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A rapid and flexible microneutralization assay for serological assessment of influenza viruses

Presenter: Kalee Rumfelt - ACOR0006

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Background

Serological responses from influenza vaccination or infection are often investigated using either the Hemagglutinin Inhibition (HAI) assay or conventional Microneutralization (MN) assay. However, these tests present issues surrounding feasibility, reproducibility, and sensitivity.

Method

Sera were provided by individuals enrolled in the Household Influenza Vaccine Evaluation (HIVE) study, a longitudinal cohort of households with children in Southeast Michigan. All serum samples were evaluated by HAI. The LMN assay was performed by mixing dilutions of patient serum with recombinant viruses containing HA/NA segments from influenza test strains and the other six influenza segments. A luciferase reporter was expressed off of the PA segment, which was measured as Relative Light Unit (RLU) output in infected cells. Neutralization titers were calculated based on input virus and the RLUs produced above the cell only background for each serum dilution. Back titering of the virus was performed to validate input virus concentration (100 infectious units). We related the LMN assay's neutralization activity against egg-adapted H3N2 targets to the HAI assay activity for the same targets and sera. Cell-adapted targets were used to compare the cell-adapted and the egg-adapted neutralization responses.

Result

333 sera that were collected between May 2016 and July 2018 were included in this study. The sampled individuals ranged from 7 to 68 years of age and 81.5-86.4% had received the influenza vaccine prior to each season. The HAI assay and the LMN assay titers were significantly correlated for two previously used egg-adapted influenza vaccine strains, A/H3N2/Hong Kong/4801/2014 (p=0.45) and A/H3N2/Singapore/INFIMH-16-0019/2016 (p=0.75). The LMN detected differences in neutralization titer for egg- and cell-adapted strains of A/H3N2/Hong Kong/4801/2014 (mean fold difference= -2.66) and A/H3N2/Singapore/INFIMH-16-0019/2016 (mean fold difference= -3.15). All samples had higher neutralization titers for the egg-adapted strains except two samples for A/H3N2/Hong Kong/4801/2014 and one sample for A/H3N2/Singapore/INFIMH-16-0019/2016.

Conclusion

The LMN assay was able to produce similar results to the gold-standard HAI assay, as well as detect differences in neutralization response for egg- and cell-adapted influenza strains. Together, these results demonstrate the utility of this assay for studies of immune correlates of protection and highlight key antigenic differences between egg-adapted vaccine strains and their circulating counterparts.

ImmunoSpot assays enable in-depth assessment of B cell reactivity against SARS-CoV-2 and seasonal influenza

Presenter: Greg Kirchenbaum - ACOR0018

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Background

Memory B cells constitute a critical component of humoral defense and are efficiently recruited into secondary immune responses upon antigen re-encounter. Expansion and subsequent differentiation of memory B cells into antibody-secreting cells (ASC) serves to rapidly increase antibody levels and limit dissemination of infectious agents. In contrast to the short half-life of secreted antibody and its eventual decline in the absence of continuous replenishment by ASC, memory B cells are long-lived and their precursory frequencies are stably maintained in the absence of antigen re-encounter. Consequently, measurement of antigen-specific memory B cell frequencies and characterization of their fine antigen specificity can provide unique insights into their recall potential and may additionally offer a more reliable and predictive correlate of protection.

Method

Presently there are few techniques that enable facile identification of antigen-specific B cells at single-cell resolution and with high-throughput capacity. Unlike flow cytometric techniques, which depend upon surface B cell receptor-mediated acquisition of fluorescently-conjugated probes, the ImmunoSpot approach permits identification of rare antigen-specific B cells based on the secretory foot-prints generated by individual ASC. Utilization of fluorescently-conjugated detection reagents in the ImmunoSpot assay also facilitates multiplexing and parallel assessment of antigen-specific B cell Ig class or IgG subclass usage. Moreover, inverted ImmunoSpot assays, in which soluble antigen is utilized as detection probe for revealing ASC-derived secretory foot-prints, enables assessment of functional affinity through titration of the antigen concentration. Additionally, incorporation of multiple antigen probes in the context of inverted ImmunoSpot assays also permits evaluation of ASC cross-reactivity at single-cell resolution.

Result

In addition to evidencing underlying B cell memory against SARS-CoV-2 and seasonal influenza in subjects exhibiting low levels of circulating antibody reactivity, ImmunoSpot assays are also economical in cell utilization and enabled parallel assessment of multiple antigens and Ig class usage using minimal precious cell material. Moreover, inverted FluoroSpot assays recapitulated both ASC reactivity and their relative precursor frequency, and facilitated assessment of functional affinity through titration of soluble antigen probes representing SARS-CoV-2 or influenza.

Conclusion

Our data illustrate the utility of ImmunoSpot assays for detailed assessment of memory B cell reactivity and the requirement for cellular immune monitoring efforts to reveal what serum antibody may not.

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Integrated Drivers of Basal Immunity and Acute Responses to Influenza Infection in Diverse Human Populations

Presenter: Aisha Souquette - ACOR0020

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Background

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

Method

Utilizing samples from healthy or influenza-infected subjects from 8 populations in 5 countries, we examined putative determinants of immune profiles, including: single nucleotide polymorphisms, ancestry informative markers, herpesvirus (HV) status, age, and sex. We modeled the effects of these factors on immune outcomes in vitro and in vivo. Immune measures included cytokine levels, leukocyte phenotypes, gene expression, and antibody titers. For influenza-infected subjects, illness outcomes include disease severity, viral load, and duration of virus shedding.

Result

In healthy subjects, we found significant differences across populations in basal cytokines, leukocyte phenotypes, and gene expression. The transcriptional immune response post in vitro challenge varied by cohort and was dependent on the baseline transcriptional profile (significant for 74% of gene expression models), ancestry (83.3%), HVs (66.7%), and biological variables, age and sex (31.1%). Analysis of 94 SNPs shows genetic differences by opposing major alleles (across diverse cohorts) in immune related genes, with potential for transcriptional effects, as 38 are expression quantitative trait loci. In subjects with acute influenza, we found two distinct disease severity cytokine profiles, largely driven by age. Analyzing acute cytokine levels with a regression model to account for age, sex, genetics, and HVs, we found that HVs have unique and interactive effects on cytokines that are high in magnitude, result in distinct signatures of immune modulation, and are specific to anatomical location during infection. Additionally, 8 cytokines had significant genetic influence during flu infection, each of which were identified as a correlate of severity in these studies.

Conclusion

These results, comprising 123,178,117 data points from 1,705 individuals, provide novel insight into the scope of basal and acute immune heterogeneity across diverse groups, the influence of the basal setpoint on acute immunity, the integrative effects of factors which drive

immune variation, and the consequences for illness outcome. Collectively, this underscores the need for future studies to search for correlates of protection or severity across diverse populations and to consider the factors which contribute to their variation in order to identify the number of severe immune profiles, the most broadly applicable treatment, and practical therapeutic options (i.e. those more affected by factors we can control or target).

5

Antigenic analysis of influenza hemagglutinin using a streamlined neutralization assay IRINA

Presenter: Larisa Gubareva - ACOR0032

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Background

Hemagglutinin (HA) is the major surface antigen of influenza viruses. Characterization of recent A(H3N2) viruses requires the use of neutralization methods (e.g., focus reduction assay (FRA)) and high content imaging-based neutralization test (HINT). While useful, these assays are lengthy and require specialized equipment. To facilitate antigenic analysis, we developed a streamlined version of HINT, where the time consuming indirect immuno-staining of nucleoprotein (NP) and cell imaging were replaced with measuring enzyme activity of neuraminidase (NA) molecules expressed on virus-infected cells. This assay was named IRINA (Influenza Replication Inhibition Neuraminidase-Based Assay).

Method

Influenza A(H3N2) viruses submitted from WHO GISRS laboratories were sequenced using NGS analysis and HA clades were determined using maximum likelihood phylogenetics. MDCK-SIAT1-grown viruses were analyzed using HINT (Jorquera et al. 2019) and IRINA (Patel et al. 2022). For IRINA, normalization of test viruses was done based on enzyme activity of cell-associated NA. In both assays, supernatants containing residual virus and antiserum were removed at 3-4 h following the addition of cell suspension to prevent interference from anti-NA antibodies. Ferret post-infection antisera were raised against the vaccine prototype virus A/Darwin/6/2021.

Result

Viruses from clade 2a.2 circulated widely in 2021-2022 and continued to circulate during the2022-2023 season. Many of them, including A/Darwin/6/2021, shared an HA amino acid substitution H156S at the dominant antigenic site B. Ferret antiserum raised against A/Darwin/6/2021 reacted well (<3-fold down) with all viruses carrying S156 (n=15), regardless of the assay used. However, its reactivity was somewhat reduced (HINT median fold reduction of 3.9; IRINA median fold reduction of 3.3) against viruses (n=6) with H156 that had also acquired S205F + A215T. Moreover, reactivity of this antiserum was reduced 4.3- and 4.8-fold as measured by HINT and IRINA, respectively, against broadly circulating viruses (n=22) with H156 that acquired E50K+ F79V+I140K (e.g., reference virus A/Florida/57/2022). Antiserum reactivity was ferret-specific as another antiserum tested reacted well (≤2 fold) with all viruses tested.

Conclusion

Results generated using IRINA were consistent with those of the HINT assay while its turnaround time was much shorter compared to HINT and other currently used neutralization methods. IRINA offers an attractive new tool to improve throughput for antigenic characterization and serological studies.

6

A rapid distinction of the potential zoonotic risk of avian influenza virus by a modified hemagglutination test

Presenter: Jie-Long He - ACOR0038

Jie-Long He[1]

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Background

With the great advantage of being simple, inexpensive, and result visualization, the hemagglutination test (HA test) has been widely used for quantifying the avian influenza virus (AIV).

Method

The principle of this rapid method is based on the sialic acid (SA) receptors on the red blood cell surface binding to the hemagglutinin glycoprotein of AIV thus causing hemagglutination. The threshold of hemagglutination is thought to be related to the affinity of different SA types on the blood cells to the HA of AIV.

Result

In this study, red blood cells from various poultry and animals were used to accurately compare the thresholds of the HA test for our standard H6 AIV strain (A/chicken/Taiwan/2838V/00), including chicken, domestic goose, common pheasant (Phasianus colchicus) and pig (Figure 1). In addition, the different type of SA or N-linked oligosaccharides on the hemagglutinin glycoprotein of chicken red blood cell was removed by enzymatic deglycosylation to clarify the mechanism of hemagglutination. It benefits the accurate quantification of the HA test application.

Conclusion

Here we describe a modified quantitative hemagglutination test platform for the rapid distinction of the potential zoonotic risk of the avian influenza virus. We will integrate the HA test with a modular microfluidic technology to apply real-time AIV surveillance in the field.

Antibody landscapes of H7N9 patients from wave 1 to wave 5 in China and its association with autoimmune markers

Presenter: Sook-San Wong - ACOR0046

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Background

H7N9 has resulted in at least 1,568 human cases between 2013 and 2018. Here we conducted a serological analysis of H7N9 patient serum to study the antigenic characteristic of the viruses from the five different waves, assess the age-specific antibody profiles, and their associated risks with autoimmune markers.

Method

42 serum samples from 21 H7N9 AIV-infected patients between 17 to 76 years old admitted between 2013 to 2018 were used in this study. The antigenic relationship of viruses in each wave was described based on hemagglutination-inhibition (HAI) assays. The longitudinal kinetics and age-specific antibody landscapes were described using ELISA against a panel of hemagglutinin (HA), neuraminidase (NA), nucleoprotein (NP), and matrix (M). Specific IgM, IgG, and IgA antibody responses and antibody avidity were measured to determine binding affinity. We also examined the association of broad antibody reactivity with autoimmune markers. Sera from individuals with severe H1N1 influenza virus infection (n=14) were used as comparators.

Result

The HAI results showed that H7N9 patients from wave 1 to 5 have a higher increase in HI titers against A/Anhui, virus from wave 1 but lower increases against A/Qingyuan and A/GD17SF003 viruses from wave 5. A significant correlation was observed between microneutralization titers and HAI and neuraminidase-inhibition (NAI) antibody titers against A/Anhui. Compared to infection with seasonal influenza viruses, H7N9 AIV infection induced significantly greater NAI-antibody titers compared to HAI-antibody titers with broader cross-group IgG, but not IgA, reactivity. This was especially evident in those below 40 years of age, who developed greater H7-specific IgM, IgG, and IgA antibody titers. In addition, those aged>60 showed lower antibody avidity to H3, and H7 than younger groups. Three months after infection, HAI and IgG titers remained stable, while NAI titers waned in H7N9 AIV cases with the highest post-infection NAI antibody titers. Markers of autoimmunity such as GM1, GQ1b, Myeloperoxidase, Beta 2-Glycoprotein, PCNA, and Jo-1 correlated with the broad antibody reactivity associated with infection with subtype H7N9 AIV infection but not seasonal influenza virus infection.

Conclusion

Post-infection human sera confirmed the antigenic changes observed with ferret anti-sera for the Wave 5 viruses, and this only affected HAI-antibodies. The breadth and avidity of antibody response after H7N9 infection differs according to age. The induction of specific autoimmune antibodies was associated with the broadly reactive antibodies in H7N9 infections. Clinical implications of this should be further investigated.

8

Quantifying the breadth of cross-reactive antibody responses to influenza A(H3N2)

Presenter: Bingyi Yang - ACOR0055

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Background

Influenza viruses were responsible for around 100,000 deaths annually, while the effectiveness of vaccine effectiveness is interfered by complex interactions between previous exposure history and the circulating strains. Such cross-reactions are often hard to quantify due to the unknown exposure history and different strains encountered by birth cohorts. A recent cohort studies found a long-term periodicity in hemagglutination inhibiting (HAI) antibodies to A(H3N2) strains spanning between 1968 and 2014 in individuals, suggesting HAI antibodies gained from previous exposures may only interfere with strains that were isolated within a time intervals. As such, the breadth of HAI titers induced from different A(H3N2) strains may be quantified and predicted using time intervals between strains were isolated, which could aid disentangling and modelling the cross-reactions from immune history in immune responses to influenza.

Method

Anti-serum data from ferret against A(H3N2) strains circulating from different calendar years were extracted from the literature. We extract the following information, including the infected and tested strains, the HAI titers of an anti-sera against the infected and tested strains. We first calculated the mean and standard error of fold changes between HAI titers to infected and tested strains, stratifying by time intervals of isolation. We then fitted the generalized additive model (GAM) to estimate the non-linear relationship between the mean fold changes in titers and time intervals of isolation. Models were fitted to each individual data set and predictions were validated both within and across data sets.

Result

We found that for strains that were isolated 4.7 (4 to 5.6) years before and 5.6 (5.0 to 6.2) years after infected strains were isolated, the fold of reductions in HAI titers would be within 16-fold (i.e., 4 log-unit). The patterns were held across infected strains that were isolated in different years and across different data sets. Correlation of predictions are highly associated within (Pearson's correlation: 0.95 to 0.96) and across (Pearson's correlation: 0.63 to 0.85) data sets.

Conclusion

Our results suggested that HAI titers generated from a single exposure could interfere the strains isolated within 6 years. Measuring HAI response to circulating strains should consider the immune history to at least previous 6 years. Limited cross-reactions in HAI titers were detected beyond 6 years since the infected strains were isolated.

9

An integrated serological platform for immunogenicity readouts and the elucidation of potential correlates of protection for influenza haemagglutinin- and neuraminidase-based vaccines

Presenter: Nigel Temperton - ACOR0060

Nigel Temperton[1]

[1]University of Kent

Background

With the rapid expansion of new universal influenza vaccine technologies, there is an acute need to match this with a holistic evaluation of immunogenicity against multiple viral targets (HA head, HA stalk and NA), subtypes and strains, and to define new correlates of protection.

Method

A best-in-class influenza hemagglutinin (HA) pseudotype library encompassing Influenza A subtypes HA1-18, Influenza B (Vic/Yam), Influenza C and Influenza D has been developed using lentiviral vector platform technologies and co-expressed proteases.

These viruses have been evaluated in influenza pseudotype microneutralization (pMN) assays with post-vaccine sera and HA1/HA2-directed mAbs. HA2-specific responses are delineated using HA pseudotypes bearing immunologically unreactive heads (H11) with a mismatched stalk. In parallel lentiviral vectors pseudotyped with an avian H11 HA and the NA of all influenza A (N1-N9) subtypes and influenza B (Vic/Yam) were produced and used in a pseudotype-based lectin assay, pELLA. An open-source R Shiny web application and R library (AutoPlate) was developed to accelerate data analysis of dose-response curve immunoassays, pMN, pMN-HA2 and NA making use of established international standards.

Result

The pMN and pELLA are highly sensitive and specific for detecting virus-specific neutralizing antibodies against influenza virus HA (HA1 and/or HA2) and NA and can be used to safely assess antibody functionality in vitro, and in high-throughput systems. We demonstrate their utility in detecting serum responses to infection and vaccination with the ability to evaluate cross-subtype neutralizing responses elicited by specific vaccinating antigens. Additionally, we demonstrate their sensitivity for the study of broadly neutralizing mAbs including CR9114 and CD6. Using the assembled HA and NA response data sets we show that AutoPlate improves on available analysis software in terms of ease of use, flexibility, speed and standardisation.

Conclusion

Our findings will inform further preclinical studies involving (universal, pan-subtype) immunization dosing regimens in animal models and may help in the creation and selection of better antigens for vaccine design. It will additionally be applicable to the study of potential correlates of immunity for current and next generation influenza vaccines. This library is the most comprehensive available globally and can be harnessed to meet strategic objectives that contribute to expansion of seasonal and zoonotic influenza prevention and control policies via vaccination and therapeutic mAbs and strengthening pandemic preparedness and response.

10

Towards an Improved Wild-type Sequence Based Hemagglutination Inhibition Assay for the Evaluation of Influenza Vaccines: Challenges and New Developments.

Presenter: Vivek Shinde - ACOR0066

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Background

The utility of the hemagglutination-inhibition (HI) assay as a tool to assess vaccine immunogenicity has recently been threatened. Due to evolutionary changes in receptor specificity, current A(H3N2) strains are largely incapable of agglutinating avian red blood cells (RBCs). Additionally, the assay may fail as a clinically-relevant immunologic surrogate of protection against wild-type circulating influenza viruses, due to historical reliance on egg-adapted virus reagents which increasingly demonstrate altered antigenicity relative to their wild-type counterparts. Egg-derived reagents introduce egg-adaptation bias, artificially showing favorable responses to egg-derived vaccines, with unintended consequence of hampering development of improved next-generation recombinant vaccines. Consequently, we developed a wild-type sequence-based HI assay (WT-HI) to coincide with the development of a recombinant hemagglutinin vaccine; and evaluated assay performance against reference antisera, and sera from a recent vaccine trial.

Method

The WT-HI assay employs virus-like-particles (VLPs)-expressing wild-type sequence HAs-as the agglutinating agent, and human type-O RBCs as the indicator particle. We tested CDC reference ferret antisera using cell- and egg-derived historical seasonal influenza viruses (no viruses were known to have undergone egg-adaptive mutations which are now common) versus wild-type VLP equivalents as agglutinins. We then tested sera from a Phase-I trial comparing a recombinant HA-nanoparticle vaccine versus a high-dose egg-derived influenza vaccine using the WT-HI assay.

Result

When tested with reference ferret antisera, the WT-HI assay using wild-type VLP reagents yielded titers which closely matched those seen with classical egg- or cell-grown virus reagents, with no homologous virus titer differences greater than 2-fold. Against a panel of five contemporary A(H3N2) drift variants, the WT-HI assay detected significant increases in post-vaccination antibody responses against wild-type HA sequences that were inapparent with egg-derived reagents.

Conclusion

The WT-HI assay rehabilitated the ability of the assay to interrogate clinically-relevant HI responses against contemporary A(H3N2) strains, and revealed potential advantages of the recombinant vaccine.

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Assessment of Neuraminidase Antibody Responses to an Octavalent Influenza mRNA vaccine using a Multiplex Neuraminidase SeroAssay

Presenter: IRINA V USTYUGOVA - ACOR0083

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Background

Seasonal influenza remains a serious public health concern. Sanofi is currently developing new influenza vaccine formulations which aim to increase vaccine effectiveness. Novel approaches include an addition of neuraminidase (NA), the second most abundant influenza surface glycoprotein, to influenza vaccine formulations currently containing mostly hemagglutinin (HA). The final product is envisioned as an octavalent formulation of mRNAs, encapsulated in a lipid nanoparticle (LNP), and delivered as 4 HA + 4 NA antigens of influenza subtypes H1, H3, and B (2 subtypes).

As the immune response to NA has not yet been fully characterized, we initiated an assessment of anti-NA breadth using two techniques: 1) an enzyme-linked lectin assay (ELLA) measuring functional NA inhibiting (NAI) antibody titers, and 2) a custom VaxArray kit for NA SeroAssay, developed by InDevR, measuring total binding antibodies against 16 unique NAs of N1, N2 and NB subtypes, spanning years 2009-2017.

Method

To assess NA-specific antibody responses, serum samples from ferrets immunized twice with an octavalent, 4 NA + 4 HA, mRNA-LNPs at 1µg/Ag or 15µg/Ag doses were evaluated. Functional homologous and heterologous NAI titers were measured by ELLA, and total binding NA-specific IgG antibodies were evaluated by a multiplex NA SeroAssay.

Result

An octavalent mRNA-LNP formulation induced detectable homologous NAI titers against N1, N2 and the 2 B subtypes. A dose-dependent statistically significant increase in NAI titers at 15µg/Ag dose was observed for both B strains in comparison to 1µg/Ag dose. An analysis of N2 heterologous responses demonstrated a decrease in NAI titers with increasing number of amino acid differences from the homologous A/Singapore/INFIMH-16-0019/2016 vaccine strain. In line with functional antibody titers, an evaluation of total binding antibody titers by NA SeroAssay also confirmed higher homologous titers over the heterologous titers for both N1 and N2 panels. Binding antibodies induced by N2 A/Singapore/INFIMH-16-0019/2016 were able to recognize N2s which were up to 14 amino acids distant from the homologous strain, while 63 amino acid difference resulted in a complete abrogation of recognition.

Conclusion

Data obtained with ELLA and NA SeroAssay allowed for the evaluation of anti-NA immune response and pointed to the presence of cross-reactivity between the strains within the subtype which was dependent of amino acid distance. Similar trends between functional NAI titers and total binding antibody titers measured by SeroAssay suggest that it could be used in upcoming preclinical and clinical studies to select the best vaccine formulation and also to determine the optimal vaccine valency and dose.

Funding by Sanofi.

12

Transcriptomic profiling of vaccination versus Post-Influenza infection

Presenter: Stephany Sanchez-Ovando - ACOR0090

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Background

Vaccination with inactivated influenza vaccine is effective. However, breakthrough infections escape the immune response elicited by vaccination. Mechanisms induced by influenza vaccine and infection are largely mediated by B cells and antibodies against the hemagglutinin protein yet differences remain largely unknown. In depth, analysis of transcriptomic profiles and BCR will provide insights into differences between vaccination and infection and will inform new vaccine development.

Method

This study investigated single cell transcriptome profiling, and BCR analysis on HA-reactive B cells on cohort participants from the Ha Nam Cohort (Viet Nam), who received inactivated influenza vaccine, but nevertheless were infected with A(H3N2) virus in the subsequent season. PBMCs collected pre and post vaccination and infection were assessed and B memory cells were sorted as CD19+, CD27+IgD+ or CD27- IgD- cells, naïve B cells were excluded. Genes expressed by HA-reactive B cells in day 7 and d21 post-vaccination and post-infection samples were compared to their pre-vaccination sample.

Result

Differentially expressed genes and pathways between day 7 post-vaccination and day 7 post-infection revealed mechanisms associated with neutrophil aggregation, toll-like receptor signaling and MHC class II receptor activity. Pre-vaccination compared with day 7 post-infection presented upregulation of genes associated with activation of NF-kappa complex and PD-1 signaling. We are currently performing an analysis aiming to identify differences in the VDJ sequence of BCRs following vaccination and post-infection samples (day 7 and day 21).

Conclusion

Investigations are underway to understand how the transcriptomic profiles and BCR sequences differ between post-vaccination and post-infection time points.

13

Dissecting the longevity and cross-reactive antibody responses after quadrivalent inactivated influenza vaccine immunization in children

Presenter: Sarah Larteley Lartey Jalloh - ACOR0093

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Background

Influenza is a major public health concern especially among vunerable individuals such as young children under 5 years old. Young children experience high rates of influenza infection during seasonal epidemics, with an estimated attack rate of 20-30%. As children are the main transmitters of influenza, vaccinating them against seasonal influenza has the potential to reduce the burden of disease in both vaccinated and unvaccinated individuals. Furthermore, the influenza virus continuously undergoes antigenic drift and thus ecaping the host's acquired immunity to influenza. Annual seasonal vaccination is recommended for individuals at high-risk of complications from influenza, these includes infants >6 months, young children under 5 years old and the elderly. While significant progress in understanding the influenza virus has led to the development of multiple vaccines over the past 50 years, there has been limited progress in improving the breadth and length of the protection conferred by influenza vaccines. We conducted a phase IV non-randomized influenza vaccine trial to evaluate the longevity and cross-reactive antibody responses after influenza vaccine vaccination in children.

Method

This phase IV vacine trial was conducted during the 2021/2022 influenza season, where fifty healthy children (aged 3-11 years old) were enrolled and vaccinated wiith the quadrivalent IIV (QIV). Clotted blood and saliva samples were collected prior to vaccination at day 0 (D0) and five consecutive timepoints at 3-7, 30, 58, 180 and 360 days after vaccination from the study participants. All children >9 years old (n = 41) received one vaccine dose, whereas children ≤9 years (n = 5) received a second dose at 4 weeks interval. We used the hemagglutination inhibition (HI) and microneutralization assays to assess hemagglutinin-specific neutralizing and functional antibody responses against the vaccine strains, past and future circulating influenza strains. Enzyme-linked immunosorbent assay (ELISA) was used to quantify influenza-specific binding antibody response in serum and salivary IgA responses

Result

The preliminary data show that the QIV rapidly elicited significant increases in HI and MN antibody titres against all the vaccines strains tested as early as 3-7 days post-vaccination. The antibody titres were maintained above the protective threshold at ï€ +40 (HI) and +80 (MN) up to one year after vaccination. Analysis of the cross-reactive responses, binding and salivary IgA antibody responses is ongoing.

Conclusion

Our preliminay result shows the vaccine is capable of rapidly induing HA and MN-specific antibody responses even in young children and will help better understanding the immunogenicity of influenza vaccination in this age group.

14

Study of oligonucleotide polymorphisms of the influenza virus using high-resolution melting (HRM) analyzing of PCR products.

Presenter: Galina Landgraf - ACOR0075

***Galina Landgraf*[2], *Yulia Desheva*[1]**

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Background

The high resolution melting (HRM) analysis generates melting curves profiles that are specific and sensitive enough for distinguishing DNA with slight differences in sequence that makes it possible to scan the mutations

Method

The cold-adapted (ca) reassortant influenza virus Ð/17/herring gull/Sarma/2006/887 (H6N1) was obtained by the genetic reassortment

Result

Analysis of the gene segments showed that the reassortant Ð/17/herring gull/Sarma/2006/887 (H6N1)

Conclusion

PCR combined with the high-resolution melting curves analysis (HRM-analysis)

Attachment: Introduction.

The high resolution melting (HRM) analysis generates melting curves profiles that are specific and sensitive enough for distinguishing DNA with slight differences in sequence that makes it possible to scan the mutations, analyze methylation, and perform genotyping. HRM-analysis can be used for the characterization of the samples based on their CG-composition and complementarity of the DNA sequences.

Materials/Methods.

The cold-adapted (*ca*) reassortant influenza virus A/17/herring gull/Sarma/2006/887 (H6N1) was obtained by the genetic reassortment of the cold-adapted A/Leningrad/134/17/57 (H2N2) master strain in chicken embryos. The genome composition of the obtained reassortant was analyzed by means of real-time PCR with the high resolution melting (HRM) analysis using the intercalating fluorescent dye EvaGreen.

Results.

Analysis of the gene segments showed that the reassortant A/17/herring gull/Sarma/2006/887 (H6N1) contained the internal proteins coding genes (PB2, PB1, PA, NP, M, and NS) of the master donor virus and the surface antigens coding genes of the A/herring gull/Sarma/51c/2006 (H6N1) avian influenza virus. Specific restriction analysis method and partial sequencing confirmed the genome composition of the reassortant Len17/H6, which was determined by HRM-analysis. The restriction enzymes cut the DNA fragments genes of the vaccine candidate that indicates the presence of specific nucleotides typical for the master donor strain. These findings confirm the results obtained with HRM-

analysis and prove that the melting of the PCR products does not destroy the DNA duplex structure, which enables the analysis of the amplified fragments by other methods.

Conclusions.

PCR combined with the high-resolution melting curves analysis (HRM-analysis) saves time compared to the visualization of results by electrophoresis in the course of PCR analysis and lowers the risk of contamination with amplicons. Furthermore, this method is relatively cheap because it does not require the use of expensive fluorescent oligonucleotide probes.

15

B CELL MEMORY RESPONSES TO FOUR DIFFERENT COVID-19 VACCINE PLATFORMS

Presenter: Camila Coelho - ACOR0004

***Camila Coelho*[1], *Zeli Zhang*[2], *Alessandro Sette*[2], *Daniela Weiskopf*[2], *Shane Crotty*[2]**

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Background

A better understanding of immune memory to COVID-19 vaccines may allow for the identification of multiple immunological correlates of protection.

Method

We examined vaccine-specific human memory B cells and durability of antibody responses after Moderna mRNA-1273, Pfizer/BioNTech BNT162b2, Janssen Ad26.COV2.S or Novavax NVX-CoV2373 immunization, assessed longitudinally for 6 months post-immunization.

Result

RBD- and spike-specific memory B cell frequencies increased over time for all four different platforms, although a significant increase was only determined for mRNA-1273 and BNT162b2. RBD-specific memory B cell immunoglobulin isotypes were comparable among the different vaccines and timepoints. Levels of activated memory B cells (CD21-CD27+) were higher in response to mRNA-1273 and BNT162b2 compared to Ad26.COV2.S or NVX-CoV2373 vaccines at 3 and 6 months postvaccination. Notably, CXCR3+ spike memory B cell levels were substantially higher in response to Ad26.COV2.S compared to mRNA vaccines and were positively correlated with Ad26.COV2.S neutralization titers.

Conclusion

COVID-19 vaccine characterization might be useful to establish vaccine correlates of protection.

16

Rapid detection of SARS-CoV-2 neutralizing immunity

Presenter: Kei Miyakawa - ACOR0007

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Background

An indicator of vaccine efficacy is the quantification of its ability to induce neutralizing antibodies (nAbs). Currently, the only reliable method available for this purpose is the quantitative plaque reduction neutralization test (PRNT) using live viruses. Recently, to address the need of the hour in the COVID-19 pandemic, several modifications of the PRNT and other novel methods have been developed to demonstrate nAbs to SARS-CoV-2. However, the practical application of these highly specific tests has drawbacks such as low throughput, long turnaround time, and the need for specialized laboratory settings with biosafety level 3 facilities to handle the live viruses used in these tests.

Method

To overcome these hurdles, we developed a HiBiT-based virus-like particle neutralization test (hiVNT) system, which detects SARS-CoV-2 nAb in serum in within 3 hours. It uses lentivirus-based virus-like particles incorporated with the NanoLuc fragment peptide HiBiT. Upon the virus entry into reporter cells expressing LgBiT intracellularly, the viral HiBiT fuses with the LgBiT to reconstitute whole NanoLuc luciferase, which is readily detected by a luminometer. The hiVNT assay is high-throughput and can be easily carried out in a low-biosafety setting. The assay can measure the amount of nAb qualitatively or quantitatively. Qualitative hiVNT can be used to rapidly determine the percentage of nAbs retention in a large number of samples. Quantitative hiVNT assay correlates well with the PRNT using live SARS-CoV-2.

Result

Qualitative hiVNT was used to evaluate the efficacy of the BNT162b2 mRNA vaccine on a panel of SARS-CoV-2 variants. The results showed that almost all the participants acquired neutralizing antibodies after two vaccinations against VOCs other than the Omicron strain, whereas at least three vaccinations were required to acquire neutralizing antibodies against the Omicron strain.

Conclusion

Qualitative hiVNT would be useful for large-scale community-wide testing to detect protective immunity.

17

OVERVIEW OF HUMORAL IMMUNE RESPONSE TO SARS-CoV-2 VARIANTS IN PATIENTS AND VACCINEES FOLLOWING HOMOLOGOUS AND HETEROLOGOUS VACCINATIONS

Presenter: Claudia Maria Trombetta - ACOR0016

Claudia Maria Trombetta[5], Serena Marchi[5], Margherita Leonardi[6], Alessandro Manenti[7], Giulia Piccini[7], Francesca Dapporto[7], Linda Benincasa[6], Angela Stufano[4], Eleonora Lorusso[4], Emilio Bombardieri[3], Antonella Ruello[2], Simonetta Viviani[5], Nicola Buonvino[1], Emanuele Montomoli[5], Nicola Decaro[4], Piero Lovreglio[4]

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Background

Since the first isolation of SARS-CoV-2 in 2020, several variants have been detected worldwide, some of which designated as "variants of concern" (VOCs).

Vaccination has proved to be the most valuable intervention in the fight against

SARS-CoV-2. This study aimed to assess the humoral immune response to the original wild-type (WT) SARS-CoV-2 virus and some VOCs in patients and vaccinees following homologous and heterologous vaccinations.

Method

Serum samples were grouped into 8 different cohorts: 37 hospitalized COVID-19 patients; 50 subjects vaccinated with 2 doses of homologous mRNA vaccine and negative for nucleocapsid (N); 23 subjects with 2 doses of homologous mRNA vaccine and positive for N; 44 subjects who received 3 doses of homologous mRNA vaccine; 35 subjects who completed a 2-dose course of adenovirus-based vaccination followed by a booster dose with an mRNA vaccine (heterologous vaccination); 342 subjects who received 2 doses of mRNA vaccine; 48 and 27 subjects collected a mean of 6 months after homologous and heterologous vaccinations, respectively.

All samples were tested in duplicate by ELISA for the detection of antibodies against N and by the virus neutralization (VN) assay to the WT SARS-CoV-2 and some VOCs.

The research protocol was approved by the Ethics Committee of the University Hospital of Bari (n. 6955, prot. N. 0067544-02082021).

Result

COVID-19 patients show the most drastic reduction in Omicron-specific antibody response in comparison with the response to the WT virus. Antibodies elicited by a triple homologous/heterologous vaccination regimen or following natural SARS-CoV-2 infection combined with a two-dose vaccine course, result in highest neutralization capacity against the Omicron variant BA.1.

The majority of subjects who received 2 doses of mRNA vaccine retained their neutralizing activity toward the Alpha and Gamma variants, respectively. By contrast, the most striking reduction in comparison with the WT virus was found in the antibody response toward the Beta and Omicron variants. In addition, subjects who had undergone primary vaccination and had previously been naturally infected had higher neutralizing antibody titers toward the 4 variants than negative subjects.

Six months post-vaccination, almost all subjects showed detectable neutralizing antibodies against the WT virus and Omicron sublineages BA.2 and BA.5 , albeit to a lower extent.

Conclusion

These findings confirm that booster dose is the correct strategy to enhance the antibody cross-protection against Omicron variant. By contrast, two doses induce neutralizing antibodies mainly toward the WT virus.

18

Intranasal vaccine with ODN2006 as an adjuvant induces cross-protective secretory IgA antibodies against SARS-CoV-2 variants, reducing the potential risk of lung eosinophilic immunopathology.

Presenter: Takuya Hemmi - ACOR0022

Takuya Hemmi[3], Akira Ainai[3], Takao Hashiguchi[6], Sora Ishikawa[3], Minoru Tobiume[2], Takayuki Kanno[2], Naoko Iwata[2], Shun Iida[2], Sho Miyamoto[2], Akira Ueno[4], Kaori Sano[7], Koji Tamura[1], Ryosuke Suzuki[5], Hideki Hasegawa[7], Tadaki Suzuki[2]

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Background

An intranasal vaccine that can induce cross-protective secretory IgA antibodies would play an important role in controlling COVID-19. However, it has been concerned that the COVID-19 vaccine carries a risk of Vaccine-Associated Enhanced Respiratory Disease (VAERD), a lung eosinophilic immunopathology in post-vaccination infection. This phenomenon has been shown to be induced by the induction of a Th2-dominant immune response by vaccination. Therefore, this potential risk should be well addressed in the development of future vaccines. This study assessed the cross-protective capacity against SARS-CoV-2 variants and the risk of VAERD in intranasal COVID-19 vaccine.

Method

Mice were vaccinated with recombinant S protein three times at 2-week intervals via intranasal or subcutaneous route. ODN2006 and alum were used as mucosal adjuvant and classical Th2 adjuvant, respectively. S-specific antibody responses were evaluated in serum, nasal, and lung washes collected from the mice one week after the last vaccination. In addition, S-specific IgG1 and IgG2a subclasses were quantified as indicators of Th2 and Th1 responses, respectively. Mouse-adapted SARS-CoV-2, QHmusX, was intranasally administered to vaccinated mice, and viral load in nasal/lung samples at 3 days post-infection (dpi) and body weight changes for 10 days were evaluated. Eosinophilic infiltration of the lungs was assessed by flow cytometry at 6 dpi. Finally, to clarify cross-protective effect of ancestral vaccine against variants, viral loads were evaluated in nasal and lung washes collected from vaccinated mice challenged with the SARS-CoV-2 variants (α, β, γ, BA.1 or BA.5).

Result

Intranasal vaccine combined with ODN2006 induced not only serum IgG antibodies but also nasal and lung IgA antibodies. In addition, mice immunized with this vaccine survived without significant body weight loss for 10 days after the virus challenge. S-specific IgG1/IgG2a ratios in mice intranasally vaccinated with ODN2006 were significantly lower than those subcutaneously vaccinated with Alum. The IgG1/IgG2a ratio correlated with the

degree of lung eosinophil infiltration, and the infiltration of eosinophils into the lung was rare in mice vaccinated with ODN2006. The virus loads of variants were reduced in the nasal cavity, where IgA antibodies are mainly induced, suggesting that nasal IgA antibodies induced by intranasal vaccination are superior to serum IgG antibodies in cross-protective capacity.

Conclusion

Our results suggest that intranasal vaccines, inducing well-balanced immune reactions between Th1 and Th2 responses, would be highly effective against SARS-CoV-2 variants reducing the risk of VAERD.

19

Protection Against COVID-19 Outpatient Illness by Level of SARS-CoV-2 Receptor Binding Domain Binding Antibody at the Time of Illness, U.S. Flu VE Network

Presenter: Kelsey Sumner - ACOR0037

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Background

Neutralizing antibodies likely contribute to protection against SARS-CoV-2 infection, but precise correlates of protection are not yet established. Using data collected by the U.S. Influenza Vaccine Effectiveness (Flu VE) Network, we estimated pre-existing protection against outpatient illness by concentration of SARS-CoV-2 index spike protein receptor binding domain (RBD) binding antibody at the time of acute illness.

Method

From October 2021-June 2022, individuals ≥18 years seeking care for an acute respiratory illness (ARI) that included fever, cough, and loss of taste or smell were enrolled within 10 days of illness onset at sites in seven states. Participants provided respiratory and dried blood spot specimens. Respiratory specimens were tested for acute SARS-CoV-2 infection using real time reverse-transcription polymerase chain reaction. Blood spot specimens were tested for immunoglobulin G antibodies against the SARS-CoV-2 RBD antigen using a multiplex bead assay measuring binding antibody units (BAUs) as determined by the World Health Organization SARS-CoV-2 serological standard. To assess the association of outpatient illness with concentration of RBD BAUs, we used a test-negative case-control design of SARS-CoV-2-positive case-patients and test-negative controls with ARI. Odds of outpatient illness across RBD BAU concentration was estimated using a logistic regression model adjusted for COVID-19 vaccination status (unvaccinated, two, or three doses), age, sex, race/ethnicity, study site, illness onset week, self-reported presence of ≥1 chronic medical condition, and high-risk SARS-CoV-2 exposure (healthcare worker or contact of lab-confirmed COVID-19 case). The percent odds reduction by RBD BAU/mL was calculated as (1-adjusted odds ratio)x100, using the odds ratio produced by regression.

Result

A total of 2,253 individuals were included in analyses, and 742 (32.9%) tested positive for acute SARS-CoV-2 infection. Accounting for a 1:300 dilution factor, a mean of 1,922.9 (standard deviation [SD]: 1,703.7) RBD BAU/mL were present in case-patients and 2,234.4 (SD: 1,843.3) RBD BAU/mL in test-negative controls at the time of ARI. Adjusted percent odds reduction of SARS-CoV-2 infection were 7.1% (95% CI: 4.2-10.0%) per 500-unit increase in RBD BAU/mL. A detection of 4,675 BAU/mL in the multiplex bead assay was associated with 50.0% (95% CI: 33.3-62.5%) protection against outpatient illness.

Conclusion

Increased concentration of SARS-CoV-2 index strain RBD binding antibody was associated with reduced likelihood of outpatient COVID-19 illness. Future research could investigate antibody responses against Omicron lineages to estimate strain-specific binding antibody correlates.

20

Immune responses to COVID-19 vaccines among healthcare workers in Hong Kong

Presenter: Benjamin Cowling - ACOR0042

***Benjamin Cowling*[1], *Wey Wen Lim*[1], *Samuel Cheng*[1], *Loretta Mak*[1], *Malik Peiris*[1]**

[1]The University of Hong Kong

Background

COVID-19 vaccines against the earlier strains of SARS-CoV-2 are now available. However, breakthrough infections can still occur due to waning antibodies and immune escape by new variants. Neutralizing antibody levels has been proposed to be a correlate of protection for COVID-19 vaccines. We assessed humoral immune responses to the mRNA (BNT162b2) and inactivated (CoronaVac) vaccines in our healthcare worker cohort (HCW). We then compared the antibody levels generated by vaccination to binding and neutralising antibody levels generated by natural infection observed in individuals infected with SARS-CoV-2 during the first wave of infections in Hong Kong before vaccines were available for COVID-19.

Method

We recruited HCWs from public and private healthcare institutions across Hong Kong and collected blood samples at enrolment and every 6 months from June 2020 to June 2022. A subset of volunteers provided blood samples between 10 - 42 days after each dose of vaccine. Immune responses to vaccination were measured as SARS-CoV-2 binding antibodies detected by an enzyme-linked immunosorbent assay (ELISA) and SARS-CoV-2 neutralising antibodies by surrogate virus neutralization test (sVNT) and plaque reduction neutralization test (PRNT). Binding and neutralizing antibodies from individuals who had been infected with the SARS-CoV-2 virus during the first wave of infections in Hong Kong was extracted from data published in a separate study.

Result

Among the 1,736 HCWs enrolled in our cohort, 252 HCWs provided pre- and post-vaccination blood samples after each dose of either vaccine. Two doses of BNT162b2 generated levels of neutralizing antibodies (sVNT inhibition = 96.8%, range = 42.8%, 98.2%) comparable to those generated by natural infections in the first wave (sVNT inhibition = 84.0%, range = 32.9%, 93.8%). Similar levels were achieved with three doses of CoronaVac (sVNT inhibition = 95.3%, range = 64.7%, 98.3%) and heterologous vaccination with two doses of CoronaVac followed by a booster dose of BNT162b2 vaccine (sVNT inhibition = 97.0%, range = 85.8%, 97.7%). These antibody levels waned faster after the second dose of COVID-19 vaccination and slower after the administration of the third dose for both vaccines.

Conclusion

The BNT162b2 mRNA vaccine and CoronaVac inactivated vaccine can generate robust antibody responses comparable to natural infections. Three doses of the CoronaVac vaccine, or a heterologous boost with the BNT162b2 vaccine following two doses of the CoronaVac vaccine are required to achieve similar levels of neutralising antibodies in vaccinees who received two doses of the BNT162b2 vaccine.

21

SARS-CoV-2 antibody responses are better for mRNA versus adenoviral vector (AdV) vaccines: results from a cohort of Australian healthcare workers

Presenter: Sheena Sullivan - ACOR0047

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Background

mRNA and adenoviral vector (AdV) vaccines against SARS-CoV-2 have high efficacy in clinical trials, but there has been limited direct comparison of immune responses induced. mRNA (BNT162b2, Pfizer Comirnaty®) and AdV (ChAdOx1 nCov-19, Oxford-Astrazeneca Vaxzevria®) vaccines were provided free by the Australian government commencing March 2021. This study compares antibody and B cell responses among SARS-CoV-2-naïve Australian Health Care Workers (HCWs) who received 2 doses of either AdV or mRNA vaccine.

Method

We collected pre and post-vaccination sera and PBMC from a cohort of health care workers who received a primary course of either BNT162b2 or ChAdOx1. Sera were assessed using in-house surrogate virus neutralization (sVNT) assays against ancestral and Omicron S1 and S2 ECD and RBD. PBMCs were collected from a subset of vaccinees to assess B cell responses.

Result

We recruited 486 HCW of whom 456 had blood collected within 10-48 d of dose 2. 84% were female. 183 received ChAdOx1 and 273 received BNT162b2. Median ages were 42 and 39 years, and median interval between vaccine doses was 84 for ChAdOx1 vaccinees and 21 days for BNT162b2. sVNT titres against ancestral strain RBD declined with age, and increased with interval between dose 1 and 2 for BNT162b2 recipients. Median post-vaccination sVNT antibody titres were 4.2 times lower among ChAdOx1 compared to BNT162b2 vaccine recipients (age-adjusted $p<0.001$). Median spike binding antibody titres were 2.2 fold lower for ChAdOx1 compared to BNT162b2 vaccinees ($p=0.0005$). Assessment of end-of-year sera is currently underway to determine whether these differences persist.

Eight ChAdOx1 and 34 BNT162b2 vaccinees provided blood for PBMC collection 7 and/or 14 days post vaccination. Less than 0.1% of memory B cells recognized fluorescent-labelled Spike or RBD on day 0 compared to up to 3.7% after vaccination. Median percentages of Spike+ B cells were 2.9 times higher for participants who received BNT162b2 compared to ChAdOx1 vaccine on day 7, and median percentage RBD+ was 8.3 times higher. Additionally, the proportion of Spike+ B cells that targeted RBD, and the percentage of memory B cells that were RBD+ plasmablasts were substantially higher among BNT162b2 vaccinees on day 7.

Conclusion

The results demonstrate that the mRNA vaccine induces greater B cell expansion and differentiation into plasmablasts, with more of the response directed towards RBD so that sVNT titres are higher than with ChAdOx1 vaccine. It will be important to determine whether this is due to anti-vector immunity, the conformation of Spike or other factors.

22

Antibody and B cell cross-reactivity and kinetics following asymptomatic versus symptomatic SARS-CoV-2 infection

Presenter: Sheena Sullvan - ACOR0049

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Background

Primary SARS-CoV-2 infections are often asymptomatic. This study examines whether symptom status is associated with the trajectory and isotype of antibodies and B cells against SARS-CoV-2 and human coronaviruses (H-CoVs).

Method

Samples were from 19 asymptomatic and 26 symptomatic SARS-CoV-2-infected adults, followed up to 5 times from 14-31 days post-symptom onset until 12-months. These were compared with pre-infection samples from 20 naïve health care workers. Sera were assessed by IgG, IgA, and IgM ELISA against the spike and nucleocapsid proteins of SARS-CoV-2, and human coronavirus (h-CoV) viruses, 229E, NL63, HKU1, and OC43. PBMCs were assessed by FACS and ELISPOT to characterize CoV-protein reactive B cells and immune-cell subsets.

Result

SARS-CoV-2-reactive antibody and B cell levels were significantly higher among asymptomatic SARS-CoV-2 cases at enrolment, and peaked earlier than for symptomatic cases. H-CoV-reactive antibody levels were similar among asymptomatic and symptomatic cases, but high compared to uninfected controls, although B cells were predominantly SARS-CoV-2 specific. Activated DC, NK, CD8, and T follicular-helper (TfH) cell frequencies were high among infected cases compared to uninfected controls but only TfH were associated with symptom status.

Conclusion

Both asymptomatic and symptomatic infection induce robust B cell and antibody responses against SARS-CoV-2 proteins, coincident with NK, cDC and TfH cell activation. Asymptomatic SARS-CoV-2 infection was associated with relatively rapid production of SARS-CoV-2 specific antibody and not with recall of H-CoV reactive B cells. However, there appears to be some delay in IgG production among those who developed symptoms.

23

Estimating the health-related quality of life benefit of prophylactic treatment for COVID-19 in immunocompromised people

Presenter: Michael Watt - ACOR0056

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Background

Despite the availability of COVID-19 vaccines, immunocompromised (IC) patients remain at increased risk of serious illness from COVID-19 and therefore many continue to either shield or make lifestyle modifications to avoid COVID-19. This non-interventional study aimed to estimate the potential health-related quality of life (HRQL) benefit of pre-exposure prophylactic treatment (PrEP) against COVID-19 in IC patients. Interim results are reported herein.

Method

The HRQL impact of PrEP for COVID-19 in IC patients was estimated using three methods: 1) interviews with IC patients to assess their HRQL using the EQ-5D-5L and to estimate their HRQL based on a vignette describing treatment; 2) time-trade off (TTO) interviews with UK general population valuing three health states (IC patient who is either shielding, not shielding but making lifestyle modifications, or has received PrEP for COVID-19 ['protected']); 3) general population EQ-5D-5L valuation of the same three vignettes. The vignettes were developed from a literature review and interviews with clinicians (N=4) and IC patients (N=10). Eligible patients had a diagnosis of an IC condition defined as high risk according to NHS guidelines and were interested in receiving PrEP for COVID-19. The EQ-5D-5L data was mapped to EQ-5D-3L using a mapping function reflecting UK preference weights.

Result

In total, 48 IC patients and 83 members of the general population completed the vignette valuation. The general population group reflected UK demographics (mean age: 43 years,

51% female). The patient group had a mean age of 46 years and had a wide range of underlying IC conditions, 41 (85%) were making lifestyle modifications and 3 (6%) were shielding. The patient group had a mean EQ-5D-5L score = 0.574, the estimated utility gain from PrEP was 0.082 (mean value for 'protected' health state= 0.656); most (75%, 30/40) of patients asked said that PrEP described would lead to behaviour change. The general population TTO and EQ-5D-5L values ranged from 0.616 (EQ-5D-5L 'shielding' health state) to 0.940 (EQ-5D-5L 'protected' health state). The TTO and EQ-5D-5L valuation were well aligned. From the general population sample the utility gain from shielding to protected was 0.316 (TTO)/0.324 (EQ-5D-5L) and from lifestyle modifications to protected was 0.108 (TTO)/0.156 (EQ-5D-5L).

Conclusion

This study demonstrated a potential HRQL gain of up to 0.324 for IC patients who can stop modifying their behaviour to avoid COVID-19. This is the first study to show the potential value of PrEP for COVID-19 on HRQL of IC individuals who are currently exhibiting lifestyle modifying or shielding behaviour, and show anticipated positive behavioural changes following PrEP.

24

Protective effectiveness of prior SARS-CoV-2 infection and hybrid immunity against Omicron infection and severe disease: a systematic review and meta-regression

Presenter: Harriet Ware - ACOR0063

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Background

We aimed to systematically review the magnitude and duration of the protective effectiveness of prior SARS-CoV-2 infection and hybrid immunity (i.e., vaccination and infection) against Omicron infection and severe disease.

Method

We searched for controlled studies in MEDLINE, Embase, Web of Science, ClinicalTrials.gov, Cochrane Central Register of Controlled Trials, WHO COVID-19 database, and Europe PMC, using keywords related to SARS-CoV-2, reinfection, protective effectiveness, previous infection, antibodies, and hybrid immunity from January 1, 2020, to June 1, 2022. The risk of bias (RoB) was assessed using the Risk of Bias In Non-Randomized Studies of Interventions Tool. We used random-effects log-odds meta-regression to estimate the magnitude of protection at 1-month intervals.

Result

Eleven studies of prior infection and 15 studies of hybrid immunity were included. Prior infection effectiveness against hospitalization or severe disease was 74.6% [63.1-83.5%] at 12 months. Prior infection effectiveness against reinfection waned to 24.7% [16.4-35.5%] at 12 months (Figure 1). Against hospitalization or severe disease, hybrid immunity effectiveness with primary series vaccination was 97.4% [91.4-99.2%] at 12 months and with first booster vaccination was 95.3% [81.9-98.9%] at 6 months after the most recent infection or vaccination. Against reinfection, hybrid immunity effectiveness involving primary series vaccination waned to 41.8% [31.5-52.8%] at 12 months, while hybrid immunity effectiveness involving first booster vaccination waned to 46.5% [36.0-57.3%] at 6 months (Figure 1, 2).

Conclusion

All protection estimates waned within months against infection but remained high and sustained for hospitalization or severe disease. Individuals with hybrid immunity had the highest magnitude and durability of protection, and as a result, may be able to extend the period before booster vaccinations are needed compared to individuals that have never been infected. Further follow-up of the protective effectiveness of hybrid immunity against hospitalization or severe disease, the outcomes that drive most COVID-19 policy decisions, is needed to clarify how much waning of protection might occur over a longer duration, especially if new variants of concern emerge.

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Serologic correlates of protection against infection of COVID-19 vaccines: preliminary findings from a randomised trial of homologous and heterologous inactivated and mRNA third-dose vaccination (the Cobovax study)

Presenter: Nancy Hiu Lan Leung - ACOR0068

Nancy Hiu Lan Leung[1]

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Background

We conducted a randomized trial of homologous and heterologous inactivated and mRNA third-dose COVID-19 vaccination in adults (the Cobovax study). We showed that during the period which the Omicron BA.2 subvariant was circulating, the risk of infection was similar across all study arms despite significant differences in the neutralizing antibody responses.

Method

Participants who had previously received two doses of either CoronaVac (C) and BNT162b2 (B) vaccine were randomised to receive homologous or heterologous third-dose vaccination, i.e. four study arms CC-C, CC-B, BB-C and BB-B. We tested the day 0 and 30 sera with ELISA against the Spike and Nucleocapsid proteins, and surrogate neutralisation test and plaque reduction neutralisation test against Omicron BA.2. We compared the post-vaccination antibody response between infected and non-infected participants in each study arm.

Result

We identified 58 (15%) infections from 378 participants within 4-6 months of receipt of third dose vaccination. Significant difference in neutralizing antibodies against BA.2 between infected and non-infected participants were only observed in the BB-C but not other study arms.

Conclusion

We are expanding laboratory testing to additional potential serologic correlates of protection against different SARS-CoV-2 viral proteins and continuing active surveillance of SARS-CoV-2 infection within one year after receiving third-dose vaccination.

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Immune-focused SARS-CoV-2 nanoparticles interrogate antibody specificity and protection

Presenter: Kylie Konrath - ACOR0073

Kylie Konrath[1]

[1]University of Pennsylvania

Background

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) vaccines may target epitopes that reduce durability or propensity for escape from variants. Glycans can be employed to alter antibody specificity induced by infection and vaccination. To comprehensively identify novel sites of glycosylation on the SARS-CoV-2 receptor binding domain (RBD), we created a computational modeling pipeline called "Cloaking with Glycans" and screened single glycan variants in vitro for expression and folding. Glycan combinations were designed to focus antibody responses to three neutralizing epitopes-the ACE2 binding site (antibody classes I and II) or one of the two RBD core epitopes (antibody classes III and IV).

Method

Binding studies with monoclonal antibodies with mapped epitopes demonstrated that the glycans reduce off-target antibody binding in vitro. The immune focused RBDs were then engineered onto self-assembling nanoparticles and assessed in vivo. Competition assays and electron microscopy suggest the antibody specificity was shifted by the glycan additions compared to a full spike immunogen.

Result

The breadth and potency of polyclonal antibodies induced by the different epitope focused vaccines were assessed by binding, neutralization, and through viral challenge. The ACE2 binding site focused RBD nanoparticle vaccine provides full protection against lethal WA-1 and delta variant challenge with a single dose in K18.hACE2 mice, but lose potency and breadth against Omicron lineages. In contrast, the Site III and Site IV focused RBD

nanoparticle vaccines provided cross-reactive titers to WA-1 and Omicron variants BA.1, BA.2, BA.4, and BA.5 but Site III focused vaccines provided more potent neutralization and protection against non-lethal challenge of BA.2 challenge in K18.hACE2 mice. We are assessing correlates of protection from each of the different variants and epitope specific titers.

Conclusion

Correlates of protection based on antibody specificity is in critical need to understand pan-coronavirus protection. We have interrogated the relationships between antibodies specific to different epitopes and their potential for protection.

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Antibody correlates of protection against symptomatic SARS-CoV-2 infection for Alpha, Delta, Gamma and Zeta variants: analysis from two randomized controlled trials in the UK and Brazil

Presenter: Elaine Shuo Feng - ACOR0078

Elaine Shuo Feng[8], Seth Seegobin[1], Kathryn Shoemaker[2], Elizabeth Kelly[7], Ian Hirsch[1], Tonya L. Villafana[6], Sagida Bibi[3], Teresa Lambe[4], Sir Andrew Pollard[5], Merryn Voysey[5]

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Background

Correlates of protection against SARS-CoV-2 infections has been investigated by a few phase-3 clinical trials before the emergence of Delta and Omicron variant. However, it is insufficiently understood that if established correlates sustained across SARS-CoV-2 variants.

Method

Data from two randomized efficacy trials of ChAdOx1 nCoV-19 (ChAdOx1, AZD1222) vaccine in the UK (COV002, NCT04400838) and Brazil (COV003, NCT04536051) were analyzed to determine if antibody responses predict protection against SARS-CoV-2 infection for Alpha and Delta variants in the UK, and Gamma and Zeta variants in Brazil.

We assessed the antibody responses associated with SARS-CoV-2 infection risk for four variants using a case-cohort design among participants who received two doses of ChAdOx1 and had antibody data available at day 28 post the second dose. Immune markers included anti-spike IgG and pseudovirus neutralising antibody. All samples from infected participants (cases) were tested while a subset of samples from non-infected participants (non-cases) were pseudo-randomly selected for testing. A proportion of infected samples were sequenced to determine variants.

To account for potential selection bias due to sample selection procedures, we adjusted for the probability of having samples selected for testing based on baseline participant and trial characteristics, and incorporated the inverse predicted probability weights when modelling correlates of risk. We predicted absolute risk of infection using weighted generalised

linear/additive models adjusting for baseline risk of exposure to infection. We will further assess the correlates using survival analysis incorporating the time since vaccination.

Result

From August 2020 to August 2021, there were 67, 41, 64 and 64 symptomatic SARS-CoV-2 cases characterized as Alpha, Delta, Gamma and Zeta variants respectively. Anti-spike IgG was significantly associated with protection against infection with Alpha variants ($p < 0.05$), whereas pseudo-neutralizing antibody titers were significantly associated with protection against Gamma and Zeta variants (all $p < 0.05$). No significant relationship was found between anti-spike IgG or pseudo-neutralizing antibody and Delta variant. There were no observable differences in the relationships between protection and antibody values across the four variants. We will further investigate if time since vaccination would affect the correlates.

Conclusion

Understanding the correlates of antibody with protection against variants of SARS-COV-2 contribute to optimize future vaccine strategies.

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Assessment of the correlation between SARS-CoV-2 serum neutralization titers and post-vaccination infections among healthcare workers from the Prospective Assessment of Seroconversion (PASS) Study

Presenter: Carol Weiss - ACOR0080

Carol Weiss[13], Wei Wang[14], Emilie Goguet[3], Richard Wang[14], Cara Olsen[6], Si'Ana Coggins[5], Tonia Conner[3], Russell Vassell[14], Monique Hollis-Perry[1], Gregory Wang[2], Yolanda Alcorta[2], David Saunders[12], Roshila Mohammed[9], Hannah Haines-Hull[4], Matthew Moser[4], Julian Davies[8], Orlando Ortega[7], Edward Parmelee[7], David Tribble[7], Timothy Burgess[11], Christopher Broder[3], Simon Pollett[10], Eric Laing[3], Edward Mitre[3]

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Background

Identification of COVID-19 vaccine correlates of protection is important for ongoing SARS-CoV-2 countermeasure development and implementation. We explored the correlation between neutralizing antibodies titers against SARS-CoV-2 and post-vaccination infections in a prospective cohort of healthcare workers.

Method

Serum samples from 176 generally healthy adult volunteers from the Prospective Assessment of SARS-CoV-2 Seroconversion (PASS) study were collected between October and December 2021 and assessed for neutralization activity against SARS-CoV-2 D614G, Delta (617.2), and Omicron BA.1 and BA.1.1 variants using a lentiviral pseudovirus neutralization assay. Thirty-two participants had post-vaccination infections during the observation period from October 1, 2021 through April 1, 2022.

Result

Of the 15 infections that were genotyped, 12 were from the BA.1 lineages and one each were from BA.2, BA.2.12, and AY.25 lineages. Post-vaccination, pre-infection geometric mean titers (GMTs) in samples from those who had post-vaccination infections and those who did not were as follows: (1) 2674 and 3883, respectively against D614G pseudoviruses ($p = 0.0055$); (2) 972 and 1450, respectively against Delta pseudoviruses ($p = 0.0034$); (3) 286 and 494, respectively against BA.1 pseudoviruses ($p = 0.0061$); and (4) 303 and 552, respectively against BA.1.1 pseudoviruses ($p=0.0013$).

Conclusion

This preliminary analysis suggests a possible correlation between neutralization titers and infection risk; analytical studies are underway to further evaluate this relationship.

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Cellular and Humoral Immune Responses and Breakthrough Infections After Two Doses of BNT162b Vaccine in Healthcare Workers (HW) 180 Days After the Second Vaccine Dose

Presenter: Paolo Cantaloni - ACOR0081

Paolo Cantaloni[1]

[1]Vismederi

Background

Immunity and clinical protection induced by mRNA vaccines against SARS-CoV-2 have been shown to decline overtime. To gather information on the immunity profile deemed sufficient in protecting against hospitalization, we tested IgG levels, interferon-gamma (IFN-) secretion, and neutralizing antibodies 180 days (d180) after the second shot of BNT162b vaccine, in HW.

Method

A total of 392 subjects were enrolled. All received BioNTech/Pfizer from February 2020 to April 2021. The vaccine-specific humoral response was quantitatively determined by testing for IgG anti-S1 domain of SARS-CoV-spike protein. Live virus

microneutralization (MN) was evaluated by an assay performing incubation of serial 2-fold dilution of human serum samples, starting from 1:10 to 1:5120, with an equal volume of Wuhan strain and Delta VOC viral solution and assessing the presence/absence of a cytopathic effect. SARS-CoV-2-spike protein-specific T-cell response was determined by a commercial IFN-release assay.

Result

In 352 individuals, at d180, IgG levels decreased substantially but no results below the assay's positivity threshold were observed. Overall, 22 naive (8.1%) had values above the highest threshold. Among COVID-naive, the impact of age, which was observed at earlier stages, disappeared at d180, while it remained significant for 81 who had experienced a previous infection. Following the predictive model of protection by Khoury, we transformed the neutralizing titers in IU/ml and used a 54 IU/ml threshold to identify subjects with 50% protective immunity. Overall, live virus MN showed almost all subjects with previous exposure to SARS-CoV-2 neutralized the virus as compared to 33% of naive double-dosed subjects ($p < 0.0001$). All previously exposed subjects had strong IFN- secretion (>200 mIU/ml); among 271 naive, 7 (2.58%) and 17 (6.27%) subjects did not show borderline or strong secretion, respectively.

Conclusion

In naive subjects, low IgG titers are relatively long-lasting. Only a third of naive subjects maintain neutralizing responses. After specific stimulation, a very limited number of naive were unable to produce IFN- . The results attained in the small group of subjects with breakthrough infection suggest that simultaneous neutralizing antibody titers <20, binding antibody levels/ml <200, and IFN- < 1,000 mIU/ml in subjects older than 58 may identify at-risk groups.

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Lack of correlation between pre-Omicron wave antibody titers and Omicron infection measured by pseudovirus neutralization activity against the original vaccine strain

Presenter: Emily Martin - ACOR0100

Emily Martin[4], Matthew Smith[1], Emily Bendell[2], Adam Lauring[2], Jefferson Santos[3], Scott Hensley[3], Arnold Monto[3]

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Background

Whether antibodies elicited from vaccination and/or prior provide sufficient protection against subsequent SARS-CoV-2 variants is a critical question for vaccine design, strain selection, and debates about the deployment of vaccines with updated strains. Upon variant emergence, initial reports of antibody evasion are primarily based on neutralization studies in absence of disease outcomes. Our objective is to measure antibodies against SARS-CoV-2

and subsequent infection outcomes in a longitudinal household cohort in a community with high vaccination uptake.

Method

The HIVE study is a longitudinal household cohort study that includes active surveillance for acute respiratory illness with laboratory confirmation of etiology and regular blood draws including pre- and 28-day-post-vaccination draws. The study conducts weekly follow-up for illnesses meeting a standard case definition, which include mild to moderate symptoms. For this analysis, we evaluated antibody levels against the SARS-CoV-2 original, monovalent vaccination strain by pseudovirus neutralization for all individuals with blood drawn within 90 days prior to the emergence of the Omicron wave. Median titer for individuals with a subsequent Omicron infection were compared by Wilcoxon rank sum to those with known household exposure but no documented infection.

Result

799 individuals from 216 households were enrolled during the emergence of the Omicron wave. 242 study participants were infected with SARS-CoV-2 between January 1 and October 30. 23 of 85 participants with pre-Omicron serum had a documented subsequent infection. Overall vaccine uptake in this subgroup was 76%. Median antibody titer was similar between infected and uninfected groups (1810 vs 1810, p=0.86) and to uninfected participants with household exposure (n=16; 773, p=0.38)

Conclusion

The lack of correlation between high antibodies to the vaccine strain and subsequent infection with Omicron in our analysis corresponds to data supporting vaccine updates. Testing against Omicron strains by pseudovirus neutralization and by binding assays is underway and will be combined with these results to develop a time-dependent model of antibody breadth and infection outcome in this highly-vaccinated population.