This work clarifies the timing of RSV F proteolytic cleavage and offers insight into the crucial role the N-linked glycosylation sites within the Pep27 play in the biological function of F.

Xiaoyu Zhang - ARNI0122

Repurposing screen identifies lonafarnib as RSV fusion protein inhibitor

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Background

Respiratory syncytial virus (RSV) is a common cause of acute lower respiratory tract infection in infants, young children, the elderly and the immunocompromised. Effective directly acting antivirals are not available.

Method

To address this need, we screened the ReFRAME drug-repurposing library consisting of 12,993 small molecules. Around 68% of these compounds are either licensed for use in humans or in advanced stages of clinical development. Disease annotations of these molecules are dominated by cancer (43%), central nervous system (CNS, 23%), and cardiovascular/respiratory (20%). Thus, 86% of molecules target physiological processes in humans, making the library a unique resource for identification of repurposing candidates suitable as antiviral drugs and for the discovery of important host factors.

Result

Interrogating the library with a RSV GFP reporter virus, we identified primary candidates mapping to 61 distinct target categories. Expectedly, RSV F protein inhibitors accumulated the largest number of candidates, followed by HSP90, and NF-kappaB inhibitors. We selected lonafarnib, a farnesyltransferase inhibitor used for the treatment of Hutchinson-Gilford Progeria Syndrome and in phase three clinical trials for the treatment of hepatitis delta virus infections (HDV), for further follow up.

Dose-response analyses and plaque assays confirmed the antiviral activity against RSV A and B strains (RSV A IC50 of 0.02 μM, CC50 of 13.74 μM). Tipifarnib, another farnesyltransferase inhibitor, was antiviral against HDV but did not inhibit RSV replication, suggesting that lonafarnib blocks RSV independently of interfering with the farnesyltransferase. Passaging of RSV with increasing concentrations of lonafarnib selected for a phenotypically resistant virus population accumulating non-synonymous mutations in the RSV F protein (T335I and T400A). Lentiviral pseudotypes harboring parental or variant RSV F proteins confirmed that lonafarnib inhibits RSV cell entry and that these mutations confer lonafarnib resistance. Surface plasmon resonance revealed RSV F protein binding of lonafarnib, but not tipifarnib. Oral administration of lonafarnib modestly reduced RSV virus load in a murine infection model.

Conclusion

Collectively, this work provides an overview of RSV drug repurposing candidates and establishes lonafarnib as a bona fide F protein inhibitor.

Presence of neutrophils in the lungs prior to infection with respiratory viruses influences disease severity in mice

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Background

In most cases infection with Respiratory Syncytial Virus (RSV) causes mild upper airway symptoms, however in susceptible individuals such as infants and the elderly, RSV can often cause severe disease. The individual's immune response to the virus is thought to influence susceptibility, but the exact determinants are unclear. Recently, the presence of neutrophils in the respiratory tract of both humans and mice prior to RSV infection was linked with exacerbated disease. In mouse models, recruitment of neutrophils, driven by intranasal CXCL1, prior to RSV infection resulted in exacerbated weight loss associated with higher viral load at 18h post infection and increased CD8+ T cell recruitment by day 8 post infection. As neutrophils are known to be short lived cells, we investigated how extending the time between neutrophil recruitment and RSV infection would alter disease outcomes.

Method

Mice were intranasally pre-treated with a single dose of CXCL1 0.5, 2, 4 or 7 days prior to infection with RSV. Weight loss was monitored and the immune response at different time points post infection was studied using flow cytometry, ELISA and qPCR.

Result

As shown before, neutrophils recruited to the lungs 12h before RSV infection resulted in enhanced weight loss. However, increasing the time between neutrophil recruitment and RSV infection to 2-4d did not result in any weight loss following RSV infection. While viral load at 18h post infection did not differ in mice pre-treated with CXCL1 2-4d prior to infection compared with RSV only controls, levels of proinflammatory cytokines such as IFN-a trended lower. Protection from weight loss was not associated with any alterations in the adaptive immune response at 8d post infection. Interestingly, at 18h post infection, alveolar macrophages (AMs) were found to be significantly increased in mice pre-treated 2-4d prior to infection compared with controls. Increased numbers of AMs were associated with an increase in a subpopulation of neutrophils, Siglec-F+ neturophils. These neutrophils also appeared to be smaller in size and more autofluorescent compared to Siglec-F- neutrophils, both attributes common to apoptotic cells.

Conclusion

Over time following recruitment to the lungs, the neutrophil population appears to change, potentially undergoing apoptosis due to their short-lived nature. This was associated with an increase in the AM population and an amelioration in the inflammatory response to RSV. Our findings suggest a finely balanced relationship between neutrophil recruitment and disease exacerbation or amelioration dependent on the time between recruitment and RSV infection

Justine Oliva - ARNI0135

Strong remodeling of RSV-induced transcriptional signature in the early stages of bacterial superinfection in human airway epithelium

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Background

RSV is the most common cause of bronchiolitis in children less than 1-year-old and is responsible for acute lower respiratory tract infection in the elderly and in immunocompromised adults. The disease is often associated with a simultaneous or secondary infection, involving bacteria such as S. pneumoniae, H. influenzae or S. aureus. Recent studies suggested a most prevalent association between RSV and S. aureus, especially methicillin-resistant S. aureus strains (MRSA). Clinical retrospective studies indicate that bacterial superinfections often correlate with more severe disease in susceptible people than simple RSV infection. However, compared to bacterial superinfections in the context of influenza virus infection, those in the context of RSV have been relatively less explored and our knowledge of the underlying mechanisms is still rather limited.

Method

In this study, we aimed to decipher the molecular mechanisms involved in the early stages of S. aureus superinfection after a primary RSV infection in a model of reconstituted human airway epithelia (HAE). RSV-infected HAE (6 days post-infection) were superinfected with S. aureus (MRSA strain) for 4h or 12h. After comparing the effect of simple infections and superinfection on epithelial integrity and ultrastructural organization by classic transmission electron microscopy, we performed the host transcriptional profiling of single infections (RSV or S. aureus) and (RSV+ S. aureus) superinfection compared to non-infected controls using RNAseq.

Result

While single RSV infection induced a mild effect on epithelium integrity, as observed with the trans-epithelial resistance (TEER) and transmission electronic microscopy (TEM), single S. aureus infection led to a strong impact on epithelium integrity, especially with a long time of infection (12h). Compared to single RSV infection, superinfection presented a stronger impact on epithelium integrity characterized by a sharp decrease in TEER of about 25% at 6dpi + 12h, as well as more cellular damages. Interestingly, superinfection led to a lower decrease of TEER compared to S. aureus infection (25% and 87% of TEER decrease, respectively). Comparison of transcriptomic signatures revealed a profound remodeling of the single RSV infection signature in the context of S. aureus superinfection, as early as 4h. While the RSV signature is distinguished by a very strong deregulation of genes involved in the antiviral response and the cilia machinery, the RSV+ S. aureus signature is rather distinguished by an upregulation of genes involved in bacterium entry into host cell, as well as apoptosis mechanisms.

Conclusion

This study has shown that the impact of RSV on the host response is profoundly affected during bacterial superinfection, even at a very early stage. The high specificity of this superinfection signature could contribute to a better understanding of the mechanisms underlying the severity of RSV/bacterial co-infections, but also constitute a starting point for the identification of markers or the development of more effective therapeutic approaches.

Dhanushika Ratnayake - ARNI0136

Single-molecule imaging unravels real-time transcription and replication dynamics of RSV

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Background

Respiratory syncytial virus (RSV) is an important human pathogen imposing a significant burden on global health and economy. Despite extensive research, molecular events that occur during the first hours of infection remain poorly understood. For example, it is unclear how viral transcription and replication are coordinated on single viral genomes and it is unknown what fraction of infecting viral genomes are able to successfully generate viral progeny. The lack of knowledge on early infection is due to low viral protein and RNA abundance in early infection and limitations in detection sensitivity of current methods to study RNA.

Method

To overcome current limitations we developed a comprehensive toolkit enabling the detection of viral RNA species and proteins at single-molecule resolution, allowing viral detection from the moment of cell entry. Single-molecule fluorescence in situ hybridisation combined with antibody staining allowed individual viral genomes, antigenomes and transcripts to be identified in fixed cells. For live analysis of infection, novel imaging modalities were established to visualise and track viral genomes/antigenomes and transcripts at a single-molecule resolution; the RSV genome was engineered to encode a tag that enabled the visualisation of its corresponding transcripts and single genomes were labelled by expression of a fluorescently-tagged protein that specifically bound the RSV nucleocapsid.

Result

Our single-molecule toolkit enabled the detailed characterisation of early RSV infection. We found that a substantial fraction of RSV virions contained multiple genomes, with 2 genomes/virion being the most common. Interestingly, a significant percentage of infected cells failed to demonstrate infection progression. Even in cells with progressing RSV infections, viral transcription and replication were highly variable both in absolute quantity and kinetics with only a small fraction of cells displaying very high infection burden.

Conclusion

We have successfully developed technologies to visualise cell entry and early viral transcription and replication. Using these tools, we characterised RSV transcription and replication dynamics at a single-molecule and single-cell resolution, which provided insights into the early rate-limiting steps of RSV infection.

Eric Simoes - ARNI0139

NIRSEVIMAB FOR THE PREVENTION OF RSV: POOLED EFFICACY FROM PHASE IIB AND PHASE III TRIALS IN PRETERM AND TERM INFANTS

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Background

A single prophylactic injection of the monoclonal antibody nirsevimab has been demonstrated to reduce the incidence of medically attended respiratory syncytial virus (RSV) lower respiratory tract infection (LRTI) in two double-blind, placebo-controlled studies over the first RSV season (Phase IIb [NCT02878330]: healthy preterm infants ≥29 to <35 weeks' gestational age, efficacy 70.1%; Phase III: MELODY [NCT03979313], healthy term and late preterm infants ≥35 weeks' gestational age, efficacy 74.5%). We report a pooled efficacy analysis of nirsevimab in term and preterm infants ≥29 weeks' gestational age from these trials, up to Day 151.

Method

Infants were randomised 2:1 to receive either an intramuscular injection of nirsevimab or placebo before their first RSV season. Data were pooled from the Phase IIb and MELODY studies for those infants under the optimised dosing regimen (i.e. infants <5 kg at dosing who received the 50 mg dose from Phase IIb and all infants in MELODY [infants <5 kg at dosing received 50 mg; infants ≥5 kg received 100 mg]) to evaluate efficacy (relative risk reduction versus placebo) against varying severities of medically attended RSV LRTI, including hospitalisation due to RSV LRTI or severe RSV (requiring supplemental oxygen or intravenous fluids).

Result

In total, 860 infants from Phase IIb (median age at randomisation: 1.60 [range 0.1-6.4] months; female: 47.6%) and 1490 infants from MELODY (median age at randomisation: 2.60 [0.03-11.10] months; female: 48.4%) were included. Demographics were comparable across studies, except for gestational age and age at randomisation. Nirsevimab demonstrated an efficacy of 79.5% against medically attended RSV LRTI, 77.3% against RSV LRTI hospitalisation, and 86.0% against very severe RSV LRTI up to Day 151 (Figure). Consistent efficacy was observed across the subgroups age at randomisation, sex, ancestry, weight, and geographical region, and across endpoints of differing disease severity.

Conclusion

In a pooled analysis of two randomised, placebo-controlled studies, prophylaxis with nirsevimab demonstrated consistent efficacy across severities of RSV LRTI up to Day 151.

Estimates of the National Burden of Respiratory Syncytial Virus in Kenyan Children Aged Under 5 years, 2010-2018

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Background

Respiratory syncytial virus (RSV) is among the leading causes of viral pneumonia worldwide. Establishing RSV-associated morbidity and mortality is important in informing the development, delivery strategies and evaluation of impact of interventions.

Method

Using data collected during 2010-2018 from population-based surveillance studies in western Kenya and the Kilifi Health and Demographic Surveillance Study, we estimated age-specific base rates of acute respiratory illness (ARI), severe acute respiratory illness (SARI - defined as hospitalization with cough or difficulty breathing with onset within the past 10 days) and SARI-associated deaths. We extrapolated the base rates to other regions of Kenya while adjusting for risk factors of ARI and healthcare seeking behaviour, and finally applied the proportions of RSV positive cases to these rates to obtain regional age-specific rates of RSV-associated ARI and SARI. We applied age-specific RSV case fatality ratios on SARI to obtain estimates of RSV-associated in- and out-of-hospital deaths.

Result

Among Kenyan children aged <5 years, the annual incidence of outpatient and non-medically attended RSV-associated ARI was 206 (95% Credible Interval, CI; 186-229) and 226 (95% CI; 204-252) per 1,000 children, respectively. The annual rates of hospitalized and non-hospitalized RSV-associated SARI were 349 (95% CI; 303-404) and 1,077 (95% CI; 934-1,247) per 100,000 children respectively. The estimated annual number of in- and out-of-hospital deaths associated with RSV infection in Kenya were 539 (95% CI; 420-779) and 599 (95% CI; 420-839), respectively. Children aged <1 year had the highest burden of RSV; 302 (95% CI; 272-336) per 1,000 children for outpatient ARI, and 1,303 (95% CI; 1,122-1,507) and 35 (95% CI 24-51) cases per 100,000 children for hospitalized SARI and in-hospital deaths, respectively.

Conclusion

Our findings suggest a substantial disease burden due to RSV infection, particularly among younger children. Prioritizing development and use of maternal vaccines and affordable long-lasting monoclonal antibodies could help in reducing this burden.

Elisabeth Robinson - ARNI0150

Trans-epithelial migration is essential for neutrophil activation in the airway during RSV infection

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Background

Neutrophils are the predominant immune cell recovered from the airway during acute severe RSV infection, comprising 80% of cells recovered in BAL, but their contribution to recovery or disease severity remains unclear. Previous clinical studies have shown these BAL neutrophils demonstrate high levels of activation. This study aims to image neutrophil trans-epithelial migration and investigate whether activated neutrophils selectively migrate across RSV infected airway epithelial cells (AECs), or whether trans-epithelial migration is sufficient and necessary for neutrophil activation.

Method

Human nasal AECs were cultured on porous (3um or 0.4um) PET inserts (Greiner) in a coverslip bottomed 24 well plate. AECs were infected with RSV or 'mock' media control 72 hours prior to migration. Neutrophils were obtained from healthy volunteer venous blood by negative immunoselection (Stem Cell) then stained with a fluorescent dye. Neutrophils were then added to the basolateral side of AECs and imaged using a fluorescence spinning disk over a 70µm z-range for 1 hour. Neutrophils were then collected and activation markers CD11b, CD62L, CD64, NE and MPO determined by flow cytometry.

Result

Numbers of neutrophils adherent to RSV infected AECs (791.1 ± 106.8 cells/cm2) were significantly (p = 0.0135) greater than mock infected AECs (449.8 ± 81.82 cells/cm2). In RSV infected AECs, neutrophil recruitment and adherence to AECs, with clustering, occurred after 15-18 minutes. Neutrophil clusters were observed to form at the location of loss of viability of a single neutrophil.

We found that the expression of CD11b was significantly (p<0.05) higher in neutrophils recovered basolateral, adherent, apical to both mock and RSV infected AECs compared to neutrophils exposed to media alone (non-AEC). Neutrophils recovered from the basolateral compartment of both mock (36,708 ± 3,563) and RSV infected AECs (54,389 ± 3,863) had 24 and 30-fold greater (p<0.05) CD11b expression, respectively, compared to non-AEC neutrophils (1,583.7 ± 34.3). With AECs grown on pores which do not permit neutrophil migration expression of neutrophil CD11b and MPO did not increase and remained consistent with no AEC controls.

Conclusion

We observed that neutrophils show distinct, measurable patterns of movement during trans-epithelial migration through RSV infected AECs, including the formation of neutrophil clusters and upregulation of CD11b expression. We present tentative evidence of bidirectional movement of neutrophils during RSV infection that could have important systemic implications for severe disease sequalae.

Early detection and prediction of respiratory syncytial virus (RSV) outbreaks using wastewater surveillance

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Background

Wastewater-based epidemiology (WBE) has the capacity to determine levels of health-associated biomarkers and enables effective surveillance of entire communities. Traditionally, WBE has been used to investigate the infection trends of gastrointestinal disease and non-enveloped virus transmitted via the fecal-oral route. More recently it has been employed as a key metric in determining community prevalence of SARS-Co-V2. To date, the application of WBE for the surveillance of respiratory viruses has been poorly studied. Respiratory syncytial virus (RSV) is an enveloped RNA virus that can cause severe infections in infants, immunocompromised or elderly individuals. RSV is a seasonal outbreak disease with infections typically peaking in October; however, due to Covid-19 pandemic, unexpected seasonal RSV outbreaks were reported in 2021. The aims of this study were to determine if RSV can be detected in wastewater (WW), monitor the circulation of RSV in the community and investigate if increasing detection of RSV in WW precedes onset of clinical cases.

Method

WW samples from 33 WW inlet treatment sites across Northern Ireland (NI) were collected between August 2021 - January 2022. Each sample was concentrated using a CP Select TM, nucleic acid was extracted by the MagNA Pure 96 instrument and tested using an RSV specific RT-qPCR assay targeting the N gene. The gene copies/L were subsequently normalised based on the rainfall flow rate and population and compared to the clinical case rate. A Susceptible-Exposed-Infectious-Removed (SEIR) model based on the normalised RSV gene copies/L in WW was developed to predict the number of infected individuals in the community.

Result

SV was detected in 27.0% of WW samples during this period in NI. We report an increasing RSV detection in WW samples from across NI beginning 6th September until 27th September 2021. This mirrored the rise in RSV detections in clinical samples, with WW surveillance leading clinical diagnostic testing by 2 weeks.

Conclusion

These results highlight that WW surveillance is a valuable tool to detect and monitor outbreaks of circulating and clinically relevant respiratory viruses. Identifying outbreaks of RSV in the community is important for targeting the administration of RSV immunoprophylaxis products for high-risk patients. Therefore, WBE has the potential to establish guidelines for diagnostic testing and preventative measures and to assist with clinical resource planning.

Tina Hartert - ARNI0172

Timing of infection with respiratory syncytial virus and risk of childhood asthma

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Background

It is unknown whether RSV infection in infancy causes childhood asthma. Prior studies of the relationships of early-life RSV and asthma have focused almost exclusively on the association of severe infection with later pediatric asthma phenotypes. However, severe RSV infection is not an exposure, it's a clinical phenotype, and the association between severe viral infection and asthma is likely confounded by heredity, with severe RSV serving as a marker of asthma risk. Thus, study designs of severe viral infection and asthma risk cannot address a causal association. To overcome this we have previously shown that birth in relationship to RSV circulation is associated with asthma risk; however, no prospective study has addressed this question.

Method

To better address the issue of causality we designed a population-based, birth cohort of natural (quasi-random) RSV infection among healthy, term children (n=1,946). We first validated the framework of our study design by testing the effect of host genetics on RSV infection in infancy to delineate potential confounding as with severe disease. We ascertained RSV infection in infancy through a combination of active and passive surveillance using RSV molecular and serologic testing. Children were followed prospectively for annual recurrent wheeze and the development of asthma at age 5 years.

Result

A GWAS of infant RSV infection demonstrated no common variants contributing to infection, cumulative small genetic effects, or association with 54 candidate genes associated with asthma or severe RSV infection. In models stratified by child's age, children not infected with RSV in infancy, but infected post-infancy, compared with those infected during infancy had a lower risk of recurrent wheeze annually at age 2- to 4-years (aRR=0.54, 95% CI=0.42-0.70, p<0.001 and aRR=0.78, 95% CI=0.61-0.99, p=0.04, respectively) and ~25% lower risk of 5-year current asthma (adjusted RR=0.74, 95% CI=0.58-0.94, p=0.01). The estimated proportion of 5-year current asthma that could be prevented by delaying RSV infection until after infancy was 13.54% (95% CI=3.23%-24.89%).

Conclusion

We demonstrate for the first time that children not infected with RSV in infancy have a decreased risk of developing recurrent wheeze and childhood asthma. We also show an age-dependent effect of RSV infection on later development of recurrent wheeze and childhood asthma throughout the preschool years compared to those infected with RSV in the first year of life. We found no genetic predisposition to infection that might confound results. Our results suggest that delaying initial RSV infection could be a strategy to decrease risk of childhood asthma at the population level.

Immunocompromised cotton rat model of human metapneumovirus (hMPV) infection

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Background

Human metapneumovirus (hMPV) is an important cause of acute respiratory disease that can take on a particularly severe form in children, elderly, and immunocompromised individuals. hMPV infection has not been modeled before in immunocompromised animal models.

Method

In this work, we evaluated hMPV infection in cotton rats S.hispidus immunosuppressed via repeat cyclophosphamide administration. Normal and immunosuppressed cotton rats were infected with hMPV and sacrificed on days 5, 7, and 9 post-infection for analysis of hMPV load in the lungs and nose, pulmonary histopathology, and cytokine expression. Efficacy of prophylactic and therapeutic administration of anti-hMPV antibody MPV467 was evaluated in the model.

Result

Immunosuppressed animals had higher pulmonary and nasal titers of hMPV on day 5 post-infection. Large amounts of hMPV were still present in the respiratory tract of immunosuppressed animals on days 7 and 9 post-infection, while normal animals cleared infection by day 7. Immunosuppression was accompanied by reduced pulmonary histopathology in hMPV-infected cotton rats. MPV467 treatment administered to immunosuppressed animals 3 days post-infection was able to protect both upper and lower respiratory tracts against hMPV infection and reduced pulmonary expression of IP-10 and MIP-1α mRNA.

Conclusion

Immunosuppression is associated with increased hMPV replication in the cotton rat S.hispidus and delayed viral clearance from both lungs and nose. Effective antibody treatment can be administered as late as 3 days after hMPV infection. These results indicate that immunosuppressed cotton rats represent a useful model to study hMPV pathogenesis and to evaluate therapeutics that can alleviate hMPV-induced disease in immunocompromised subjects.

Thomas Mariani - ARNI0178

Assessment of pediatric lung respiratory syncytial virus infection at the single cell level

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Background

Respiratory syncytial virus (RSV) is the leading cause of severe respiratory disease in infants. We recently described novel airway transcriptional responses associated with clinical severity in RSV-infected infants. Here we leverage a novel, physiological in vitro model of bona fide infant and pediatric airway epithelial cells to characterize responses to viral infection at the single cell level.

Method

Pediatric human lung epithelial (PHLE) cells from organ donor infant lung tissues were grown and differentiated at Air-Liquid Interface (ALI). ALI-differentiated PHLE cultures were infected apically with RSV GFP-A2 strain at a multiplicity of infection of 1. After 48 hours of infection, cells were harvested for single-cell capture and library preparation on the 10X Chromium system. Sequencing was performed on a HiSeq4000 and reads were aligned to GRCh38. Gene expression data were analyzed to identify clusters, marker genes and gene expression patterns using Seurat. Functional annotation of marker genes and pathway analysis of gene sets was performed using Toppgene functional analysis software (ToppFun).

Result

We present a dataset including 8,627 cells from 2 control and 2 RSV infected samples. Lung epithelial cell sub-populations identified include secretory cells (~43%), cycling cells (~23%), stratified cells (~17%), basal (~13%), ciliated cells (~3%) and ioncytes (< 1%). Viral transcripts were expressed predominantly, but not exclusively, in cycling cells. We identified a number of genes (n=721 at FDR<0.05) and pathways that were significantly associated with viral infection. Importantly, multiple genes associated with severe clinical symptoms in vivo were significantly increased in multiple cell populations (CCL20, CXCL2, etc.). Activation of interferon signaling (IFIT1, RSAD2, etc.) was evident in all cell types. Conversely, reductions in extracellular matrix gene expression were noted only in cycling cells. We also noted the proportion of cells productively infected with RSV in vitro was lower in cells displaying a robust interferon signaling response. We subsequently found a significant inverse correlation between viral gene expression and measures of host cell interferon response in independent infections (n=10).

Conclusion

We have generated a single cell transcriptomic atlas for pediatric human airway epithelial cells infected with RSV. Our data provide novel insights into the heterogeneity of RSV infection, and identify potential mechanisms contributing to susceptibility.

Kevin Groen - ARNI0180

Human metapneumovirus lacking M2-2 protein expression accumulates defective interfering particles and hypermutated genomes that induce type-I interferon production upon infection.

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Background

Human metapneumovirus (HMPV) is a major cause of respiratory illness. Fundamental knowledge of viral evasion of innate immune responses may facilitate design of novel antiviral therapies. Roles for the HMPV G, SH, and M2-2 proteins as interferon antagonist have been reported but are conflicting possibly due to variation in experimental designs for deletion mutants. Deletion of genes including gene-start (GS) and gene-end (GE) signals could disrupt the transcriptional gradient leading to altered gene expression. We aimed to investigate how HMPV subverts the type-I interferon (IFN) response with deletion mutants designed in three ways.

Method

Viruses lacking G, SH, or M2-2 expression were generated by introduction of nonsense mutations, leaving the genome length intact, or by deletion of open reading frames (ORFs) with or without the GS and GE signals. These viruses were assessed for replication kinetics and induction of IFN production.

Result

No effect on virus replication was observed between mutant viruses generated with different knock-out designs. Upon inoculation of A549 cells, M2-2 deletion mutants induced significantly more IFN production, while G and SH deletion mutants induced similarly low IFN levels, as the wild type virus. No differences were observed in induction of IFN between viruses generated with the three different knock-out designs. Upon inoculation of A549 cells lacking RIG-I or MAVS expression, M2-2 mutant viruses did not induce IFN production, confirming the role of the RIG-I-MAVS signaling pathway in sensing of M2-2 mutant viruses. However, genome sequence analysis of M2-2 mutant viruses revealed hypermutation throughout the genome. The mutation patterns indicated a role for ADAR, which was confirmed by generating M2-2 mutant viruses in cells with ADAR-1 knockdown by CRISPR-interference. In addition, Northern blot analyses of M2 2 mutant viruses revealed the presence of defective interfering RNAs (DIs), potent inducers of the IFN response. The role of the M2-2 protein as IFN antagonist was further investigated using recombinant respiratory syncytial virus (RSV) in which the IFN antagonists NS1 and NS2 were replaced by the HMPV M2-2 gene (RSVΔNS1+2HMPV-M2-2), or by the NS1 gene of Influenza A virus (RSVΔNS1+2IAV-NS1) as control. Upon inoculation of HEp-2 cells, RSVΔNS1+2HMPV-M2-2 induced similar IFN levels as RSVΔNS1+2, while RSVΔNS1+2IAV-NS1 induced significantly lower levels of IFN than RSVΔNS1+2.

Conclusion

Our data indicate that neither the G and SH proteins, nor the M2-2 protein of HMPV are robust canonical IFN antagonists. The IFN inducing phenotype of M2-2 deletion mutants can be explained by the hypermutated genomes and presence of DIs in virus stocks.

Julia Rogers - ARNI0189

Streptococcus pneumoniae carriage density and associated respiratory virus coinfections in a congregate shelter population in King County, WA

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Background

Sheltered people experiencing homelessness have a high prevalence of chronic diseases and increased risk of respiratory syncytial virus (RSV), influenza and other viral respiratory infections that may contribute to Streptococcus pneumoniae disease. We investigated S. pneumoniae nasal carriage in a shelter population and assessed whether carriage density is correlated with viral detection and comorbidity.

Method

We analyzed data from a respiratory viral surveillance study in 23 homeless shelters in the Seattle metropolitan area between October 2019-May 2021. Research assistants recruited self-selecting shelter residents aged >3 months with acute respiratory illness 3-6 days/week; mid-turbinate nasal swabs were collected and tested by PCR for respiratory pathogens including RSV, influenza A and B and S. pneumoniae. After April 1, 2020, all residents and staff were eligible regardless of symptoms. Generalized estimating equations were used to test for differences in S. pneumoniae relative cycle threshold (Crt) values.

Result

Of 14,464 nasal specimens collected from 3,281 participants, S. pneumoniae was detected in 1,321 (9%) from 571 unique participants. 295 (22%) also had ≥1 viral pathogen detected (RSV=6; SARS-CoV-2=9; influenza A/B=11; adenovirus=26; seasonal coronavirus=27; enterovirus=31; and rhinovirus=202). Median participant age was 44 (range: 3 months - 74 years), 24 (range: 12-42), and 11 years (range: 5-27) for those with S. pneumoniae alone and RSV and influenza co-detected, respectively. All participants with RSV or influenza detected reported ≥1 symptom. Chronic conditions were present in 33% (n=334) and 23% (n=69) of participants with S. pneumoniae only versus viral codetection. Of participants with codetection compared to those without, 47% and 30% reported ≥1 symptom (p<0.001). Mean S. pneumoniae Crt values for participants with viral codetection were lower (20.6, SD: 4.8) compared to those without (22.4, SD: 3.8, p<0.001). S. pneumoniae Crt values for specimens with influenza A/B (p=0.005), enterovirus (p=0.003), adenovirus (p=0.01), and rhinovirus (p<0.001), detected were lower compared to those without these viruses detected, and higher among SARS-CoV-2 (p=0.01); no difference was found among RSV-positive specimens. Presence of chronic conditions did not affect the Crt value of S. pneumoniae in participants overall or stratified by viral codetection.

Conclusion

Overall, S. pneumoniae and viral codetection was significantly associated with higher pneumococcal density. Among those with RSV and S. pneumoniae codetection, we did not observe a difference in carriage density. Strategies to decrease burden of respiratory viral infections may reduce risk for pneumococcal carriage in a high-risk population.

Sex-Dependent Enhancement of Respiratory Syncytial Virus Infection by Toll-Like Receptor 7

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Background

Respiratory syncytial virus (RSV) is the leading cause of infant mortality worldwide, and the disease burden of RSV is greater for males than females. There are no vaccines or therapeutic options for RSV because the immune response and host cell interactions during infection are incompletely understood. RSV infection is detected by pattern recognition receptors, such as toll-like receptors (TLRs), to initiate an antiviral immune response. Viruses like RSV have evolved to co-opt TLR signalling to enhance viral replication. Moreover, RSV infection is detected by TLR7, which is encoded on the X-chromosome. Due to incomplete X-chromosome inactivation, females express higher levels of TLR7. Here, we describe how RSV uses TLR7 in bronchial epithelial cells (BECs) to drive optimal viral replication.

Method

Sucrose-purified RSV-A1-GFP was used to infect 1HAEo- and primary human BECs in the presence of TLR7 agonists or inhibitors. RSV infection was enumerated using an immuno-plaque assay or by RT-qPCR and the effects on host gene expression were determined by Nanostring nCounter assay. We observed the interaction of RSV replication complexes with TLR7 in BECs using confocal immunofluorescence microscopy. The expression of TLR7 in primary BECs was supported by western blot, RT-qPCR and by single cell RNA-SEQ of human lungs. RT-qPCR was used to measure TLR7, HPRT housekeeping input genes, and RSV viral load in residual nasopharyngeal (NP) swabs collected from clinical labs.

Result

We detected TLR7 in BECs that colocalized to RSV replication complexes during infection. Pharmacological activation of TLR7 enhanced RSV replication in BECs and Nanostring gene expression analysis identified new TLR7 response genes during RSV infection. Experimental blockade of TLR7 or inhibition of ERK-MAP kinase signalling abrogated TLR7-enhancement of RSV replication. Consistently, we observed higher TLR7 expression and higher RSV viral loads in female NP swabs compared to males. In male NP swabs, we found that TLR7 expression was closely correlated with RSV viral load.

Conclusion

TLR7 is expressed in BECs and regulates RSV replication. Inhibition of TLR7 activation and signalling reduced RSV replication in BECs. This research examines a novel sex-dependent pathological role of TLR7 during RSV infection and yields potential therapeutic targets to inhibit RSV replication.

Yan Yung Ng - ARNI0221

Comparing the cell-specific innate immune response to RSV between preterm and term infants

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Background

Respiratory syncytial virus (RSV) is the most common cause of childhood lower respiratory tract infection globally. A common risk factor for severe RSV disease includes premature birth, with a higher morbidity and mortality rate in preterm infants. As the adaptive immune response of preterm infants is still developing, the immaturity in innate immune response is postulated as an important factor for the increased risk of severe RSV disease. However, there is limited data on the susceptibility of specific innate immune cells to RSV infection.

Method

We stimulated cord blood mononuclear cells (CBMCs) from 25 moderate preterm and 25 term infants with RSVA for 24 hours. Innate immune cell-specific intracellular cytokine production, including IL-6, IL-1b, IL-8, IL-10, TNF-a, IFN-a, IFN-g was examined by high-dimensional flow cytometry.

Result

Our interim analysis revealed that RSV predominantly infects myeloid dendritic cells (mDCs) (50% of total mDCs) and monocytes (61% of total monocytes). RSV stimulation significantly increased the production of IL-6, IL-1b and TNF-a in both mDCs (8-fold, 5-fold and 5-fold increase respectively) and monocytes (5-fold, 2-fold and 7-fold increase respectively) compared to unstimulated CBMCs. A similar level of IL-8 production was observed between RSV stimulated and unstimulated CBMCs. In contrast, a reduced level of IL-10 (4-fold) was observed in monocytes after RSV stimulation. The analysis of the differences in cell-specific cytokine response to RSV between preterm and term infants is ongoing.

Conclusion

RSV can infect a major proportion of innate immune cells and influence their cytokine production. These differences may help to explain why some infants develop severe RSV disease and could be used to inform new strategies to protect high-risk groups.

Rachel Hartman - ARNI0232

Viral infections and co-infections among American Indian and Alaska Native children with acute respiratory illness, 2019-2022

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Background

Historically, American Indian/Alaska Native (AI/AN) children have experienced high rates of acute respiratory illness (ARI).

Method

We conducted surveillance for ARI in inpatient and outpatient AI/AN children age <5 years in the Southwest United States (Navajo Nation and White Mountain Apache Tribal lands) and Alaska (Yukon Kuskokwim Delta and Anchorage) over three years (November 2019-May 2022). Mid-turbinate nasal swabs were tested by PCR for RSV, SARS-CoV-2, influenza viruses A, B and C, human metapneumovirus (hMPV), rhinovirus, adenovirus, and parainfluenza viruses (PIV) 1-4. The proportion of children with respiratory viruses and with co-infections pre-pandemic (Nov 1, 2019-Mar 14, 2020) and during the COVID-19 pandemic (Mar 15, 2020-May 31, 2022) were compared using a chi-square test.

Result

ARI decreased with pandemic onset; 362 children with ARI were enrolled pre-pandemic and 212 during the pandemic. Most children had a single virus detected (Table 1). Pre-pandemic, RSV was the most commonly detected pathogen; during the pandemic, RSV, rhinovirus, and SARS-CoV-2 were most common. In the Southwest, the proportion of co-infections was similar pre-pandemic and during the pandemic, while in Alaska it was higher during the pandemic (Table 2). Co-infections pre-pandemic most commonly included RSV in both sites. Co-infections during the pandemic most commonly included rhinovirus.

Conclusion

In these settings, the predominant cause of ARI among AI/AN children changed during the COVID-19 pandemic, with a notable decline in RSV. However, RSV remained one of the most commonly detected pathogens in both single infections and co-infections.

Claire Chottin - ARNI0242

Immunomodulation strategy against Respiratory Syncytial Virus infection by using lung primo-colonizing bacteria

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Background

The respiratory syncytial virus (RSV) is the main etiological agent of bronchiolitis in infants. Few therapeutics are available to prevent or treat RSV infection. The neonatal lung environment is responsible for the high susceptibility of infants to RSV infection. Indeed, at birth the lung is a complex environment characterized by an evolving immune system continuously exposed to environmental stimuli including microbiota colonization. We assume that primo-colonizing bacteria are involved in the maturation of lung immunity and thus in the susceptibility of neonates to pulmonary infection. The neonatal period constitutes a window of opportunity to immunomodulate lung immune responses. The use of bacterial strains during this period could help directing the local immune response towards a protective immunity to RSV infection. The aim of our project is to demonstrate that primo-colonizing bacterial strains isolated from neonatal lungs could reduce the severity of RSV pathology.

Method

In a first part, we used neonatal mouse lung explant to i) characterize the immunostimulant property of the primo-colonizing bacterial strains by measuring cytokines secretion (Luminex assays) ii) to evaluate the capacity of these strains to interfere with the replication of a recombinant RSV expressing luciferase.

Finally, we confirmed the anti-RSV activity of these bacteria by infecting Air-Liquid Interface human cell culture (Mucilair, Epithelix) with a recombinant RSV expressing mCherry.

Result

Twenty-five primo-colonizing strains were characterized on neonatal mouse lung explants for their capacity to stimulate cytokine secretion and to interfere with RSV replication. We identified several non-cytotoxic bacterial strains that could stimulate lung explants for the secretion of type-I cytokine, such as IL-12 and IFNÉ¤. These strains were also able to reduce RSV viral replication in lung explants. The bacteria 17 was selected for its original cytokine signature (Type-I immunity and IL-9) in addition with its antiviral capacity on neonatal mouse lung explants. The antiviral activity of this particular bacterial strain was confirmed with Air-Liquid Interface epithelial human cell culture pre-exposed to the bacteria 17 then infected with the mCherry-RSV.

Conclusion

The bacteria 17 exhibit anti-RSV properties on ex vivo mouse lung explants and on human airway epithelial cells. Our next goal is to establish if this particular primo-colonizing bacterial strain is able to reduce RSV pulmonary disease. To achieve it, this bacterium will be administered as a preventive treatment in a relevant neonatal mouse model of RSV infection. Such beneficial bacteria with immunomodulatory properties could provide a new potential therapeutic strategy for bronchiolitis.

Respiratory Syncytial Virus prefusion F (RSVpreF) vaccination: antibody persistence and revaccination

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Background

RSVpreF has demonstrated high neutralizing titers 1 month after vaccination. Information on the persistence of antibody levels and potential revaccination intervals will be of high interest by recommending bodies and prescribers.

Method

In a phase 1/2 randomized, placebo-controlled, observer-blind, dose-finding, first-in-human study, participants 18-85 years old who had received an initial dose of 240 µg RSVpreF with or without aluminum hydroxide before the northern hemisphere RSV season (Sep 2018/19) were revaccinated 12 months later with the same dose level and formulation of RSVpreF alone or concomitantly with seasonal inactivated influenza vaccine and followed-up for 12 months. Immunogenicity, local reactions, systemic events, and adverse events were assessed. Sera were assayed for RSV subgroup A (RSV A) and RSV subgroup B (RSV B) neutralizing titers. Results were stratified by age (younger group, 18−49 years; older group, 65−85 years).

Result

Among 263 participants in the younger and older groups, one month after the first RSVpreF dose, RSV A neutralizing titer showed robust geometric mean fold-rises (GMFRs) that ranged from 9-18 across different dose levels and formulations across the age groups (Figure). RSV neutralizing titers slowly declined over time, but remained approximately 2-4 fold higher than pre-vaccination levels at 12 months post-vaccination. Revaccination at 12 months provided a modest increase in titers, with RSV A GMFRs ranging from 1−2 fold higher than pre-revaccination levels. Neutralizing titers were slightly lower 1 month after revaccination vs 1 month after the initial vaccination, but titers remained 5-8 fold higher compared with before the first vaccination. Neutralizing titers decreased at a slower rate after revaccination compared with the first RSVpreF dose. Similar trends in GMFRs and neutralizing titer kinetics were obtained for RSV B. The safety and tolerability profile was acceptable, most events being mild or moderate.

Conclusion

One month after the first vaccination, RSVpreF elicited high RSV neutralizing responses, which declined over time but remained higher than pre-vaccination levels through 12 months. Neutralizing titers increased again upon revaccination with RSVpreF, but increases were generally lower than the first 1-month postvaccination levels. Revaccination is safe and boosts neutralizing antibodies.

In vitro coinfection by respiratory syncytial virus and influenza A virus generates hybrid virions with altered structure and tropism

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Background

Interactions between respiratory viruses occur at multiple levels -from cells to populations- and impact virus transmission and clinical outcomes. Using qPCR diagnostic data from a large population in Scotland, we showed that respiratory syncytial virus (RSV) is frequently detected in virus coinfections. Here, we characterised interactions between RSV and other respiratory viruses at the cellular level, by performing coinfections with prototypical strains of RSV and influenza A virus (IAV) in a human lung-derived cell line and in air-liquid interface (ALI) cultures of bronchial cells.

Method

We characterised the structure of virions budding from A549 cells coinfected with RSV (A2 strain) and IAV (Puerto Rico/8/34) using super-resolution microscopy, live-cell imaging, scanning electron microscopy, and cryo-electron tomography (cryo-ET). To determine changes in virus antigenicity in virions harvested from coinfected cells, we performed virus neutralisation assays using anti-IAV and anti-RSV antibodies. To assess changes in virus tropism in viruses obtained from coinfections, we infected A549 cells depleted of sialic acids with supernatants and cell-associated fractions collected from coinfected cultures. To determine if RSV and IAV could infect the same cells within the respiratory tract, we coinfected ALI cultures of bronchial epithelium.

Result

We observed the presence of extracellular and membrane-associated filamentous structures consistent with hybrid viral particles (HVPs). Cryo-ET showed that HVPs harbor surface glycoproteins and genomes of IAV and RSV. Neutralisation assays showed that HVPs use the RSV fusion glycoprotein to evade anti-IAV antibodies. Furthermore, we showed that HVPs facilitate IAV infection and spread among cells lacking IAV receptors. Finally, we showed that IAV and RSV can coinfect the same cells in the bronchial epithelium.

Conclusion

We define a previously unknown interaction between RSV and IAV that might affect virus pathogenesis by expanding virus tropism and facilitating immune evasion. Our in vitro results show that the formation of HVPs is biologically feasible and the observation of coinfected cells within the bronchial epithelium suggests that they might also occur in the human respiratory tract.

PK, SNA, and Efficacy to Prevent RSV MALRI from a Phase 1b/2a Study of Monoclonal Antibody Clesrovimab (MK-1654) in Infants

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Background

MK-1654 is an investigational RSV-neutralizing monoclonal antibody targeting site IV of the RSV F protein currently in phase 3 development for the prevention of RSV medically-attended lower respiratory tract infection (MALRI) in infants.

Method

This phase 1b/2a double-blind, randomized, placebo-controlled study evaluated the safety, tolerability, PK, and serum neutralizing antibody (SNA) titers of MK-1654 in pre-term (born 29-35 weeks gestational age) and full-term infants. The study randomized participants (n=181) 2 weeks to 8 months of age in a 4:1 ratio within five separate panels (pre-term: 20, 50, 75 or 100-mg, full-term: 100mg) to receive a single intramuscular dose of MK-1654 or placebo. Blood samples were collected to quantify MK-1654 serum concentrations and SNA titers. A preliminary population PK (popPK) model was developed to describe PK of MK-1654 in infants. The efficacy of MK-1654 was predicted using clinical trial simulations which were based on the popPK model and a published model-based meta-analysis. An exploratory efficacy analysis of observed RSV MALRI through day 150 was conducted.

Result

Concentration data from 111 pre-term infants and 32 full-term infants through at least 150 days post-administration were available. The PK of MK-1654 were best characterized by a linear two-compartment popPK model with first-order absorption and elimination. Clearance and volume of distribution (Vd) scaled allometrically with time-varying body weight. The half-life of MK-1654 was approximately 42 days. A linear relationship was observed between increasing concentrations of MK-1654 and increasing SNA. Clinical trial simulations predict that a single dose of 100 mg of MK-1654 will provide >76% efficacy for the prevention of RSV MALRI for a duration of 5 months in infants. Exploratory analysis of the phase 1b/2a study data yielded an observed efficacy of 74.2% (95% CI: -92.9%, 96.5%) for all dose groups (20-100 mg) vs. placebo and 80.6% (95% CI: -141.2%, 99.6%) for the 100 mg dose group vs. placebo (Table).

Conclusion

Model-based efficacy predictions aligned with the observed MK-1654 efficacy to prevent RSV MALRI in the phase 1b/2a study. The data support the continued evaluation of MK-1654 in ongoing Phase 3 studies.

Nirsevimab for the prevention of RSV disease in healthy late-preterm and term infants: follow-up through second RSV season

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Background

Nirsevimab is a highly neutralizing monoclonal antibody against RSV pre-fusion F protein with extended half-life of approximately 70 days. It was shown to protect term and late preterm infants through their first respiratory syncytial virus (RSV) season against medically attended (MA) RSV lower respiratory tract infections (LRTI) in the Phase 3 MELODY trial (≥35 weeks gestational age, efficacy 74.5%; NCT03979313).

Antibody-dependent enhancement (ADE) could theoretically occur when non-neutralizing antibodies or antibodies in sub-neutralizing concentrations bind to viral antigens without blocking or clearing infection. Infants were followed through a second RSV season without redosing to evaluate the theoretical risk of ADE in the setting of low nirsevimab concentrations. We report the incidence and disease severity of MA RSV LRTI during the second RSV season.

Method

Infants were randomized 2:1 to receive one intramuscular injection of nirsevimab (infants <5 kg at dosing: 50 mg; ≥5 kg: 100 mg) or placebo prior to their first RSV season. Infants were followed for the detection of cases of MA RSV LRTI for a period of 510 days post-dose. Cases of MA RSV LRTI met predefined clinical criteria of disease severity and were confirmed by real-time reverse-transcriptase polymerase chain reaction. "All cause" referred to any medically attended LRTI or respiratory illness and includes cases of MA RSV LRTI.

Result

Overall, 1490 infants were randomized and included in the intent-to-treat population (994 nirsevimab and 496 placebo), including 1446 (964 nirsevimab and 482 placebo) who were followed through the second season. In the first season (2019-2020), the incidence of MA RSV LRTI was 1.2% and 5.0% in the nirsevimab and placebo recipients, respectively. In the second season (2020-2021) the incidence was lower than in the first season, occurring in 0.7% and 0.4% of recipients in the nirsevimab and placebo groups, respectively (Table). There were no cases of MA RSV LRTI requiring hospitalisation in the second season. The incidence of MA LRTI of any cause and hospitalisation for respiratory illness of any cause was balanced between treatment groups in the second season.

Conclusion

Among participants in the MELODY trial, incidence of MA RSV LRTI in the second RSV season was low across treatment groups and no case required hospitalisation. There was therefore no evidence to support ADE in nirsevimab recipients.

Ann R Falsey - ARNI0276

Efficacy of Ad26.RSV.preF/RSV preF Protein Vaccine Against RSV in a Phase 2b Study of Adults Aged ≥65 Years Over 3 Seasons

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Background: Respiratory syncytial virus (RSV) can cause serious lower respiratory tract disease (LRTD) among older adults. There is no licensed RSV vaccine. In CYPRESS (a randomized, double-blind, placebo-controlled, phase 2b proof-of concept trial; NCT03982199), an Ad26.RSV.preF/RSV preF protein vaccine demonstrated 80% efficacy for prevention of RSV LRTD and 70% efficacy for prevention of any RSV acute respiratory infection (ARI) in adults aged ≥65 years through the first RSV season in the primary analysis. We report the final vaccine efficacy (VE) analysis for Ad26.RSV.preF/RSV preF protein among CYPRESS participants through 3 RSV seasons.

Methods: Participants (N=5782) were randomized 1:1 to receive vaccine or placebo before the first RSV season. The primary endpoint was first occurrence of RT-PCR–confirmed RSV LRTD after the first RSV season. To assess VE durability for a single dose of Ad26.RSV.preF/RSV preF protein, the first occurrence of RSV LRTD or ARI was evaluated in the overall study population during the first, second, and third RSV seasons.

Results: Through season 2, 2124 participants in the vaccine group and 2126 participants in the placebo group remained enrolled in the study and were included in the per-protocol efficacy (PPE) set. Through season 3, 864 participants in the vaccine group and 881 participants in the placebo group were followed and included in the PPE set. In seasons 2 and 3, RSV LRTD was observed in 4 participants in the vaccine group and 17 participants in the placebo group (RSV ARI: 9 and 21 participants, respectively). In the study population overall, VE for prevention of RSV LRTD for seasons 2 and 3 combined was 76.1% (95% CI: 26.9%, 94.2%); across 3 seasons, VE for prevention of RSV LRTD was 78.7% (95% CI: 57.3%, 90.4%). VE for prevention of any RSV ARI was 56.6% (95% CI: 1.2%, 82.5%) in seasons 2 and 3 combined and 65.7% (43.5%, 79.9%) across 3 seasons.

Conclusions: Data from CYPRESS support that a single dose of the Ad26.RSV.preF/RSV preF protein vaccine is efficacious against RSV LRTD and any RSV ARI through at least 3 RSV seasons in adults aged ≥65 years.