EMERGEX
VACCINES

The Case for T-Cell Vaccines for Elimination, and for Control of Transmission, of COVID-19

Herd immunity requires individual immune responses. This process can only occur if natural infection is allowed to proceed. Sterilizing vaccines are contra-indicated for the control of COVID-19.

The “T prime” strategy employed by Emergex is designed to reduce the Shannon diversity index and the bottleneck size of the ‘door inoculum’ (infected individual) and thus will reduce or eliminate the ability of an infected individual to pass on the virus. This will simultaneously synergize with Muller’s ratchet leading to decrease in viral fitness resulting in herd immunity being achieved at a lower population threshold.

At the same time, subclinical disease/ no disease should be the outcome.

In order for an antibody-based vaccine to “prevent” COVID-19 infection (sterile immunity), a long term respiratory mucosal IgA response is required. No current antibody-generating vaccine is designed to produce this type of response: IgG has no access to mucous membranes except in the genital and vaginal tract

It is been demonstrated that giving young children flu jabs (antibody-generating) and preventing natural exposure to flu prevents T-Cell responses from evolving and thus limits lifetime immunity and memory T Cell response to flu exposure. This loss of ‘birth cohort’ flu immunity should not be repeated with COVID-19 treatment strategies.

When a virus infects a cell, the infected cell reactively produces a peptide ‘code’ that is then expressed on its surface. In healthy uninfected cells, the ‘self-code’ is derived from endogenous proteins and provides for recognition of “self” and prevents the immune system from eliminating that cell as foreign. For infected cells, the newly expressed ‘viral code’ originates from internal (in contrast to structural) conserved viral proteins, displaces the ‘self-code’ and thereby is recognized as foreign (not self): the

human immune system will kill and eliminate those infected cells via cytotoxic T lymphocytes (CTLs) called CD8+ T-Cells which target Class I HLA molecules that express the viral codes.

It is becoming increasing evident that vehicles (vaccines) that deliver the viral 'code' can be a medicament that primes the immune system to perform its kill-and-clear functions. The data is now substantial that, similarly to other RNA viruses, both short and long term immunity to COVID-19 will occur via the activation of naïve T-Cells that transform into T effector cells to control the later stages of an initial infection (natural killer cells -- NK cells, i.e. pre-primed T cells -- are the first line of defence) and then naturally transform into long-lived central memory T-Cells that can be recalled from lymph nodes (reactivated and expanded) upon subsequent infection. Importantly, "re-infection" is an absolute requirement for boosting cellular immunity since the T-Cells do not recognize nor interact with intact virions (viral particles), but only recognize their presence once a cell is infected (via re-expression of the "viral code" in the Class I molecules) and then act to 'clear' those infected cells.

It is important to note that neither natural immunity nor live attenuated vaccines such as polio, measles, yellow fever, etc. are sterilizing. That is, secondary infection of a limited number of susceptible cells will always occur on re-exposure (it is impossible to prevent re-infection since bottlenecks of just one virion can result in infections) and are a necessary component of immunity as these secondary infections provide viral antigen boosts throughout a person's lifetime. Importantly these secondary infections do not give rise to illness nor to transmissible virus.

1. COVID-19 is a quasi-species RNA virus; transmission and disease outcome therefore is determined by the Shannon diversity index within the transmission microdroplet and the bottleneck size. The 'bottleneck' governing infectious disease transmission describes the size of the pathogen population transferred from the donor to the recipient/host. Bottleneck size is important for rapidly evolving pathogens as narrow bottlenecks reduce the amount of transferred viral genetic diversity and thus may decrease the rate of viral adaptation. Importantly T-Cell immunity is able to reduce diversity and consequently to reduce the efficiency of viral transmission. This process contrasts with antibodies that can increase diversity via forcing the creation of antibody escape mutants which are then transmitted to the next individual. This sequence of events is considered the leading cause for loss of flu vaccination efficiency within a given year/cycle – flu vaccine efficiency decreases by 16%/month as the flu season progresses.

2. It is well established that the CD8+ cytotoxic T-Cell response to a virus is determined by the frequency of pre-existing naïve T-Cells to Class I MHC expressed epitopes. The greater the frequency of the background T-Cell (and repertoire size), the greater the CTL response on primary encounter with a viral pathogen. These naïve T-Cells consist of public and private specificities of unique T-Cell repertoires generated by random DNA recombination events in each individual human. T-Cell responses to viral infections are also influenced by memory T-Cells generated in response to unrelated pathogens. This result is called 'heterologous immunity'. A combination of the above events leads to variation in immunopathology to a given viral infection. This outcome is clearly the case for the kernels of COVID-19 symptomology, i.e. asymptomatic extending to ARDS. The innate immune response (NK cells, T delta/gamma, nIgM [natural IgM]) which is the initial response to a viral infection, is also under genetic control and thus a private specificity. The IgG/IgA antibody adaptive arm of the immune response plays no role in the clearance of an initial RNA viral infection and a neutralizing antibody only occurs as result of B-Cell maturation well after the viral illness has resolved.

3. Emergex' 'T prime' strategy is not a "T-Cell-only" vaccine. The "prime" event serves to increase the frequency of public specificities of the naïve T-Cells against MHC Class I expressed epitopes present on COVID-19 infected cells. The "army" therefore is present on Day 1 of infection in contrast to Days 7-10 in a natural infection. As Emergex is stimulating only CD8+ naïve T-Cells and not CD4 T-Cells, the CD4 suppression of the critical NK cell immune response is not compromised on subsequent infection. Upon subsequent infection of a T-Cell primed individual, the normal immune response runs its course and a genetically matched adaptive immune response is generated in each individual. That is, private specificity T-Cells can emerge as well as a broadly neutralizing polyclonal antibody response to the specific infecting variants that have emerged in this individual infection. This process

is critical to achieving herd immunity. Significant literature exists for the effects of IgG pre-immunization in both animals and in humans by antibody-based vaccines. As discussed above, a pre-existing antibody will transiently reduce the diversity index, but this result quickly rebounds by generating a new set of antibody escape mutants. Consequently, individuals primed with epitope-specific vaccines will transmit (become a donor for) a different set of variants (to a recipient) to those viral variants to which the persons have been infected. This process is the main cause of the failure of antibody-based flu vaccines.

Predicted Failure of Antibody-based Vaccines

As of April 29, 2020, two major clades and six subclades of the SARS-CoV-2 virus are evident in the analysis of 5349 whole genomes that are unevenly distributed across the world. The key goal of antibody platform-based vaccines is the induction of broadly neutralizing antibodies (**bnAbs**). These specialist antibodies are generated by antibody affinity maturation and are expanded to have cross-clade neutralization activity. To date, no vaccines have been developed to induce bnAbs. This current lack of results has been previously seen as a failure to develop cross-clade vaccines for both HIV and influenza. The generation of bnAbs will be imperative if an antibody platform vaccine is to provide immunity to COVID-19, which infection has already split into multiple clades in the first four months.

1. Failure to generate bnAbs after vaccination appears to be a tolerogenic process. Many antibodies against viruses are cross-reactive against auto-antigens and these antibodies have the characteristics of bnAbs. Therefore, the generation of high affinity bnAbs after vaccination needs to be limited in the same way that it is limited after many natural infections. This process is called “affinity reversion” and basically establishes an upper set point on the permissible affinity of the antibody – striking a compromise between auto-antibody activity and anti-viral activity. For example, in a SARS context, there is cross-reactivity between the nucleocapsid protein and the human cytokine IL-11. Also, there is a high statistical association between the incidence of rheumatoid arthritis and coronavirus infections: nearly 10% of all rheumatoid arthritis cases appeared at the time of a coronavirus respiratory infection. It is noteworthy to mention the relationship between autoimmune diabetes as a co-morbidity with COVID-19. If any cross-reactive autoimmunity between diabetes and COVID-19 really exists, then it would result in affinity reversion with the consequence of placing those patients at increased risk to the COVID-19 infection. The concept of affinity reversion could make it impossible to make an antibody-based vaccine against COVID-19 – this limitation now appears to be the root cause of the earlier failures of HIV and hepatitis C vaccines (i) to generate bnAbs and (ii) to consequently control the infection. These non-results also suggest that CD8+ T-Cell immunity is critical to get around the fundamental limitation of antibody affinity maturation that is restricted by potential autoimmunity.

2. Putting aside the potential autoimmunity issues, conditions needed to promote bnAb activity have been described in the literature and, in general, can be achieved by serial vaccination with different vaccine formulations such that each vaccination has specific immunologic characteristics and different viral antigens. For example, serial vaccination of ferrets with four different strains of influenza generates an immunologic response which is able to neutralize a fifth strain that the ferret has never previously seen, a desirable outcome. Please note that immunization with each of the influenza strains individually does not generate this cross-clade response. This serial vaccination approach is impractical, however, in the real world as it requires multiple, costly, time-consuming vaccinations and a significant degree of patient compliance. In contrast, it has been shown that repeat vaccination with the same influenza strain in humans in two successive years reduces antibody-affinity maturation and probably contributes to lower vaccine effectiveness of seasonal influenza vaccines in humans.

Potential Complications of Viral Vector Vaccines Using Adenovirus Constructs

The 2008 HIV STEP vaccine trial was halted since vaccinated individuals had an increased incidence of HIV infection compared to unvaccinated persons. This trial used an adenovirus vector to deliver a

number of HIV proteins that should have produced a cell-mediated response against HIV infected cells. The increased incidence of HIV infection was only seen in the cohorts that were already seropositive for an adenovirus infection. Since 2008, various proposals have been made to explain the increase in HIV incidence secondary to vaccination. The most obvious explanation was that the adenovirus vector induced a strong recall of adenovirus specific CD4 Cells (new targets for HIV infection). There are 65 known adenoviruses and, at the T-Cell level, many of these would be cross-reactive; hence there is an expectation that the vaccine either increased the CD4 targets or it changed their permissiveness to HIV infections in the adenovirus seropositive cohort. These hypotheses could not be experimentally confirmed on circulating blood CD4 cells. Also, the hypothesis required a persistence of a pool of CD4 cells to be infected after vaccination. Nevertheless, the important observation that the cohort which was seronegative for adenovirus did not show an increased HIV incidence highly suggested that some form of adenovirus-dependent recall response was contributing to the increased HIV incidence in seropositive individuals.

Natural infections with adenoviruses are linked to **mucosal** surfaces – the same site as HIV infection. In the vaccine study, adenovirus vaccination was to **muscle**. Subsequent experimental studies suggested that boosting recall to mucosal-targeting CD4 cells (usual site of HIV infection) was occurring in the seropositive individuals and that muscle vaccination did not generate the same subset of mucosal-homing CD4 Cells. However, it is not clear how muscle vaccination with adenoviruses was able to stimulate CD4 cells that then homed to mucosal surfaces. Reactivation of previous mucosal-targeting CD4 cells during passage through [i] the antigen presenting adenovirus-infected muscle or [ii] reactivation of previous T memory cells with APC in lymphoid tissues were proposed explanations.

The above vaccine trial illustrates the dangers of using viral vectors as vaccine platforms since these vectors can remain active potentially for the life of an individual. Volunteers in this study potentially carry with them an increased risk of HIV for the rest of their lives. Persistent non-replicating viral vectors also can stimulate both CD4 and CD8 T-Cell exhaustion. In the above STEP trial, it was not considered as a mechanism, but T-Cells to epitopes on the three HIV proteins in the construct could have been rendered tolerogenic via T-Cell exhaustion. In addition, the persistence of the adenovirus vector could generate a continuous pool of CD4 T effector cells that do not die off and do not revert to T memory cells which normally occurs upon antigen withdrawal.

COVID-19 infects mucosal surfaces, presumably, of the respiratory tract. Adenovirus also have as its main target infectivity of the mucosal respiratory tract and has been linked to fatal adenovirus infections of the respiratory track in children and also the pathogenesis of chronic obstructive pulmonary disease. The potential co-localization of SARS-CoV-2 and latent adenoviruses or active adenoviral infection suggests caution in the use of adenovirus vectors for immunization in COVID-19. The current adenoviral vaccines for COVID-19 all use **intramuscular** administration as per the failed, unfortunate HIV STEP trial.

Importance of T cell Immunity in Transmission Dynamics

Since COVID-19 is a positive strand RNA virus it will exhibit quasi-species dynamics during infection and subsequent transmission. The bottleneck governing infectious disease transmission describes the size of the pathogen population transferred from the donor to the recipient host. Transmission is governed by a loose, but highly variable, transmission bottleneck whose size is positively associated with the severity of infection of the donor. During a pandemic the virus spreads to new areas and countries that were previously uninfected. Founder effects will have a significant impact on mutation frequencies. Paradoxically the smaller the bottleneck the greater the chance of new variants appearing in a recipient and as such any mutations present in the initial small inoculum will rapidly become common even if they are rare in the particular geographic area which seeded the

transmission. Essentially “mini-epidemics” are constantly being formed from novel founder populations which quickly either dissipate or enlarge depending on subsequent bottleneck events.

Transmitted /founder viruses can potentially rapidly escape from CD8+ T cell responses to a particular variant. In general, the rapid escape dynamics (peptide mutations) are associated with the higher-magnitude(affinity) CD8+ T cell responses – that is, the immunodominant T cell responses. This could be detrimental to the virus since subdominant T cell responses are thought to be critical in elimination of an infection and
the dominate T cell clones are involved in disease chronicity. Further, there is a fundamental difference between the consequences of antibody escape mutants and T cell escape mutants. The T cell escape mutants, whilst losing HLA binding affinity in the initial
recipient (unique HLA type), will on subsequent transmission to a new recipient (Founder effect) be faced with a different HLA haplotype in the population. Therefore, HLA haplotype heterogeneity in the population ensures that the T escape epitopes generated in one individual will be reactive in other members of the population as a whole. This “spreading” of HLA Class I reactivity is probably critical in ensuring that rare HLA haplotypes in the population are not left unresponsive (immunologically silent) to the virus upon infection.

In summary Emergex T prime strategy as a population concept sets in motion Muller’s ratchet. That is, for a vaccinated population, each new bottleneck of founder viruses upon transmission will be met with a pre-existing T cell response resulting is smaller viral populations in the recipient. Muller’s ratchet is an important concept in RNA viral population genetics and differs from the herd immunity paradigm. The Muller’s ratchet concept predicts that when mutation rates are high (quasi-species) and a significant proportion of mutations are deleterious, an irreversible ratchet mechanism will gradually decrease mean fitness of small viral populations (threshold). Experimentally it has been shown that the most regular and sever fitness losses occurred during virus passages on a new host cell type. Muller’s ratchet could therefore have significant implications for variability of disease severity (directly proportional to transmission efficiency) during virus outbreaks, since genetic bottlenecks must often occur during respiratory droplet transmission and during the spread of low-yield RNA viruses from one body site to another.

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