Influenza (flu) Vaccine White Paper

In this White Paper we explore influenza virus epidemiology and the challenges of developing an effective flu vaccine for both seasonal and pandemic flu. We review a number of current approaches to vaccine development and explore the scientific rationale and business case for a promising new approach using synthetic T cell stimulating vaccines.

The Flu Vaccine Challenge

The factors that allow interspecies transmission (zoonosis) of influenza, but limit human-to-human transmission, are unknown (Zaraket et al). Influenza infection in its natural host (e.g. chickens) is generally asymptomatic and generates high genetic diversity – quasispecies (Vinuzzi et al.). The bottleneck governing infectious disease transmission describes the size of the pathogen population transferred from the donor to the recipient host (Leonard et al.). Narrow bottlenecks reduce the amount of transferred viral genetic diversity and thus may decrease the rate of viral adaptation. A tight genetic bottleneck during avian-to-human transmission has been shown to be a limiting factor for avian H7N9 influenza virus adaptation to mammals (Zaraket et al.). Genetic bottlenecks are therefore critical to the emergence of a new pandemic and viral factors that affect the bottleneck size in the viral host and recipient host population are novel vaccine targets to prevent a new pandemic (Moncla et al.).

Viral Shedding and Seasonal Flu Vaccines

Aerosol viral shedding amongst cases of college aged volunteers that had confirmed influenza infection demonstrated that those volunteers that had received both the current and previous years' flu vaccines (2012-2013) had 6.3 times more aerosol shedding compared with individuals having no vaccination in the two seasons (Yan et al.). It was proposed that certain types of prior immunity may promote lung inflammation, air closure and aerosol generation. Seasonal flu vaccines induce influenza A virus - specific B lymphocytes to produce antibodies that protect against infection. Paradoxically the viral antigen-specific B cell receptors generated after seasonal flu vaccination sensitizes B cells to future infection by influenza virus (Dougan et al.). During a subsequent influenza infection, lung resident B cells are infected by the virus binding to B memory cells expressing the influenza specific antibody B cell receptors – viral B cell infection causes both disruption of antibody secretion and B cell death thus delaying the onset of protective antibody in the lungs (Dougan et al.). This viral strategy allows an efficient means of ensuring a window for replication and horizontal transmission; a larger viral bottleneck is generated as observed in the seasonal vaccinated cohort that shed 6-fold more virus than the non-vaccinated group (Yan et al.).

Seasonal Flu Vaccination is generating an at-risk population for a pandemic

It has also been demonstrated that, whilst annual influenza vaccination is cost-effective against seasonal flu, in children it severely hampers the development of virus-specific CD8+ T cell responses by natural infections and thus may affect the induction of heterosubtypic protection (Bodewes et al.). This could render young children who have not previously been infected with an influenza virus more susceptible to infection with pandemic influenza of a novel subtype. Specifically, protection against

strain-matched seasonal infection might lead to increased vulnerability to antigenically different influenza strains. Characterization of the human CD8+ T cell response following infection with 2009 pandemic influenza H1N1 virus demonstrated that, upon infection with influenza A virus, individuals who most likely have experienced one or more influenza infections in the past responded with a rapid recall T cell response (Hillaire et al.). Whilst T cell recall cannot prevent infection it can prevent disease with peak T cell response within 1 week of infection and subsequent rapid clearance of infection (Hillaire et al.).

Generation of herd immunity is a natural consequence of virus circulating in the environment

The majority of virus-specific CD8 T cells are directed against conserved virus internal proteins and the ligandome represents Class I peptide antigens that are shared by different subtypes of influenza A virus (Hillaire et al.). Induction of these cross-reactive T cells could be a mechanism for the development of vaccines that could induce broadly protective immunity. The magnitude, kinetics and nature of T cell responses that are observed after natural infections can afford heterosubtypic immunity which would be advantageous in the face of the continuous pandemic threat caused by influenza A viruses of novel subtypes. However, since populations contain individuals who have never been infected, are post-infectious or post-vaccinated, great caution is needed regarding the indiscriminate introduction of a vaccine that could have a selective and deleterious effect on a distinct population, especially with regards to "original antigenic sin", as occurred upon the recent introduction of Sanofi's Denvaxia in South East Asia (Midgley et al.).

Live attenuated influenza vaccine (LAIV) cannot reduce the pandemic flu risk

There is an age dependent increase in the frequency of virus-specific CD8 T cells in unvaccinated children reflecting the increase in the number of subjects who experienced an infection with an influenza virus early in life. This age-dependent increase in virus-specific CD8 T cell response is absent in children vaccinated annually (Bodewes et al.). Since non-viable seasonal flu vaccines do not give rise to T cell responses it has been proposed that live attenuated influenza vaccines be developed that could generate a similar broad protective immune response similar to that which occurs in natural infection (Bodewes et al.). Like other endemic infectious diseases (e.g. Dengue, tuberculosis, etc.), childhood, adolescent and adult populations are made up a different proportion of individuals exposed to environmental and iatrogenic exposure via vaccination to influenza antigens. Any new flu vaccine must take into account the mixed target population. The blocking theory posits that prior immunity to an infectious disease blocks replication of a live attenuated vaccine and therefore reduces efficacy (Fordham von Reyn, C.). This has been demonstrated in BCG revaccination; BCG fails to act as a booster 10-15 years after childhood vaccination with BCG, when the efficacy of BCG wanes (Brandt et al.). Dendritic cells have also failed as vaccine carriers as they are short-lived due to sensitivity to CTL-mediated elimination (blocking). Longevity of antigen presentation persists, however, due to persistence of antigen-containing exosomes that are not susceptible to CTL elimination (Luketic, L.). This has led to the proposal that exosome-based vaccines may be advantageous for booster immunization due to their resistance to pre-existing Class I specific CTLs. FluMist®, the live attenuated vaccine strain of H1N1 was removed from the market in 2016 due to efficacy failure (Schnirring L.). Failure of this LAIV is most probably due to blocking by the pre-existing CTL immune response to influenza which would prevent replication of the weakened H1N1 strain in the vaccine. LAIV can only work in individuals not previous exposed to influenza which limits its use probably to the age group of 2 to 4 years.

Unfortunately, in this age group viral shedding can become an issue similar to the problems with live attenuated polio vaccine (Onorato et al.). Therefore, use of LAIV as a booster will not achieve any better impact than naturally occurring LAIV prime during infancy as a result of exposure to influenza. Re-vaccination of children in adolescence would be required if use of LAIV became widespread and in that event LAIV could not be used for the booster similar to the failure of BCG to act as an adolescent booster for BCG priming (Fordham von Reyn, C.).

Live attenuated vaccines versus Exosome Vaccines

It is important to note that the incidence of influenza pandemics has increased since the introduction of season flu vaccines in the 1940's (Ziegler, M.). A number of authors have suggested that seasonal flu vaccine places individuals at risk for a pandemic (Bodewes et al., Hillaire et al., Yan et al.). By definition a flu pandemic is the human to human spread of a novel animal derived flu strain in which no herd immunity exists in the population. Development of a universal flu vaccine that covers all current existing strains would not prevent a new pandemic. As stated above, use of seasonal flu vaccines increases bottleneck size for horizontal transmission making the spread of a novel flu strain more efficient and also allowing for a window of replication in the recipient host so the cycle can be repeated (Yan et al., Dougan et al.). The only previous suggested alternative to live attenuated vaccines inducing a memory T cell response has been the proposed use of dendritic cell-derived exosome vaccines (Rodrigues et al., Luketic L.). These vaccines are designed to exploit nature's antigen delivery pathway (i.e. cross-presentation) (Delcayre et al., Smith VL. et al., Zitvogel et al.). The "vaccine" function is to transfer antigen-loaded MHC class I and II molecules directly to lymph node APCs where activation of naïve T cells and innate (natural killer cells) cellular immune responses occurs. The peptide antigen in the MHC binding site protects the peptide from proteolytic degradation during blood/lymph transport to the lymphoid tissue. Unfortunately, this technology, along with the living drug therapies such as CAR T cells, or TCR-T cells, etc. is not a feasible approach to a prophylactic vaccine for large populations. The use of these technologies also requires a prior knowledge of the Class I HLA expressed ligandome in addition to the magnitude, kinetics and nature of T cell responses that are observed after natural infections.

Re-engineering the adaptive immune response from post infectious individuals – use of quantum clusters as artificial exosomes for delivery of Class I antigen information directly to APC

Once the HLA expressed ligandome is determined experimentally, recall of T cell memory from immune cells isolated from post-infectious patients can be used to define a vaccine that can reproduce the adaptive immune response to a natural infection (Huang et al., Comber et al. Testa et al.). Computer-predicted viral ligandomes cannot be used since processing of viral elements to class I peptides doesn't follow the same uniform 9mer rule as that previously described for normal-self or tumour-associated class I peptides (i.e. most class I peptides derived from viral elements contain unpredicted overlap sequences on either/both the N or C terminal of the respective HLA supertype 9mer motifs -unpublished observation). Since the exosome and living drug therapies are not viable technologies for mass vaccination, an alternative cross-presentation delivery system is required. Such a delivery system must be able to target APC in immune tissue, and deliver class I peptides to class I molecules for APC presentation to naïve T cells (or central memory T cells). Gold nanoparticle quantum clusters (~1.6nm) can be synthesized that contain surface presented class I peptides that can be targeted and delivered to lymphoid APC. Dendritic cell homing molecules are used to passivate the quantum clusters and

mediate the homing and uptake by APC. High levels of intracellular glutathione release the peptides into the cytoplasm for uptake by endogenous class I molecules that are then expressed on the APC surface. This compartmentalization enhances the class I uptake by circumventing the law of mass action, i.e. cytosolic peptides compete with each other for class I presentation but they do not compete with peptides generated from the abundant full-length endogenous proteins (Lev et al.). Importantly, the quantum clusters disrupt water Brownian motion up to a distance of 22nm from the particle surface, resulting in inactivation of proteases and thus preventing peptide degradation during delivery. Gold quantum clusters, therefore, can be considered artificial exosomes that can be used to deliver HLA ligandome information to APC, thus mimicking cross-presentation of the normal antigen information transfer that occurs in natural infections. These particles contain no biological components other than synthetic peptides of the length ~9-11mer, and contain all the information required to prime naïve T cells in the same qualitative manner that occurs in a natural infection. Validation of the efficacy is obtained by recall response of central T memory cells from previously infected individuals. By analogy, the glutathione gradient is also used for the natural delivery of Class I antigens by exosomes. Disulfide-linked Class I molecules are formed on exosomes as they exit the glutathione rich cell and move into glutathione-deficient plasma. It is the Class I dimers that are novel structures for recognition by immune receptors on APCs. (Lynch et al.).

The synthetic exosome nanoparticles do not require a replication cycle to be active. Consequently, previous immunization with a natural infection or an attenuated live viral vaccine will not reduce their efficacy. They can be used to generate a primed response in an individual not previously exposed to an infectious agent, or a booster response to an individual previously vaccinated with live attenuated vaccine or exposed to the infectious agent in the environment. Class I antigens to be incorporated within the particles can be chosen to be either genus-typic (i.e. universal flu vaccine) or specific to unique Class I antigens for a specific strain. The Class I antigens can also be mixed to cover the major supertypes of HLA to give population coverage > 95%. Vaccine delivery can be via microneedle dermal patches which will not require cold chain. Vaccine compliance can be enhanced by self-administration if necessary and vaccine procurement by post or other mass-delivery methods if required.

Mechanism of T cell Vaccine Action - Bottleneck Vaccines

In order to produce a universal flu vaccine, it is necessary to have knowledge of what factors are involved in expanding an initial narrow bottleneck (animal to host) to a large bottleneck that allows subsequent host to host horizontal transmission. This is a requirement for a novel flu stain to propagate in a population without herd immunity (pandemic vaccine). That is, the quasi species variability is the critical factor and not an immunological barrier. Yellow fever vaccine is a prime example of a vaccine limiting bottleneck expansion in a recipient host (Pulendran B.). Wild type yellow fever is made up of thousands of variants that act as a population to cause disease. Yellow fever vaccine has recently been shown to contain just a single viral variant which is not present in the wild type population (Beck et al.). Despite this, this vaccine can prevent thousands of quasi species from expanding in the host post-infection and then leading to disease. Whilst significant cross-reactivity might be expected it is the ability of this vaccine to limit expansion of the wild type virus variants in the recipient host to a sufficient number of variants to cause disease that is the basis of the yellow fever vaccine efficacy. Attenuated live polio virus has also been shown to act in this manner in preventing neurologic complications of infection with wild-type polio (Vignuzzi et al). Importantly, reduction in expansion of the bottleneck is the mechanism of action of these vaccines, and serotypic specificity is irrelevant (see comments below on "original antigen sin"). As mention above studies on flu have shown that at least 192 variants must be transferred before further horizontal transmission is possible (Sobel et al.).

Bottlenecks also occur during infection, dissemination and transmission by a virus's natural enzootic vector. For example, with Venezuelan equine encephalitis virus (VEEV), the initial inoculum from host to a mosquito only gains hold in its enzootic host because of a small subset (1-5 cells) of midgut epithelial cells that are susceptible to allowing viral replication (Smith et al.). This is the initial bottleneck in the transmission cascade. Transmission bottlenecks may constrain the natural genetic diversity (genetic drift) that could potentially lead to extinction via Muller's rachet (Forrester et al.). As mentioned above previously, mammalian adaptation of influenza A(H7N9) virus is limited by a narrow genetic bottleneck. In contrast, loose bottlenecks are important in horizontal transmission of influenza (Sobel et al., Zaraket et al.).

Development of a pandemic flu vaccine to prevent horizontal transmission of novel flu strain

Emergex Vaccines has patented a novel pandemic flu vaccine containing peptide fragments derived from Influenza A that are unexpectedly coded by the genomic (negative) strand of a segment of all current human influenza A viruses including 1918 Spanish flu. The peptides are expressed in Class I HLA of flu-infected human cells and the targets for immune T cell responses. The predominant association of the novel Open Reading Frame (ORF) present in the genomic strand with human influenza viruses indicates that a putative expressed protein (currently unannotated) may only be an advantage to influenza viruses replicating in humans – a key requirement for the establishment of a new pandemic. Mutation of current circulating non-human influenza A viruses to acquire this novel ORF could have a serious implication in causing a future human pandemic. Synthetic T cell stimulating vaccines incorporating these human and potential pandemic -specific targets are currently in pre-clinical testing and clinical development of these vaccines is expected in early 2019.

Selective Key References

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