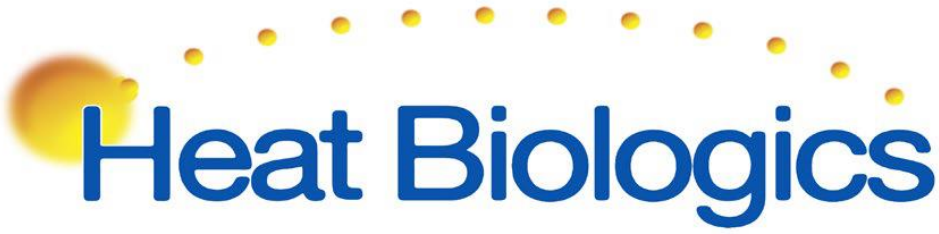

The Importance of T-Cell Immunity in SARS-CoV-2 Infection

The Importance of T-Cell Immunity in SARS-CoV-2 Infection

Antibodies Are Not Enough

Innate immunity plays a "first responder" role in infection control, followed by pathogen-specific responses that activate the adaptive arm of the immune system. Innate immunity provides a bridge to adaptive immunity, and an effective vaccine against SARS-CoV-2 should activate the two arms of the adaptive immune system, humoral (antibodies) and cellular (T cells) immunity. Antibodies block the initial infection and the spread of new virus particles by binding to key specific viral entry proteins; in the case of SARS-CoV-2, this would be the spike or "S" glycoprotein. T cells serve two major functions: help antibody-producing cells (B cells) make neutralizing antibodies and kill virus-infected cells. Most of the COVID-19 vaccines in clinical trials are focused on generating a predominantly humoral (antibody) response. There is now significant and mounting evidence that antibodies alone may not be enough to protect against SARS-CoV-2 infection.

Vaccines are often combined with an adjuvant to trigger innate immunity and enhance the antibody response. Viruses trigger innate immunity via pattern-recognition receptors such as Toll-like receptors (TLRs), which recognize double-stranded viral RNA or single-stranded viral RNA and DNA. TLRs can also be activated to boost innate and adaptive immunity via endogenous signals, such as those which may be provided by immune stimulatory protein gp96 via Heat Biologics' (Heat) SARS-CoV-2 vaccine platform, as described below. Heat's vaccine platform is unique in orchestrating all three arms of the immune response - innate, humoral, and cellular via gp96 - with a more pronounced activation of cellular or T-cell–driven immunity.

Antibodies Generated to SARS-CoV-2 Are Fleeting

Glycosylation may dampen or inhibit antibody recognition of spike glycoprotein epitopes

Since entry of SARS-CoV-2 into host cells is via the spike glycoprotein, the focus of vaccine development is to generate neutralizing antibodies targeting epitopes on this spike glycoprotein. Antibodies generated tend to wane quickly and may not be effective as key epitopes are often shielded by glycan or sugar residues that prevent easy access to the immune system (see **Figure 1**). This phenomenon is similar to what HIV does for its viral entry protein, gp120.[1, 2] **Figure 1** depicts a view of the spike glycoprotein showing the significant glycosylation that hampers recognition of epitopes on the spike protein that can generate neutralizing antibodies. It is estimated that 40% of the spike protein is shielded by glycans, hindering antibody recognition.[3]

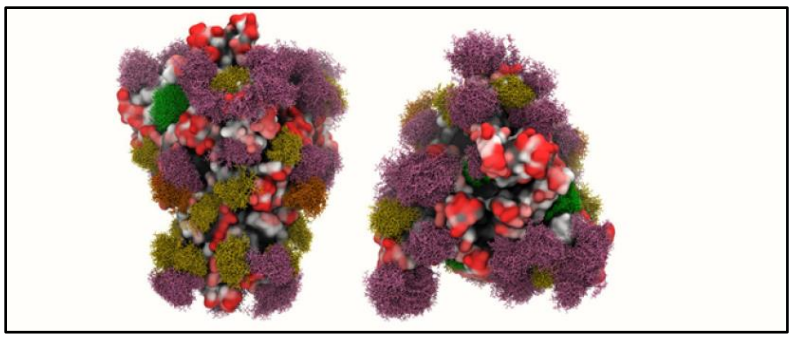

Figure 1: *Spike glycoproteins are heavily glycosylated, shielding epitopes from antibody recognition or allowing only for weak binding of antibody (adapted from Arco Biosystems).*[3]

A recent publication in the *New England Journal of Medicine*[4] sheds light on a study that evaluated 34 individuals for antibody titer after recovery from SARS-CoV-2. Blood samples were analyzed by enzyme-linked immunosorbent assay (ELISA) to detect anti–SARS-CoV-2 spike receptor-binding domain IgG. Antibody decay was observed as shown in **Figure 2**. Notably, antibody decay with SARS-CoV-2 observed in this study was quicker than that reported for the SARS infection from the 2003 outbreak.

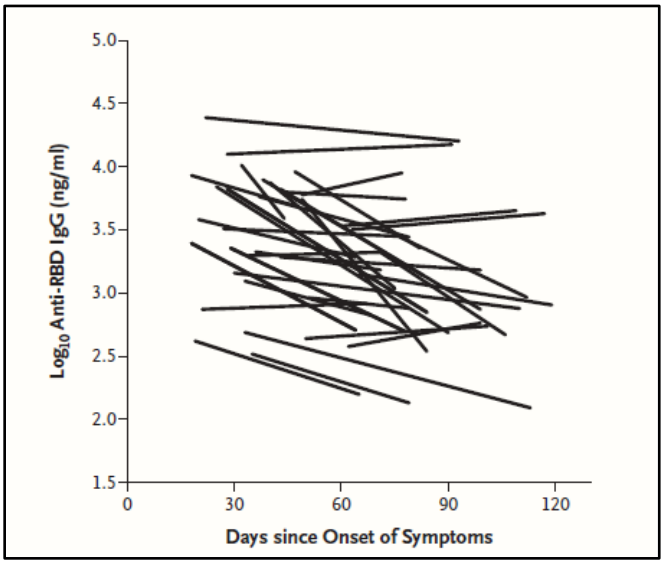

Figure 2: *Rapid decay in IgG antibody to SARS-CoV-2 spike receptor-binding domain in individuals who recovered from infection. These findings raise serious concerns for herd immunity.*

Another study, from the Chongqing Medical University in China, involving asymptomatic and symptomatic COVID-19 patients, found a 70% decline in protective antibodies after 2-3 months of natural infection, with some patients losing antibodies against the virus completely.[5] Humoral antibody responses against other coronaviruses have shown similar results for SARS-CoV-1 and MERS-CoV, lasting about a year in duration.[6, 7] The logic that herd immunity can protect populations exposed to this virus appears less likely, as protective antibody responses are barely generated even in patients who have recently recovered from SARS-CoV-2 infection.[8] Several other recent reports support the growing hypothesis that vaccines that produce more than just protective antibody responses are required to fight this pandemic, and that T-cell responses will be required to prevent the spread of SARS-CoV-2 between hosts.[9, 10]

T Cells Are Crucial in Immunity to SARS-CoV-2

Basic principles of immunology apply — CD4 T cells are essential for priming antibody-producing B cells for long-term immunity

T-cell immunity plays a pivotal role in generating a durable, immune memory response to protect against viral infection (see **Figure 3**). Prior studies have shown that memory B-cell responses tend to be short-lived after infection with SARS-CoV-1.[11, 12] In contrast, memory T-cell responses can persist for many years.[13] In addition to destroying virus infected cells directly, T cells are crucial for induction of high-affinity antibodies and immune memory.[14] Recent reports show that patients who have recovered from severe SARS-CoV-2 infection have T-cell responses against viral spike proteins, and in some patients, T-cell responses were present regardless of symptomology or antibody seropositivity.[15-17]

One reason the antibody responses in patients who recovered from SARS-CoV-2 may weaken or disappear could be the lack of optimal activation of T-cell immunity, such that CD4 T helper cells are not activated in response to SARS-CoV-2 infection. This could be a mechanism by which the virus suppresses host immunity and escapes immunosurveillance. Studies have also shown reduction in CD4 and CD8 T-cell responses during SARS-CoV-2 infection, some of which were correlated with in-hospital death and severity of illness.[18, 19]

In a study of 20 adult patients who recovered from SARS-CoV-2 infection, higher antibody titers were associated with stronger SARS-CoV-2–specific CD4 T-cell responses.[20] In addition, interferon-gamma–expressing, SARS-CoV-2−specific CD8 T cells (functional CD8 T cells) were observed in most SARS-CoV-2 patients. These interferon-gamma-positive CD8 T cells also co-expressed granzyme B, an essential lytic enzyme involved in killing virus-infected cells. In this study, the magnitude of protective IgG and IgA titers against spike protein and the extent of CD4 and CD8 T-cell responses to spike protein were all generally well corelated.[20] These findings are meaningful since protective antibody responses are dependent on CD4 T-cell help. Spike protein-specific antibodies, memory B cells, and T follicular helper cells are consistently elicited after SARS-CoV-2 infection, demonstrating robust interplay between the humoral and adaptive arms of immunity, and positive correlation with viral neutralizing antibody activity.[21]

Memory T-Cells Persist Regardless of Initial Humoral Response

Evidence from the 2003 SARS outbreak and the current SARS-CoV-2 pandemic point to protective memory T-cell responses in recovered and asymptomatic individuals. Notably, some of the memory T cells were in the absence of an antibody response

Generation of memory T cells that provide long-lasting immunity for years or even decades is a sign of an effective immune response against viral infections. A recent publication in *Nature*[13] demonstrated that SARS-recovered patients from the 2003 outbreak still possess long-lasting memory T cells, while antibody responses wane. This memory T-cell response was also frequently observed in individuals who were not

exposed to SARS or SARS-CoV-2, likely because of past exposure to related coronaviruses showing cross-reactivity.[13]

A study conducted at the Karolinska Institute in Sweden demonstrated that individuals with asymptomatic or mild SARS-CoV-2 generated highly functional and durable memory T-cell responses, and that these T-cell responses were often generated in the absence of corresponding antibody responses.[17]

Channappanavar et al studied antibody and T-cell–mediated immune response to the SARS outbreak of 2003.[11] Reminiscent of the current SARS-CoV-2 pandemic, the authors presented the following observations:

- Neutralizing antibody titers and memory B-cell responses were short-lived in SARS-recovered patients
- SARS-CoV–specific IgM and IgA response lasted less than 6 months, while virus-specific IgG titer peaked 4 months post-infection and declined markedly after 1 year
- Despite the lack of virus-specific memory B-cell response, SARS-CoV–specific memory T cells persist in SARS-recovered patients for up to 6 years post-infection

This pre-pandemic SARS study also concluded that driving T-cell immunity is essential and may be important for all respiratory virus infections in general.[11]

T-Cell Response to Viral Vaccines

An effective immune response requires coordination between cellular and humoral immunity

As depicted in **Figure 3**, generation of CD4-helper and CD8-killer T-cells is triggered when dendritic cells or antigen presenting cells, after capturing vaccine/antigen in the peripheral tissues, migrate to draining lymph nodes, where T-cell vaccine responses are elicited in parallel to B-cell responses, which are then primed to generate antibodies. CD4 helper and T follicular helper cells are important in helping B cells in germinal centers to mature and fine tune antibody strength (affinity maturation). An orchestrated immune response (as depicted in **Figure 3)** to a viral infection therefore involves CD4 T helper cells facilitating B-cell priming and maturation to develop affinity matured antibodies, along with activation of CD8 killer T cells that destroy virus infected cells. This coordinated immune response, that requires maturation of the antibody response helped by T cells, may be required in response to SARS-CoV-2 infection, as well as the broader respiratory coronavirus family, to provide long-term protection.[30]

An example of a coordinated humoral and cellular immune response is Heat's gp96/OX40L vaccine platform, described below (and in **Figure 4**). While the gp96/OX40L platform is particularly efficient in activation of a robust CD4 and CD8 T-cell response, IgG antibodies are also generated along with innate immunity.

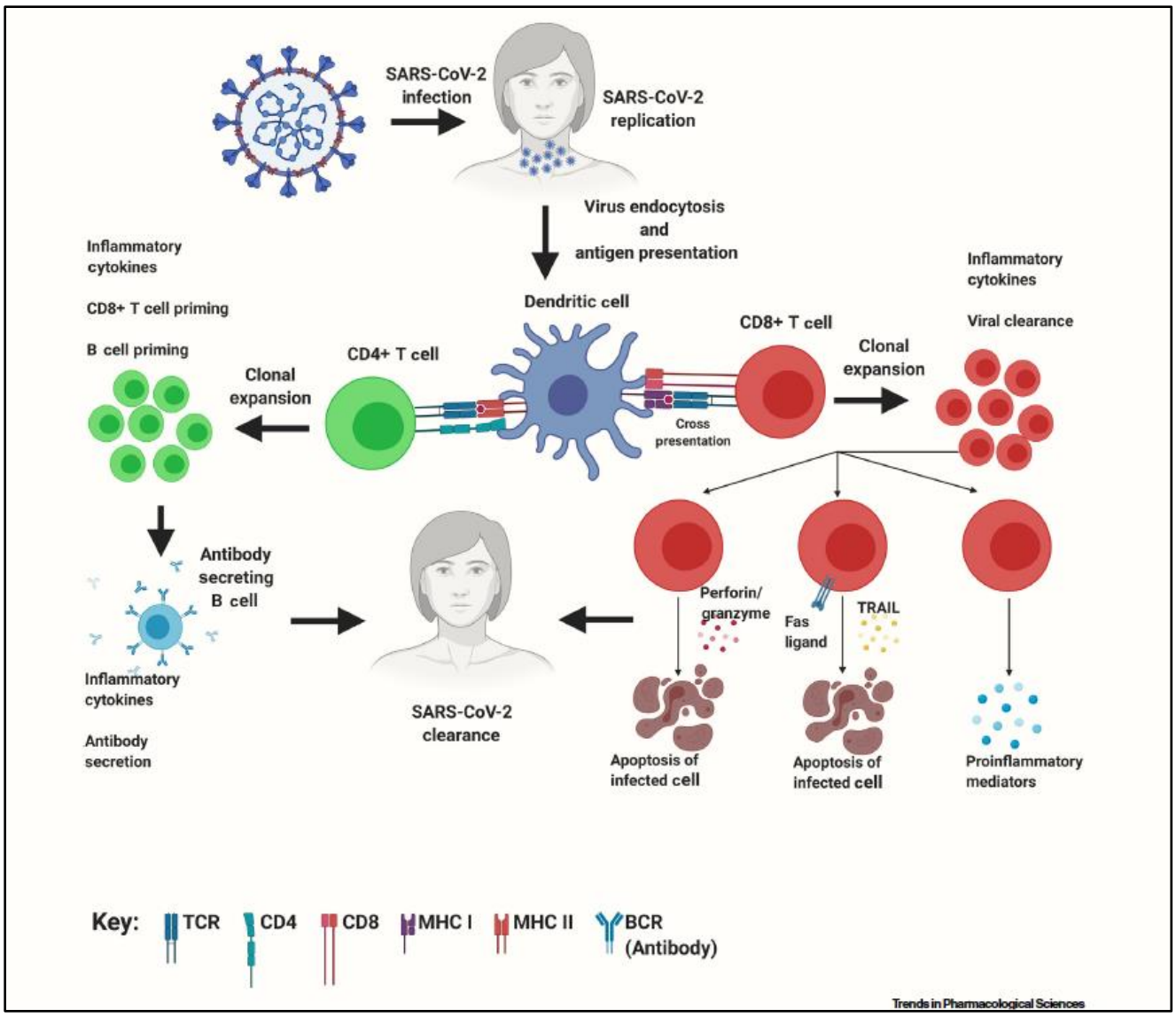

Figure 3: *T-cell–mediated response to SARS-CoV-2 infection (from Gutierrez et al, Trends in Pharmacological Sciences[31]). Infection occurs in patients after respiratory tract exposure. Subsequently, viral entry occurs via binding to angiotensin-converting enzyme 2 on the surface of various cell types and viral replication begins. Antigen presenting cells, such as dendritic cells, endocytose the SARS-CoV-2 virus and degrade them through a process called antigen processing. These antigen fragments are then presented by proteins, termed MHC class molecules, on the cell surface and allow recognition by a T cell. If a CD8 T cell is capable of binding, it will undergo clonal expansion and directly target infected cells through either perforin/granzymes, Fas ligand/tumor necrosis factor-related apoptosis inducing ligand (TRAIL) pathways, or secretion of proinflammatory mediators. If the CD4 T cell is capable of binding, it can activate B cells that recognize the antigen by causing them to clonally proliferate and secrete antibodies to target the SARS-CoV-2 virus. Abbreviations: BCR, B-cell receptor; MHC, major histocompatibility complex; TCR, T-cell receptor.*

Table 1 provides a summary of the T-cell response to a vaccine and further conveys the importance of coordination between humoral and cellular immunity in protection against viral infections. The role of T cells is multi-pronged and goes beyond providing B cell help to generate antibodies. The T-cell response to vaccines also includes the generation of a host of cytokines that regulate various aspects of the immune response including activation of dendritic cells, mucosal inflammation, and the generation of effector, central and tissue-resident memory T cells.

In a study that evaluated a SARS-CoV-2 infected patient with moderate disease that required hospitalization, orchestration of humoral and cellular immune responses was observed as the patients symptoms resolved over time.[30] Evaluation of Immune metrics showed coordination of antibody production as measured by IgM and IgG, T follicular helper cells that facilitate antibody producing B-cells, and activation of CD4 and CD8 T cells. Notably, the increase in CD8 killer T cells at days 7 to 9 preceded the resolution of symptoms, and then declined as the patient's disease resolved by day 20.[30] The early activation of both antibody and T-cell responses may be crucial in protecting patients from acute SARS-CoV-2 disease complications. A vaccine that can activate both cellular and humoral immunity is therefore ideal.[30]

Type	Mechanisms, presumed	Function
CD4 T-helper cells		
Th1	IFN-γ production	Extrafollicular B-cell help
Th1	Cell contact, IFN-γ	Activation of CD8 T cells
Th1/Th2	Cell contact, CD40L	Dendritic cell activation
Th2	IL-4, IL-5, IL-13	Extrafollicular B-cell help
Th2	Cell contact, IL-4	Suppression of CD8 T cells
Th17	IL-17, IL-21, IL-22	Mucosal inflammation
CD4 follicular T-helper cells		
Tfh1	IFN-γ	Germinal center B-cell help
Tfh2	IL-4, IL-5, IL-13	Germinal center B-cell help
CD4 regulatory T cells	Multiple mechanisms	Suppression of CD4/CD8 responses
CD8 T cells	IFN-γ, TNF-α	Killing of infected cells
Memory T cells		
Effector memory T cells	Th1/Th2 cytokines, perforin, granzyme	Rapid secondary effector responses in periphery
Central memory T cells	IL-2, IL-10, CD40L	Delayed activation/proliferation in lymph nodes
Tissue-resident memory T cells	Th1/Th2 cytokines, perforin, granzyme	Tissue localization enabling immediate-early reactivation

IFN, interferon; IL, interleukin; Th, T-helper; TNF, tumor necrosis factor.

Table 1: *T-cell responses to vaccines (adapted from Plotkin's Vaccines 7th Edition, 2018; Chapter 2 Vaccine Immunology by Claire-Anne Siegrist, pages 16-34).*[29]

Heat Biologics SARS-Cov-2 Vaccine Platform

A clinically validated gp96-based vaccine platform that drives a prominent T-cell response, an antibody response, and activation of innate immunity, orchestrating all arms of the immune system

Heat's gp96 technology platform was originally developed to activate cellular immunity, specifically killer CD8 T cells, to eliminate tumors. In a next-generation version, the costimulatory molecule, OX40L, was inserted into the vaccine, to promote helper CD4 T cells that facilitate B-cell antibody production and further enhance killer CD8 T-cell function. A salient feature of the gp96 vaccine platform is the activation of innate immunity via TLRs, as gp96 activates TLR2/TLR4 on macrophages and dendritic cells.[22] This technology platform has shown very positive data in the clinic,[23] and numerous publications using this platform have demonstrated the capacity to leverage cell-mediated immunity to fight both infectious diseases as well as cancer.[24-26]

Unlike most conventional vaccines that predominantly drive a humoral response, Heat's SARS-CoV-2 vaccine platform drives a prominent cellular immune response via CD4 and CD8 T cells, in addition to a humoral immune response, via neutralizing IgG antibody. Heat's SARS-CoV-2 vaccine targets the spike protein, and expresses gp96 and OX40L, a potent T-cell co-stimulator. OX40L co-stimulation expands CD4 helper T cells that promote B-cell differentiation and IgG/IgA antibody class switching,[27] as depicted in **Figure 4**. Phase 2 clinical studies in patients with non-small cell lung carcinoma treated with HS-110, Heat's lead gp96-based product, showed increased T-cell infiltration into the lung, resulting in tumor growth inhibition and improved overall survival. The efficacy of this gp96-based vaccine platform has also been demonstrated in numerous studies funded by the National Institutes of Health and the US Department of Defense in infectious disease models of simian immunodeficiency virus, zika virus, and malaria, wherein a robust antibody, as well as T-cell responses, have been elicited to confer long-term protection against viral and parasitic infection.[25, 28]

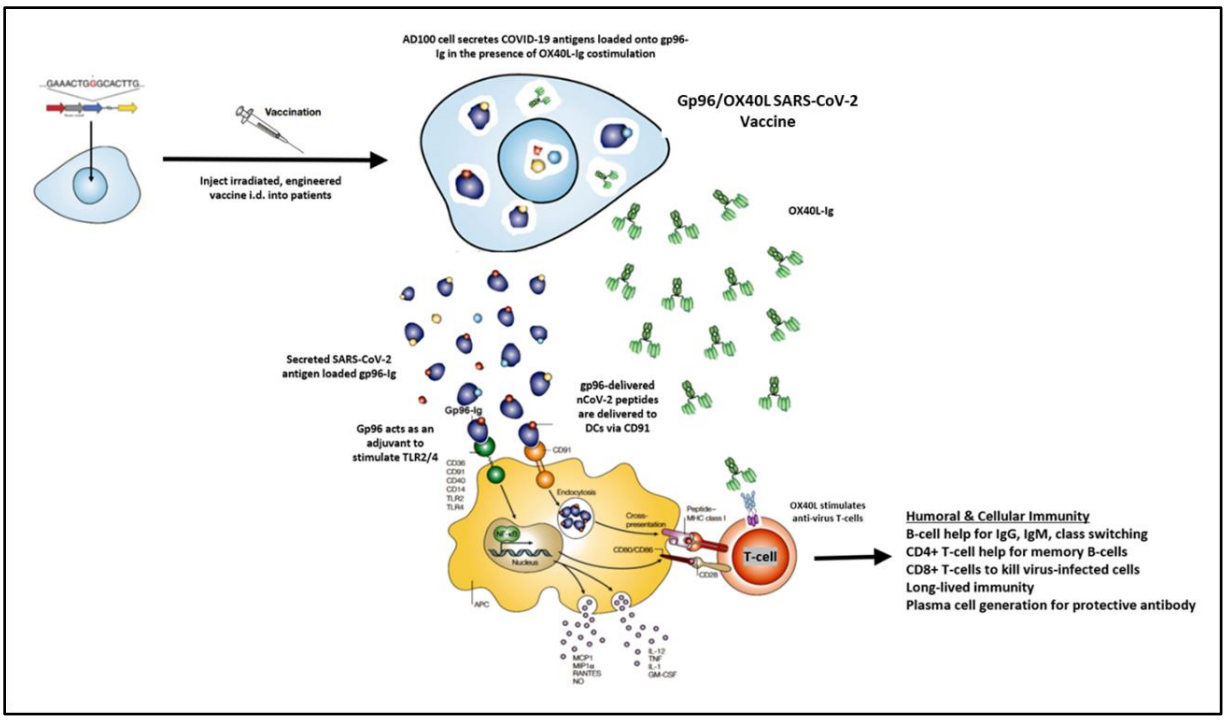

Figure 4: *Heat Biologics' gp96-based COVID-19 vaccine. The premise of Heat's gp96-based allogeneic cellular vaccine technology. gp96 is a naturally occurring intracellular protein found in normal, tumor, or virally infected cells. The gp96 cytoplasmic tail KDEL amino acid retention sequence anchors it in the intercellular compartment within the endoplasmic reticulum after sorting in the Golgi apparatus by retrograde transport. Heat developed genetically modified gp96 by replacing the retention signal with a secretion molecule, IgG1-Fc. gp96-Ig is exported together with other secreted proteins. Cells that secrete gp96-Ig generate a very powerful immune response against its target, in this case, SARS-CoV-2. Heat has further enhanced this technology by including OX40L-Ig to the gp96-Ig vaccine, and S1/S2 proteins to target SARS-CoV-2. Original figures adapted from Nicchitta CV, Nature Reviews Immunology, Vol. 3, May 2003.* [22]

Significance in the vulnerable aged population

The gp96/OX40L-Ig COVID-19 vaccine platform is designed to harness natural antigen presentation and T-cell activation pathways that may overcome immune deficits in the aged population, in patients with comorbidities, or in patients with a compromised immune system. Heat's vaccine is designed to be used as a standalone vaccine or in combination with other vaccines that drive humoral immunity to provide an added layer of cellular immunity. Immunity in the aged population is clearly compromised (see **Table 2**), which might explain the heavy toll that the SARS-CoV-2 pandemic has inflicted on the aged population and in those with comorbidities. The reduction in the reservoir of robust, naïve T cells and limited effector memory T cells in the aged population is a weakness that Heat's gp96/OX40L-Ig COVID-19 vaccine platform aims to resolve. The aged population is treated with a double dose of the flu vaccine in order to compensate for weaker immune systems. Therefore, it is likely that this compromised population may benefit most from Heat's SARS-CoV-2 vaccine alone or in combination with a conventional vaccine.

In elderly people	
Limited magnitude of antibody responses to polysaccharide	Low reservoir of IgM memory cells; weaker differentiation into plasma cells
Limited magnitude of antibody responses to proteins	Limited germinal center responses: suboptimal CD4 helper responses, suboptimal B-cell activation, limited FDC network development; changes in B/T cell repertoire
Limited quality (affinity, isotope) of antibodies	Limited germinal center responses; changes in B/T cell repertoire
Short persistence of antibody responses to proteins	Limited plasma cell survival
Limited induction of CD4/CD8 responses	Decline in naïve T-cell reservoir (accumulation of effector memory and CD8 T-cell clones)
Limited persistence of CD4 responses	Limited induction of new effector memory T cells (IL-2, IL-7)

FDC, follicular dendritic cell; Ig, immunoglobulin; IL, interleukin.

Table 2: *The aged population may need additional help in boosting immunity, in particular T-cell immunity (adapted from Plotkin's Vaccines 7th Edition, 2018; Chapter 2 Vaccine Immunology by Claire-Anne Siegrist, pages 16-34).*[29]

Historically, the Vaccine Industry Has Focused on Antibodies

For bacterial infections and most viral infections, antibodies may be reflective of an apt biomarker to determine efficacy, but this is not true for SARS-CoV-2 or even the related SARS and MERS family

Antibody or humoral immunity is only one aspect of an immune response to a viral infection. While neutralizing antibodies are an important and seemingly indispensable entity in establishing initial SARS-CoV-2 clearance, we tend to focus exclusively on antibodies because its measurement is convenient via a simple serological test. On the other hand, measures of T-cell immunity to an infection or vaccine is more complex and cumbersome. The vaccine industry is set up to evaluate immune responses based on simple antibody measures that may be reflective of efficacy in most viral infections, but that might not paint the entire picture in SARS-CoV-2 infection. For some infections, it is more helpful to measure T-cell responses than antibody responses; this is so with tuberculosis, where a T-cell test is used to diagnose latent (asymptomatic) disease. In the case of SARS-CoV-2, SARS, and MERS, T-cell response is an important metric to evaluate immunity.

Rationale for SARS-CoV-2 Vaccine Combination Approaches

Heat's SARS-CoV-2 vaccine is ideally suited to enhance durable cellular immunity in combination with conventional vaccine approaches. Given the nature of SARS-CoV-2 and the broader coronavirus family, Heat's vaccine, either as a standalone or in combination, may have utility in boosting immunity in the aged population and in those with comorbidities

There is growing evidence that combining vaccines is a rational approach, and that combination vaccines can be administered safely to induce an effective and durable immune response.[32-36] Combination vaccine approaches may be of greatest value when conventional vaccines are found lacking, as seen in the case of Ebola or generally within the aged population, or in those with underlying comorbidities when the immune system may be weakened. Heat's SARS-CoV-2 vaccine can be used as a standalone vaccine or combined with other SARS-CoV-2 vaccines currently in clinical development to provide an added layer of T-cell immunity boost to generate an effective and long-term immune response.

A combination vaccine for Ebola was recently approved and sets a precedent for combination vaccine approaches. Furthermore, multiple vaccines for pediatric use are routinely administered in a single injection with no issues of safety or efficacy.

Johnson & Johnson and Bavarian Nordic combination Ebola vaccine approved

A combination of two vaccines was approved on July 1, 2020, for Ebola in the European Union, and the Food and Drug Administration review process in the United States is ongoing.[32] The Johnson & Johnson (J&J) and Bavarian Nordic (Bavarian) vaccines are genetically modified, non-replicating viral vectors—an adenovirus (J&J) and a modified vaccinia virus (Bavarian).[33, 34] The adenovirus (adenovirus type 26 vector Ad26.ZEBOV) encodes the Ebola glycoprotein and the modified vaccinia virus encodes glycoproteins from Ebola virus, Sudan virus, Marburg virus, and Taï Forest virus nucleoprotein (MVA-BN-Filo), which encompass the filovirus family.[33-35]

The vaccination regimen is based on a priming dose of Ad26.ZEBOV vaccine, followed by a booster dose of the MVA-BN-Filo vaccine 8 weeks later. This approach of combining two vaccines as prime and boost is designed specifically to induce long-term humoral and cellular immunity against Ebola.[33, 34]

This combination vaccine effort included global collaborations and funding from the National Institutes of Health, the Biomedical Advanced Research and Development Authority (BARDA), the US Department of Health and Human Services, Innovative Medicines Initiative (IMI) in Europe, Bavarian Nordic, and J&J.

Pediatric and adult vaccines are administered as combinations in a single injection

Vaccines have been administered as a single combined injection for the pediatric population routinely. Examples include Pediarix (combines diphtheria, hepatitis B, and polio), ProQuad (combines MMR and chickenpox), and Kinrix (combines diphtheria and polio), among others.[36] These combination vaccine approaches have the advantage of a single injection for children, which results in improved compliance. No safety or efficacy issues have been observed over decades of use. The Centers for Disease Control and Prevention points out that more combination vaccines are being tested to allow children to get additional

protection with fewer shots.[36] For adults, the hepatitis A and hepatitis B combined vaccine, Twinrix, is administered in a single injection.

Heplisav for hepatitis B, a TLR9 agonist combined with hepatitis B surface antigen

Heplisav is a TLR9 agonist plus a hepatitis B surface antigen. TLR9 agonists, while considered an adjuvant, have a defined endosomal receptor-mediated biological effect that activates innate immunity and subsequently a Th1 effector response. Therefore, TLR9 agonists are not merely adjuvants and were previously in development as drug candidates for hepatis C and are currently in clinical trials for oncology in combination with checkpoint inhibitors. Heplisav was approved in a registration study that compared it with the previously approved Engerix, GlaxoSmithKline's hepatitis B vaccine that does not include a TLR9 agonist. Heplisav had a similar safety profile to Engerix, but with improved immunogenicity and fewer doses over a shorter time, plus it has the potential to provide improved seroprotection over time.[37]

Notably, Heplisav has been shown to induce higher rates of seroprotection than Engerix (without TLR9 activation) in individuals aged over 60 years with diabetes.[37-39] This finding suggests that conventional vaccines may not be suited to induce a protective immune response in the aged population or in those with comorbidities.

Concluding Remarks

T-cell immunity can complement and sometimes even compensate for a lack of antibody response. Vaccines against SAR-CoV-2 should focus on stimulating the adaptive arm of the immune system with a clear focus on generating long-term memory T cells. Natural immunity, or herd immunity, may not be feasible with SARS-CoV-2. Only a vaccine that can stimulate long-term T-cell immunity will be capable of preventing the spread of SARS-CoV-2. In high-risk populations (older individuals and those with comorbidities) the need is acute, and a vaccine that triggers a durable T-cell–driven cellular immune response may be critical to prevent spread. For SARS-CoV-2 and the related coronavirus family, vaccine approaches that can orchestrate innate, humoral, and cellular immunity are ideal candidates to generate a robust and long-lasting immune response in the fight against SARS-CoV-2.

Heat's SARS-CoV-2 vaccine may be ideally suited to compliment conventional vaccines in combination approaches by enhancing T-cell immunity, while contributing to innate immunity and antibody generation. In the more vulnerable aged population and/or those with comorbidities a vaccine combination approach may boost immunity and provide protection against SARS-CoV-2. A combination vaccine for Ebola with a prime and boost administration schedule was recently approved in Europe and is under review in the US. A prime and boost combination with Heat's SARS-CoV-2 vaccine with many of the other SARS-CoV-2 vaccines in clinic may be a rational approach.

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