COVID-19 VACCINE SAFETY CONSIDERATIONS

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ABSTRACT

The SARS-CoV-2 pandemic has produced global health and economic adverse impacts. The main measures being taken to control the spread of SARS-CoV-2 and of the virus-associated diseases (COVID-19) are conceptually those that were taken to control the spread of SARS-CoV in the previous coronavirus-driven pandemic of 2002-2003: good hygiene, facemasks, and quarantine (lockdown). The difference is the larger scale of these measures for SARS-CoV-2.

A degraded/dysfunctional immune system appears to be the main determinant of serious/fatal reaction to viral infection (for COVID-19, SARS, and influenza alike). There are four major approaches being employed or considered presently to augment or strengthen the immune system, in order to reduce adverse effects of viral exposure. The three approaches that are focused mainly on augmenting the immune system are based on the concept that pandemics can be controlled/prevented while maintaining the immune-degrading lifestyles followed by much of the global population. The fourth approach is based on identifying and introducing measures aimed at strengthening the immune system intrinsically in order to minimize future pandemics.

Specifically, the four measures are: 1) restricting exposure to virus; 2) providing reactive/tactical treatments to reduce viral load; 3) developing vaccines to prevent, or at least attenuate, the infection; 4) strengthening the immune system intrinsically, by a) identifying those factors that contribute to degrading the immune system, then eliminating/reducing them as comprehensively, thoroughly, and rapidly as possible, and b) replacing the eliminated factors with immune-strengthening factors.

A previous monograph [1] focused mainly on strengthening the immune system intrinsically, and secondarily on vaccine-related issues. It identified many hundreds of factors that contribute to weakening the immune system, as well as measures that can strengthen it.

The present monograph focuses on vaccine safety. A future COVID-19 vaccine appears to be the treatment of choice at the national/international level globally. Vaccine development has been accelerated to achieve this goal in the relatively near-term, and questions have arisen whether vaccine safety has been/is being/will be compromised in pursuit of a shortened vaccine development time.

In addition to identifying short-term adverse vaccine effects, the present monograph identifies potential mid-and long-term adverse vaccine effects that cannot be identified in short-term tests characteristic of vaccine efficacy testing. To ensure vaccine safety, long-term testing under real-life conditions (exposures to multiple toxic stimuli) is required. There is an incompatibility between the accelerated vaccine development times being pursued by government and industry and the long times required for validation of vaccine safety.

In summary, it is difficult to see how safe COVID-19 vaccines can be developed and fully tested for safety on development time scales of one or two years, as proposed presently. The only real protection against a future COVID-19 pandemic or any other viral pandemic is the one that was demonstrated to work in the SARS, MERS, and COVID-19 pandemic, and in the annual influenza pandemics: a healthy immune system capable of neutralizing incoming viruses as nature intended.

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Chapter 1 – Introduction

1A. Background

Over the past two decades, there have been at least three major coronavirus-based infectious disease outbreaks/epidemics/pandemics: Severe Acute Respiratory Syndrome (SARS), 2002-2003; Middle East Respiratory Syndrome (MERS), starting in 2012; COVID-19, starting in December 2019. There are a number of similarities among these three infectious diseases, including abnormal values of selected biomarkers (e.g., neutrophils, lymphocytes, albumin, CRP, TNF-alpha, etc.), pulmonary inflammation, pulmonary damage. The most important similarity among these infectious diseases is the demographic affected most severely. This demographic tends to be the elderly, with comorbidities and degraded/dysfunctional immune systems, and others with degraded/dysfunctional immune systems [2-12]. While there is some decline in the immune system with age, comorbidity is a stronger predictor of impaired immunity than chronological age in older adults [13-14].

There are also similarities between COVID-19 and influenza: "Both (COVID-19 and influenza) cause fever, cough, body aches and fatigue; sometimes vomiting and diarrhea; can be mild or severe, even fatal in rare cases; can result in pneumonia" [15]. Additionally, "Neither virus is treatable with antibiotics, which only work on bacterial infections; both are treated by addressing symptoms, such as reducing fever; severe cases may require hospitalization and support such as mechanical ventilation" [15]. Both COVID-19 and influenza share the demographic affected most severely, as well.

The main measures being taken to control the spread of the SARS-CoV-2 coronavirus (the virus mainly associated with COVID-19) are conceptually those that were taken to control the spread of the SARS-CoV coronavirus in 2002-2003: good hygiene and quarantine (lockdown). The difference is the scale of these measures. Currently, many countries are on lockdown (at different levels of severity), restricting many activities and businesses that involve gatherings of large numbers of people in close proximity. As of mid-September, 2020, it is unknown how long these restrictions will be in place.

1B. Treatments

There are myriad efforts being pursued to develop treatments and preventative measures for COVID-19. Some of these will now be outlined.

If treatments are defined as a set of actions that improve health, then (at least) two types of treatments are possible. The first type can be defined as positive treatments. They can be sub-divided into high-tech treatments and low-tech treatments. The high-tech are the classical treatments where drugs (or supplements) and/or radiation and/or surgery are implemented, and symptoms are alleviated. These high-tech positive treatments are basically a reactive tactical response to abnormal markers of health. They can be applied for the short-term (e.g., antibiotics for bacterial infections, antivirals for viral infections, etc.), or for the long-term (e.g., statins, blood thinners, antihypertensives, etc.). The low-tech treatments involve dietary, sleep, and other behavioral changes shown to impact the immune system positively (see section A4-C of our previous COVID-19 monograph [1] for a bibliography of low-tech immune system strengthening factors). For long-term benefit, these low-tech treatments need to be maintained indefinitely. On average, the high-tech treatments have greater risk than the low-tech treatments.

The second type can be defined as negative-negative treatments, where those factors that contribute to disease are first identified and then removed. The name derives from the mathematics world, where a negative of a negative is a positive. These negative-negative treatments are basically a proactive strategic response to abnormal markers of health, and typically involve long-term changes in lifestyle and harmful exposures for improved health.

1B1. Tactical Treatments

Much of the effort to help the most vulnerable COVID-19 demographic at this time has been searching for, and experimenting with, treatments that were/are used to combat other (mainly) viral diseases (aka repurposed treatments). These treatments include, but are not limited to:

Actemra/Tocilizumab; Avigan/Favipiravir; Azithromycin; Baricitinib/Olumiant; Bevacizumab/Avastin; Calquence/Acalabrutinib; Chloroquine; Colcrys/Colchicine; Convalescent Plasma; EIDD-2801; Fingolimod/Gilenya; Galidesivir; Hydroxychloroquine; Ilaris/Canakinumab; Ivermectin; Jakafi/Ruxolitinib; Kaletra/Lopinavir/Ritonavir; Kevzara/Sarilumab; Kineret/Anakinra; Leronlimab; Mavrilimumab; Methylprednisolone; Olumiant/Baricitinib; Otezla/Apremilast; Remdesivir; Tamiflu/Oseltamivir; Umifenovir/Arbidol; Xeljanz/Tofacitinib [16-18].

Other novel treatments could be identified using our Literature-Related Discovery and Innovation (LRDI)-based treatment repurposing methodology [19].

1B2. Strategic Treatments

Strategic treatments were the focus of our previous COVID-19 monograph [1]. Their identification is a two-step process. First, markers of immune system health (ranging from specific biomarkers to more general descriptors) are selected. Second, those substances (e.g., smoking, excess alcohol, pesticides, etc.) behaviors (e.g., sedentary lifestyle, substance abuse, etc.), and other toxic stimuli that degrade the levels of these markers (i.e., lead to immune dysfunction, immunotoxicity, immunosuppression, etc.) are then identified and recommended for elimination. The strategic treatments identified in the previous monograph are those contained within the immune system core literature. Additional novel strategic treatments could also be identified using our LRDI-based treatment repurposing methodology [19].

1B3. Reactive Tactical vs Proactive Strategic Treatments

The reactive tactical treatment approach for countering infections from viral exposure improves biomarker levels and reduces symptoms (if successful), but ordinarily does little to improve the body's resistance to disease. For viral infections, the tactical treatments will do little to strengthen the degraded/dysfunctional immune (and other) system. After tactical treatments for one viral infection, people with degraded/dysfunctional immune systems will again be vulnerable to serious infectious consequences from exposure to the next harmful virus they encounter.

The proactive strategic treatment approach will strengthen the immune (and other) system by removing those critical factors that contribute to disease and a degraded/dysfunctional immune system (unless irreversible damage has been done to the immune system, or individuals possess congenital or other hereditary damage to their immune system). These strategic treatments tend to require long-term adherence by their recipients. In turn, these recipients of strategic treatments will be less

vulnerable to infection from exposure to the next pathogenic virus they encounter (SARS-CoV-2 or otherwise). Like many healthy people who were exposed to SARS-CoV and SARS-CoV-2, these people who follow the (typically) long-term proactive strategic treatment regimen successfully may not even be aware they have been exposed to, or infected by, the coronavirus. The only indication of their infection will be coronavirus antibodies in their serum.

1C. Structure of Monograph

The remainder of this monograph is structured as follows. Chapter 2 overviews the methodology used to identify potential safety issues in successful COVID-19 vaccine development. Chapter 3 presents the potential safety issues that need to be addressed before a commercial vaccine can be viewed as intrinsically safe. Chapter 4 contains the references for the present study. Appendix 1 provides a short bibliography of vaccine adverse effects.

Chapter 2 – Methodology

A hybrid methodology was used to identify references showing potential long-term adverse effects of vaccines and vaccine/infection-induced mechanisms that could contribute to these adverse effects. Based on reading myriad vaccine adverse effects review articles, terms showing mechanisms were extracted (e.g., antibody-dependent enhancement, viral interference, route of infection, original antigenic sin, etc), and used as a Medline query to retrieve potentially relevant articles. These articles were read, and the most relevant ones extracted. Their titles were entered into the Web of Science, and the citation network was explored (citing papers, cited papers, papers that shared common references, etc). Those records were read, and the most relevant ones extracted for this monograph.

Chapter 3 – Results and Conclusions

3A. Overview

The main body of our previous COVID-19 monograph [1] addressed the first type of strategic treatment: 1) identification and removal of factors contributing to weakening the immune system (see section A4-A of the previous monograph [1] for a table of these contributing factors), and 2) identification and addition of factors contributing to strengthening the immune system (see section A4-C of the previous monograph [1] for a bibliography of low-tech immune system strengthening factors). The present chapter addresses the second type of strategic treatment: development and implementation of a COVID-19 vaccine. The prospects for such a vaccine will be addressed from three criteria perspectives: development time, efficacy, and safety.

Calina et al. evaluated the ongoing approaches to COVID-19 vaccine development, and stated: "Normally, the period of development of a vaccine is 12-15 years" [20]. Against this backdrop, SARS-CoV-2 vaccines are targeted for accelerated development, safety testing, manufacturing, and distribution by an order of magnitude [21-22]. Each of the accelerated steps listed in this reference has drastically reduced the time required from normal development.

3B. Past Coronavirus Vaccine Development History

There have been two prior coronavirus pandemics in the 21st century: SARS in 2002-2003, and MERS starting in 2012. Vaccine development for each started/accelerated during the height of each pandemic. What have been the results of these prior coronavirus vaccine development efforts?

According to a comprehensive 2019 article on MERS vaccine development [23], "To date, there is no specific treatment proven effective against this viral disease. In addition, no vaccine has been licensed to prevent MERS-CoV infection thus far ... In general, the potential vaccine candidates can be classified into six types: viral vector-based vaccine, DNA vaccine, subunit vaccine, nanoparticle-based vaccine, inactivated-whole virus vaccine and live-attenuated vaccine"

According to a comprehensive 2020 article on SARS and MERS vaccine development [24], "As of April 2020, no vaccine is commercially available for these coronavirus strains". The rationale for lack of a vaccine is given by the following: "Reasons for the lack of commercial and effective vaccines for SARS and MERS are varied. In the case of MERS, it is likely that the vaccine development was delayed because of the scarcity of suitable and cost-effective small animal models during pre-clinical experimentation. In addition, it is probable that a vaccine has not been delivered because of the low interest in investing in a vaccine for a disease that has produced relatively low and geographically centralized cases (compared with other more global and persistent infectious diseases such as influenza, HIV and tuberculosis). This last factor might have also contributed to the lack of a vaccine for SARS, in the sense that it was considered pointless to continue investing in a vaccine for a disease whose cases ceased to be reported in 2004."

While interest in a vaccine may have waned after the SARS pandemic seemed to have terminated, research on such a vaccine persisted. References in the above article showed SARS vaccine research continued for a decade or more after the pandemic had ended [25-26].

Based on the experiences with SARS and MERS, successful vaccine development was not achieved after about a decade of research, or even more. That does not bode well for COVID-19 coronavirus vaccine development/safety testing/distribution for the one-year timescales being projected.

3C. Challenges for Successful Vaccine Development

3C1. Overview

The main challenges facing successful coronavirus vaccine development can be summarized as time to development, efficacy of the vaccine and, most importantly, safety of the vaccine. A complementary perspective on some of the problems listed in [20] can be stated as follows:

"First, although the virus's spike protein is a promising immunogen for protection, optimizing antigen design is critical to ensure optimal immune response. Debate continues over the best approach — for example, targeting the full-length protein or only the receptor-binding domain.

Second, preclinical experience with vaccine candidates for SARS and the Middle East respiratory syndrome (MERS) have raised concerns about exacerbating lung disease, either directly or as a result of antibody-dependent enhancement. Such an adverse effect may be associated with a type 2 helper T-cell (Th2) response. Hence, testing in a suitable animal model and rigorous safety monitoring in clinical trials will be critical" [27].

Numerous mid- and longer-term potential adverse effects from vaccines have been identified. Their themes are summarized initially, followed by excerpts from specific cited references

1) Antibody-Dependent Enhancement (where enhanced virus entry and replication in a number of cell types is enabled by antibodies);

-1a) Intrinsic Antibody-Dependent Enhancement (where nonneutralizing antibodies raised by natural infection with one virus may enhance infection with a different virus)

-1b) Immune Enhancement (enhancement of secondary infections via immune interactions)

-1c) Cross-reactivity (an antibody raised against one specific antigen has a competing high affinity toward a different antigen.)

-1d) Cross-Infection Enhancement (infection enhancement of one virus by antibodies from another virus)

2) Vaccine-associated Virus Interference (where vaccinated individuals may be at increased risk for other respiratory viruses because they do not receive the non-specific immunity associated with natural infection);

3) Vaccine-Associated Imprinting Reduction (where vaccinations could also reduce the benefits of 'imprinting', a protection conferred upon children who experienced infection at an early age);

4) Non-Specific Vaccine Effects on Immune System (where previous infections can alter an individual's susceptibility to unrelated diseases);

5) Impact of Infection Route on Immune System (where immune protection can be influenced by the route of exposure/delivery);

6) Impact of Combinations of Toxic Stimuli (where people are exposed over their lifetime to myriad toxic stimuli that may impact the influence of any vaccine);

7) Antigenic Distance Hypothesis (negative interference from prior season's influenza vaccine (v1) on the current season's vaccine (v2) protection may occur when the antigenic distance is small between v1 and v2 (v1 ≈ v2) but large between v1 and the current epidemic (e) strain (v1 ≠ e).)

8) Bystander Activation (activation of T cells specific for an antigen X during an immune response against antigen Y)

9) Gut Microbiota (Impact of gut microbial composition on vaccine response)

10) Homologous Challenge Infection Enhancement (the strain of challenge virus used in the testing assay is very closely related to the seed virus strain used to produce the vaccine that a subject received)

11) Immune Evasion (evasion of host response to viral infection)

12) Immune Interference (interference from circulating antibody to the vaccine virus)

-12a) Original antigenic sin (propensity of the body's immune system to preferentially utilize immunological memory based on a previous infection when a second slightly different version of that foreign entity (e.g. a virus or bacterium) is encountered.)

13) Prior Influenza Infection (effects of prior influenza infection on severity of future disease symptoms)

14) Timing between Viral Exposures (elapsed time between viral exposures)

15) Vaccine-Associated Enhanced Respiratory Disease (where vaccination enhances respiratory disease)

16) Chronic Immune Activation (continuous innate immune responses)

Each of these effects will now be addressed in more detail.

3C2. Potential Mechanistic Adverse Effects from Vaccines

3c2a. Antibody-Dependent Enhancement (where enhanced virus entry and replication in a number of cell types is enabled by antibodies)

"preclinical experience with vaccine candidates for SARS and the Middle East respiratory syndrome (MERS) have raised concerns about exacerbating lung disease, either directly or as a result of antibody-dependent enhancement. Such an adverse effect may be associated with a type 2 helper T-cell (Th2) response. Hence, testing in a suitable animal model and rigorous safety monitoring in clinical trials will be critical" [27].

"Examples of vaccine-induced enhancement of susceptibility to virus infection or of aberrant viral pathogenesis have been documented for infections by members of different virus families. Several mechanisms, many of which still are poorly understood, are at the basis of this phenomenon … Certain experimental lentiviral vaccines even proved to be counterproductive: they rendered vaccinated subjects more susceptible to infection rather than protecting them. For vaccine-induced enhanced

susceptibility to infection with certain viruses like feline coronavirus, Dengue virus, and feline immunodeficiency virus, it has been shown that antibody-dependent enhancement (ADE) plays an important role … Consequently, vaccine-induced enhancement has been a major stumble block in the development of certain flavi-, corona-, paramyxo-, and lentivirus vaccines. Also recent failures in the development of a vaccine against HIV may at least in part be attributed to induction of enhanced susceptibility to infection" [28].

"for a number of viral pathogens, under certain conditions, antibodies provide an attractive means of enhanced virus entry and replication in a number of cell types. Known as antibody-dependent enhancement (ADE) of infection, the phenomenon occurs when virus-antibody immunocomplexes interact with cells bearing complement or Fc receptors, promoting internalization of the virus and increasing infection. Frequently associated with exacerbation of viral disease, ADE of infection presents a major obstacle to the prevention of viral disease by vaccination and is thought to be partly responsible for the adverse effects of novel antiviral therapeutics such as intravenous immunoglobulins." [29].

"Previous studies have shown that the immunization of mice with inactivated whole SARS- CoV, the immunization of rhesus macaques with MVA- encoded S protein and the immunization of mice with DNA vaccine encoding full- length S protein could induce ADE or eosinophil- mediated mmunopathology to some extent, possibly owing to low quality and quantity of antibody production. Additionally, we need to consider whether a vaccine is safe and effective in aged hosts.

For instance, double- inactivated SARS-CoV vaccine failed to induce neutralizing antibody responses in aged mice. Furthermore, although an alum- adjuvanted double- inactivated SARS- CoV vaccine elicited higher antibody titres in aged mice, it skewed the IgG subclass toward IgG1 instead of IgG2, which was associated with a T helper 2 (TH2)- type immune response, enhanced eosinophilia and lung pathology." [30]. Also, see [31-33].

"Under certain circumstances, a viral infection or vaccination may result in a subverted immune system, which may lead to an exacerbated illness. Clinical evidence of enhanced illness by preexisting antibodies from vaccination, infection or maternal passive immunity is available for several viruses and is presumptively proposed for other viruses…..It has been confirmed that certain infection- and/or vaccine-induced immunity could exacerbate viral infectivity in Fc receptor- or complement bearing cells-mediated mechanisms." [34].

3C2a1. Intrinsic Antibody-Dependent Enhancement (where nonneutralizing antibodies raised by natural infection with one virus may enhance infection with a different virus)

"Preexisting antibodies may enhance viral infections. In dengue, nonneutralizing antibodies raised by natural infection with one of four dengue viruses (DENVs) may enhance infection with a different virus by a process we term "intrinsic antibody-dependent enhancement" (iADE). In addition, nonprotective antibodies raised by formalin-inactivated respiratory syncytial virus (RSV) and measles virus vaccines have led to enhanced disease during breakthrough infections. Infections under iADE conditions not only facilitate the process of viral entry into monocytes and macrophages but also modify innate and adaptive intracellular antiviral mechanisms, suppressing type 1 interferon (IFN) production and resulting in enhanced DENV replication." [35].

"Using an adjuvanted and an unadjuvanted double-inactivated SARS-CoV (DIV) vaccine, we demonstrate an eosinophilic immunopathology in aged mice comparable to that seen in mice immunized with the SARS nucleocapsid protein, and poor protection against a nonlethal heterologous challenge…..In the absence of alum, DIV vaccine performed poorly in young animals challenged with lethal homologous or heterologous strains. In contrast, DIV vaccines (both adjuvanted and unadjuvanted) performed poorly in aged-animal models. Importantly, aged animals displayed increased eosinophilic immune pathology in the lungs and were not protected against significant virus replication. These data raise significant concerns regarding DIV vaccine safety and highlight the need for additional studies of the molecular mechanisms governing DIV-induced eosinophilia and vaccine failure, especially in the more vulnerable aged-animal models of human disease." [36].

"Dengue provides the most abundant example in human medicine and the greatest human illness burden caused by the phenomenon of intrinsic antibody-dependent infection enhancement (iADE). In this immunopathological phenomenon infection of monocytes or macrophages using infectious immune complexes suppresses innate antiviral systems, permitting logarithmic intracellular growth of dengue virus. The four dengue viruses evolved from a common ancestor yet retain similar ecology and pathogenicity, but although infection with one virus provides short-term cross-protection against infection with a different type, millions of secondary dengue infections occur worldwide each year. When individuals are infected in the virtual absence of cross-protective dengue antibodies, the dengue vascular permeability syndrome (DVPS) may ensue." [37].

"These symptoms are the result of uncontrolled immune activation. Macrophages and dendritic cells are the main target of dengue virus (DENV) and the cellular source of cytokines associated with this immune activation. Macrophages and dendritic cells express several innate immune receptors that have been implicated in DENV immune activation, of which, CLEC5A, RIG-I and MDA5 are most important. Notably, activation of these receptors have profound effects on adaptive immune responses against DENV. This review will focus on how innate immune receptors drive DENV immune activation by inducing inflammatory cytokines and by activating adaptive immune responses." [38].

"Antibody-dependent enhancement (ADE) of dengue virus infection has been proposed as the early mechanism underlying DHF/DSS. Dengue cross-reactive antibodies raised following a first dengue infection combine with a second infecting virus to form infectious immune complexes that enter Fc-receptor-bearing cells. This results in an increased number of infected cells and increased viral output per cell. At the late illness stage, high levels of cytokines, possibly the result of T cell elimination of infected cells, result in vascular permeability, leading to shock and death." [39].

"Epidemiologic and observational studies demonstrate that the majority of severe dengue cases, dengue hemorrhagic fever and dengue shock syndrome (DHF/DSS), occurs predominantly in either individuals with cross-reactive immunity following a secondary heterologous infection or in infants with primary DENV infections born from dengue-immune mothers, suggesting that B-cell-mediated and antibody responses impact on disease evolution. We demonstrate here that B cells play a pivotal role in host responses against primary DENV infection in mice…..In addition, we show that poly and monoclonal anti-DENV-specific antibodies can sufficiently increase viral replication through a suppression of early innate antiviral responses and enhance disease manifestation, so that a mostly non-lethal illness becomes a fatal disease resembling human DHF/DSS. Finally, treatment with intravenous immunoglobulin containing anti-DENV antibodies confirmed the potential enhancing capacity of

subneutralizing antibodies to mediate virus infection and replication and induce severe disease manifestation of DENV-infected mice. Thus, our results show that humoral responses unleashed during DENV infections can exert protective or pathological outcomes and provide insight into the pathogenesis of this important human pathogen." [40].

"2. Antibody-mediated enhancement of SARS-CoV infection with anti-SARS-CoV Spike immune-serum was observed in vitro. 3. Antibody-mediated infection of SARS-CoV triggers entry into human haematopoietic cells via an FcgammaR-dependent and ACE2-, pH-, cysteine-protease-independent pathways. 4. The antibody-mediated enhancement phenomenon is not a mandatory component of the humoral immune response elicited by SARS vaccines, as pure neutralising antibody only could be obtained. 5. Occurrence of immune-mediated enhancement of SARS-CoV infection raises safety concerns regarding the use of SARS-CoV vaccine in humans and enables new ways to investigate SARS pathogenesis (tropism and immune response deregulation)." [41].

3C2a2. Immune Enhancement (enhancement of secondary infections via immune interactions)

"To determine whether this strategy could be transposed to another animal model, and by extension, to humans, we have evaluated the efficacy of adenoviral vectors in a natural model of AIDS, infection of the cat by the feline immunodeficiency virus (FIV)…..Six weeks after the second administration, cats were challenged by the intraperitoneal route with the homologous strain, and viral burden in plasma was followed by quantitative RT-PCR. Immunisation with FIV antigens did not afford protection. Rather, viral RNA was detected at earlier time points in cats immunised against Gag than in cats immunised with a vector expressing an irrelevant antigen. Such immune-mediated enhancement did not appear to have a long-range impact on viral set point or inversion of the CD4(+)/CD8(+) ratio. Thus, in the feline AIDS model pre-existing immunity against a viral antigen exacerbated acute phase infection." [42].

"The dengue viruses exist as four antigenically distinct serotypes. These four serotypes co-circulate and interact with each other through multiple immune-mediated mechanisms. Though the majority of previous efforts to understand the transmission dynamics of dengue have assumed identical characteristics for these four serotypes, empirical data suggests that they differ from one another in important ways. Here, we examine dynamics and persistence in models that do not assume symmetry between the dengue viruses. We find that for serotype transmission rates that are only slightly asymmetric, increased transmissibility of secondary infections through immune enhancement increases the persistence of all dengue viruses in opposition to findings in symmetric models. We identify an optimal magnitude of immune enhancement that maximizes the probability of persistence of all four serotypes. In contrast to other pathogen systems where heterogeneity between serotypes in transmissibility facilitates competitive exclusion…..here we find that in the presence of Antibody Dependent Enhancement (ADE) heterogeneity can increase the persistence of multiple serotypes of dengue." [43].

"Increasing evidence points to host Th17 inflammatory responses as contributing to the severe lung pathology and mortality of lower respiratory tract infections from coronaviruses. This includes host inflammatory and cytokine responses to COVID-19 caused by the SARS-2 coronavirus (SARS CoV2). From studies conducted in laboratory animals, there are additional concerns about immune enhancement and the role of potential host immunopathology resulting from experimental human COVID-19 vaccines. Here we summarize evidence suggesting there may be partial overlap between the underlying

immunopathologic processes linked to both coronavirus infection and vaccination, and a role for Th17 in immune enhancement and eosinophilic pulmonary immunopathology. Such findings help explain the link between viral-vectored coronavirus vaccines and immune enhancement and its reduction through alum adjuvants. Additional research may also clarify links between COVID-19 pulmonary immunopathology and heart disease." [44].

"Here, an African green monkey (AGM) model was used to elucidate immune mechanisms that facilitate viral clearance but may also contribute to persistent lung inflammation following SARS-CoV infection. During primary infection, SARS-CoV replicated in the AGM lung for up to 10 days. Interestingly, lung inflammation was more prevalent following viral clearance, as leukocyte numbers peaked at 14 days postinfection (dpi) and remained elevated at 28 dpi compared to those of mock-infected controls. Lung macrophages but not dendritic cells were rapidly activated, and both cell types had high activation marker expression at late infection time points…..In SARS-CoV homologous rechallenge studies, 11 of the 12 animals were free of replicating virus at day 5 after rechallenge. However, incidence and severity of lung inflammation was not reduced despite the limited viral replication upon rechallenge. Evaluating the role of antibodies in immune protection or potentiation revealed a progressive increase in anti-SARS-CoV antibodies in lung and serum that did not correlate temporally or spatially with enhanced viral replication. This study represents one of the first comprehensive analyses of lung immunity, including changes in leukocyte populations, lung-specific cytokines, and antibody responses following SARS-CoV rechallenge in AGMs." [45].

3C2a3. Cross-reactivity (an antibody raised against one specific antigen has a competing high affinity toward a different antigen.)

The potential of cross-reactivity having adverse impact in a coronavirus environment is sufficiently serious to merit more detailed discussion in section 3C5.

"our own findings that 21 out of 50 tissue antigens had moderate to strong reactions with the SARS-CoV-2 antibodies are a sufficiently strong indication of cross-reaction between SARS-CoV-2 proteins and a variety of tissue antigens beyond just pulmonary tissue, which could lead to autoimmunity against connective tissue and the cardiovascular, gastrointestinal, and nervous systems." [46].

Concern has been raised that using the full SARS-CoV-2 protein as a vaccine antigen (as is done for myriad vaccines) could lead to cross-reactivity-induced autoimmune disease. "before considering a protein as a vaccine antigen, special care should be taken in analyzing the sequence of tissue cross-reactive epitopes in order to avoid possible future side effects…..we feel that our own findings that 21 out of 50 tissue antigens had moderate to strong reactions with the SARS-CoV-2 antibodies are a sufficiently strong indication of cross-reaction between SARS-CoV-2 proteins and a variety of tissue antigens beyond just pulmonary tissue, which could lead to autoimmunity against connective tissue and the cardiovascular, gastrointestinal, and nervous systems."[47]. See section 3C5 for a more detailed discussion of the relation between molecular mimicry and autoimmune disease.

3C2a4. Cross-Infection Enhancement (infection enhancement of one virus by antibodies from another virus)

"anti-DENV antibodies can enhance the infectivity of DENV for certain classes of immune cells, causing increased viral production that correlates with severe disease outcomes. Similarly, ZIKV has been shown to undergo ADE in response to antibodies generated by other flaviviruses. We tested the neutralizing and enhancing potential of well-characterized broadly neutralizing human anti-DENV monoclonal antibodies (HMAbs) and human DENV immune sera against ZIKV using neutralization and ADE assays. We show that anti-DENV HMAbs, cross-react, do not neutralize, and greatly enhance ZIKV infection in vitro. DENV immune sera had varying degrees of neutralization against ZIKV and similarly enhanced ZIKV infection. Our results suggest that pre-existing DENV immunity may enhance ZIKV infection in vivo and may lead to increased disease severity. Understanding the interplay between ZIKV and DENV will be critical in informing public health responses and will be particularly valuable for ZIKV and DENV vaccine design and implementation" [48].

"Anti-Flavivirus antibodies are highly cross-reactive and may facilitate Zika virus (ZIKV) infection through the antibody-dependent enhancement (ADE) mechanism. We demonstrate that dengue-specific antibodies enhance the infection of a primary Brazilian ZIKV isolate in a Fc gamma RII-expressing K562 cell line. In addition, we demonstrate that serum samples from dengue-immune pregnant women enhanced ZIKV infection. These findings highlight the need for epidemiological studies and animal models to further confirm the role of ADE in the development of congenital and neurological complications associated with ZIKV infections." [49].

3C2b. Vaccine-Associated Virus Enhancement (where vaccinated individuals may be at increased risk for other respiratory viruses because they do not receive the non-specific immunity associated with natural infection)

"Examining non-influenza viruses specifically, the odds of both coronavirus and human metapneumovirus in vaccinated individuals were significantly higher when compared to unvaccinated individuals (OR = 1.36 and 1.51, respectively) ... the laboratory data in our study showed increased odds of coronavirus and human metapneumovirus in individuals receiving influenza vaccination ... While influenza vaccination offers protection against influenza, natural influenza infection may reduce the risk of non-influenza respiratory viruses by providing temporary, non-specific immunity against these viruses ... On the other hand, recently published studies have described the phenomenon of vaccine-associated virus interference; that is, vaccinated individuals may be at increased risk for other respiratory viruses because they do not receive the non-specific immunity associated with natural infection" [50].

"We identified a statistically significant increased risk of noninfluenza respiratory virus infection among TIV {trivalent inactivated influenza vaccine} recipients, including significant increases in the risk of rhinovirus and coxsackie/echovirus infection ... Receipt of TIV could increase influenza immunity at the expense of reduced immunity to noninfluenza respiratory viruses" [51].

"prior receipt of 2008-09 TIV was associated with increased risk of medically attended pH1N1 illness during the spring-summer 2009, with estimated risk or odds ratios ranging from 1.4 to 2.5" [52].

"Among children there was an increase in the hazard of ARI {acute respiratory illness} caused by non-influenza respiratory pathogens post-influenza vaccination compared to unvaccinated children during the same period." [53].

"when influenza type A hit early, RSV {respiratory syncytial virus} outbreaks tended to be delayed, coronavirus outbreaks tended to be intensified" [54].

"Here we show that reported influenza vaccination coverage rates for 29 OECD countries are associated significantly with recently observed SARS-CoV-2 infection rates in these countries. This early result, which merits further investigation, suggests that during the current coronavirus outbreak an influenza vaccination background might be a relevant factor for SARS-CoV-2 infection" [55].

3C2c. Vaccine-Associated Imprinting Reduction (where vaccinations could also reduce the benefits of 'imprinting', a protection conferred upon children who experienced infection at an early age)

"Imprinting by the first childhood influenza infection is known to confer long-lasting immunity focused toward priming epitopes. Our findings suggest vaccine mismatch may negatively interact with imprinted immunity. The immunological mechanisms for imprint-regulated effect of vaccine (I-REV) warrant investigation." [56].

"we suggest that the potential impact of distant influenza immune imprinting on current vaccination outcomes should be considered in the design of next generation or universal vaccine candidates" [57].

3C2d. Non-Specific Vaccine Effects on Immune System (where previous infections can alter an individual's susceptibility to unrelated diseases)

"vaccines against infectious diseases have nonspecific effects on the ability of the immune system to handle other pathogens. For instance, in randomized trials tuberculosis and measles vaccines are associated with a substantial reduction in overall child mortality, which cannot be explained by prevention of the target disease. New research suggests that the nonspecific effects of vaccines are related to cross-reactivity of the adaptive immune system with unrelated pathogens, and to training of the innate immune system through epigenetic reprogramming ... diphtheria-tetanus-pertussis (DTP) vaccine, although protective against the three target diseases, increases female mortality from other infectious diseases ... and it turned out that DTP vaccine administered after the measles vaccine was the explanation for the increased female mortality observed in the high-titer measles vaccine trials ...The effects of vaccines on the immune system may be modulated by other immune-modulating factors. Interactions are found between vaccines and high-dose vitamin A supplementation ... and two vaccines may have completely different effects when administered simultaneously ... We need to explore systematically what is likely to happen when our effective interventions are administered with other vaccines, drugs, or micronutrients and in different sequences." [58].

"Epidemiological data suggest that previous infections can alter an individual's susceptibility to unrelated diseases … Substantial research efforts have expanded the classical concept of immune memory to also include long-lasting changes in innate immunity and antigen-independent reactivation of adaptive immunity. Collectively, these processes provide possible explanations on how acute infections might induce long-term changes that also affect immunity to unrelated diseases … This heightened state of alert enhances the ability of the immune system to combat even unrelated infections but may also increase susceptibility to autoimmunity. At the same time, infection-induced

changes in the regulatory compartment may dampen subsequent immune responses and promote pathogen persistence." [59].

3C2e. Impact of Infection Route on Immune System (where immune protection can be influenced by the route of exposure/delivery)

Vaccine-based infections have different routes of exposure from natural exposure, and this could lead to different impacts on the immune system. The typical vaccine is injected directly into the bloodstream, thereby bypassing much of the innate immune system, while the naturally acquired infection evolves through the time-consuming process of delay and resistance by the innate immune system. Studies have been performed examining the effects of different routes of exposure. For example:

"our study demonstrates that the identification of candidate LAVs {live attenuated viruses} and immune protection markers in an animal model can be strongly affected by the route of infection used" [60].

"vaccine formulation and route of delivery can influence outcomes as suggested by our studies ... Consideration of alternative methods rather than reliance on parenteral methods for vaccination can lead to vaccination strategies that produce improved efficacy and long-term memory response." [61].

"we have compared pulmonary and subcutaneous delivery of BCG vaccine in the tuberculosis-susceptible DBA/2 mouse strain, a model in which parenterally administered BCG vaccine does not protect against tuberculosis. Our data show that intranasally but not subcutaneously administered BCG confers robust protection against pulmonary tuberculosis challenge." [62].

3C2f. Impact of Combinations of Toxic Stimuli (where people are exposed over their lifetime to myriad toxic stimuli that may impact the influence of any vaccine)

"Toxic stimuli (stressors) exposure limits are typically based on single toxic stimuli experiments, but are presently used for both toxic stimuli in isolation and in combination with other toxic stimuli (simultaneous co-exposure or exposures separated in time). In the combination case, typically less of each constituent of the combination is required to cause damage compared to the amount determined from single stressor experiments." [63]. Also, see [64].

"The effects of vaccines on the immune system may be modulated by other immune-modulating factors. Interactions are found between vaccines and high-dose vitamin A supplementation ... and two vaccines may have completely different effects when administered simultaneously ... We need to explore systematically what is likely to happen when our effective interventions are administered with other vaccines, drugs, or micronutrients and in different sequences." [58].

3C2g. Antigenic distance hypothesis (negative interference from prior season's influenza vaccine (v1) on the current season's vaccine (v2) protection may occur when the antigenic distance is small between v1 and v2 (v1 ≈ v2) but large between v1 and the current epidemic (e) strain (v1 ≠ e).)

"variation in repeat vaccine efficacy is due to differences in antigenic distances among vaccine strains and between the vaccine strains and the epidemic strain in each outbreak…..These results have implications for the selection of influenza vaccine strains, and also for vaccination strategies for other antigenically variable pathogens that might require repeated vaccination." [65].

"Prior vaccination effects varied significantly by season, consistent with the ADH. There was no interference by v1 in 2010-2011 when v1 v2 and v1 e, with comparable VE for v2 alone or v2 + v1: 34% (95% confidence interval [CI] = -51% to 71%) versus 34% (95% CI = -5% to 58%). Negative interference by v1 was suggested in 2012-2013 with nonsignificant reduction in VE when v1 v2 and v1 e: 49% (95% CI = -47% to 83%) versus 28% (95% CI = -12% to 54%). Negative effects of prior vaccination were pronounced and statistically significant in 2014-2015 when v1 = v2 and v1 e: 65% (95% CI = 25% to 83%) versus -33% (95% CI = -78% to 1%).....Effects of repeat influenza vaccination were consistent with the ADH and may have contributed to findings of low VE across recent A(H3N2) epidemics since 2010 in Canada." [66].

"Studies in the 1970s and 1980s signaled concern that repeated influenza vaccination could affect vaccine protection. The antigenic distance hypothesis provided a theoretical framework to explain variability in repeat vaccination effects based on antigenic similarity between successive vaccine components and the epidemic strain.....A meta-analysis of vaccine effectiveness studies from 2010-11 through 2014-15 shows substantial heterogeneity in repeat vaccination effects within and between seasons and subtypes. When negative effects were observed, they were most pronounced for H3N2, especially in 2014-15 when vaccine components were unchanged and antigenically distinct from the epidemic strain. Studies of repeated vaccination across multiple seasons suggest that vaccine effectiveness may be influenced by more than one prior season. In immunogenicity studies, repeated vaccination blunts the hemagglutinin antibody response, particularly for H3N2." [67].

3C2h. Bystander activation (activation of T cells specific for an antigen X during an immune response against antigen Y)

"In the last decade, reports have accumulated on various autoimmune disorders, such as idiopathic thrombocytopenia purpura, myopericarditis, primary ovarian failure, and systemic lupus erythematosus (SLE), following vaccination. In this review, we discuss the possible underlying mechanisms of autoimmune reactions following vaccinations and review cases of autoimmune diseases that have been correlated with vaccination. Molecular mimicry and bystander activation are reported as possible mechanisms by which vaccines can cause autoimmune reactions." [68].

"Autoimmune reactions to vaccinations may rarely be induced in predisposed individuals by molecular mimicry or bystander activation mechanisms. Autoimmune reactions reliably considered vaccine-associated, include Guillain-Barre syndrome after 1976 swine influenza vaccine, immune thrombocytopenic purpura after measles/mumps/rubella vaccine, and myopericarditis after smallpox vaccination" [69].

"Three main mechanisms have been offered to explain the development of autoimmunity: molecular mimicry, epitope spreading, and bystander activation. The latter is characterized by auto-reactive B and T cells that undergo activation in an antigen-independent manner, influencing the development and course of autoimmunity. Activation occurs due to a combination of an inflammatory milieu, co-signaling ligands, and interactions with neighboring cells. In this review, we will discuss the studies performed seeking to define the role of bystander activation in systemic and organ-specific ADs." [70].

3C2i. Gut Microbiota (Impact of gut microbial composition on vaccine response)

-Chemicals

"DEHP exposure altered bacterial communities both in composition and diversity, particularly decreases in Rothia sp. and Bifidobacterium longum in the DEHP group. Furthermore, DEHP exposure significantly enhanced anti-HBsAg-IgM responses in the DEHP group (p = 0.013). Early-life DEHP exposure alter gut microbiota of newborns and may change their immune responses in later life." [71].

"Emerging evidence indicates a central role for the microbiome in immunity. However, causal evidence in humans is sparse…..However, in a second trial of subjects with low pre-existing antibody titers, there was significant impairment in H1N1-specific neutralization and binding IgG1 and IgA responses. In addition, in both studies antibiotics treatment resulted in (1) enhanced inflammatory signatures (including AP-1/NR4A expression), observed previously in the elderly, and increased dendritic cell activation; (2) divergent metabolic trajectories, with a 1,000-fold reduction in serum secondary bile acids, which was highly correlated with AP-1/NR4A signaling and inflammasome activation." [72].

-Nutrition

"In children living in low-income and middle-income countries, undernourishment and repetitive gastrointestinal infections are associated with the failure of oral vaccines. Intestinal dysbiosis associated with these environmental influences, as well as some host-related factors, compromises immune responses and negatively impacts vaccine efficacy." [73].

"Protein-deficient pigs vaccinated with oral AttHRV vaccine had lower protection rates against diarrhea post-VirHRV challenge and significantly increased fecal virus shedding titers (HIFM transplanted but not GF pigs) compared with their protein-sufficient counterparts. Reduced vaccine efficacy in protein-deficient pigs coincided with altered serum IFN-alpha, TNF-alpha, IL-12 and IFN-gamma responses to oral AttHRV vaccine and the suppression of multiple innate immune parameters and HRV-specific IFN-gamma producing T cells post-challenge. In protein-deficient HIFM transplanted pigs, decreased serum KYN, but not TRP levels were observed throughout the experiment, suggesting an association between the altered TRP metabolism and immune responses…..our findings confirm the negative effects of protein deficiency, which were exacerbated in the HIFM transplanted pigs, on innate, T cell and cytokine immune responses to HRV and on vaccine efficacy, as well as on TRP-KYN metabolism." [74].

"Oral vaccination efficacy has been found to vary considerably with differences in geographical locations and socioeconomic status. Specifically, in children living in resource-poor countries, undernourishment and chronic gastrointestinal (GI) infection are associated with the failure of OVs…..Both undernutrition and GI infection have been shown to profoundly affect the microbiota, inducing 'dysbiosis' characterized by narrowed bacterial diversity and increased frequency of bacterial clades associated with the induction of inflammation. Recent studies have demonstrated that the microbiota exerts a profound effect on the development of mucosal immune responses. Therefore, it seems likely that OV failure in resource-poor regions is affected by alterations to the immune response driven by dysbiotic changes to the microbiota." [75].

3C2j. Homologous Challenge (the strain of challenge virus used in the testing assay is very closely related to the seed virus strain used to produce the vaccine that a subject received) Infection Enhancement

"Cats were vaccinated with fixed autologous feline immunodeficiency virus (FIV)-infected cells in order to present viral proteins to the immune system of individual cats in an MHC-matched

fashion…..vaccinated cats were not protected. Instead, accelerated virus replication was found, an observation similar to what previous experiments using other vaccine candidates have shown. Here, the results of the present study are discussed in the light of enhancement of lentivirus infections as a complicating factor in lentivirus vaccine development." [76].

"Immunogenicity and protective activity of four cell-based feline immunodeficiency virus (FIV) vaccines prepared with autologous lymphoblasts were investigated…..under one condition of testing, some vaccine sera enhanced FIV replication in vitro. As a further limit, the vaccines proved inefficient at priming animals for anamnestic immune responses. Two months after completion of primary immunization, the animals were challenged with a low dose of homologous ex vivo FIV. Collectively, 8 of 20 vaccinees developed infection versus one of nine animals mock immunized with fixed uninfected autologous lymphoblasts. After a boosting and rechallenge with a higher virus dose, all remaining animals became infected, thus confirming their lack of protection." [77].

"Immunization induced production of anti-CD134 and anti-SU antibodies that significantly inhibited FIV infection in vitro. However, no vaccine combination protected cats from FIV infection, and neat serum from vaccinated cats enhanced FIV growth in vitro. CD134+SU vaccinated cats exhibited increased CD4:CD8 ratio immediately prior to challenge, and antibodies were much more efficiently generated against vaccine by-products versus target antigens. Results suggest vaccination against viral and cryptic receptor epitopes yields neutralizing antibodies that synergistically inhibit FIV infection in vitro. Factors contributing to vaccine failure may include: (1) Heat-labile serum factors that enhance viral replication, (2) changes in circulating target cell populations induced by vaccination, and (3) weak immunogenicity of neutralizing epitopes compared to off-target vaccine components. Results reinforce the need to monitor vaccine preparation components and avoid non-specific immune stimulation during vaccination." [78].

3C2k. Immune Evasion (evasion of host response to viral infection)

"Influenza viruses have evolved numerous strategies in order to evade the host innate immune response…..The viral nonstructural (NS)1 protein acts at multiple levels to prevent IFN production: NS1 can limit PAMP detection, block essential retinoic acid-inducible gene-I post-translational modifications and block the processing and export of IFN mRNAs…..NS1 may also limit IFN responses by preventing expression of antiviral proteins or by directly inhibiting their activities…..Other strategies used by influenza viruses to evade the effects of IFN include: limiting PAMP formation, general inhibition of host cell protein synthesis, replication compartment, replication speed, induction/inhibition of apoptosis and reduced sensitivity to host antiviral proteins." [79].

"Strategies of immune evasion and modulation used by PRRSV affect both innate and adaptive immune responses. In infected cells, PRRSV sequesters itself to limit PRRs detection, suppresses type I IFN production and signaling, manipulates the cytokine responses and modulates apoptosis. PRRSV structural protein (N) and nonstructional proteins (nsp1, nsp2, nsp4 and nsp11) play significant roles in attenuating signaling through the IFN induction and JAK/STAT pathway. In addition, PRRSV evolves mechanisms to inhibit the process of antigen presentation and induce proliferation and activation of Tregs, causing deficiency of subsequent adaptive immune responses. The effect of ADE also facilitates the entry and propagation of PRRSV." [80].

"This selection pressure exerted by complement on viruses has made them evolve a multitude of countermeasures. These include targeting the recognition molecules for the avoidance of detection, targeting key enzymes and complexes of the complement pathways like C3 convertases and C5b-9 formation - either by encoding complement regulators or by recruiting membrane-bound and soluble host complement regulators, cleaving complement proteins by encoding protease, and inhibiting the synthesis of complement proteins. Additionally, viruses also exploit the complement system for their own benefit. For example, they use complement receptors as well as membrane regulators for cellular entry as well as their spread." [81].

"the major mechanisms of immune evasion strategies of MERS-CoV. We have demonstrated that M, 4a, 4b proteins and Plppro of MERS-CoV inhibit the type I interferon (IFN) and nuclear factor-κB signaling pathways and therefore facilitate innate immune evasion. In addition, nonstructural protein 4a (NSP4a), NSP4b, and NSP15 inhibit doublestranded RNA sensors. Therefore, the mentioned proteins limit early induction of IFN and cause rapid apoptosis of macrophages. MERS-CoV strongly inhibits the activation of T cells with downregulation of antigen presentation. In addition, uncontrolled secretion of interferon ɣ-induced protein 10 and monocyte chemoattractant protein-1 can suppress proliferation of human myeloid progenitor cells." [82].

3C2l. Immune Interference (interference from circulating antibody to the vaccine virus)

"immune interference during co-inoculation was examined using DNA vaccines expressing lentiviral Envs and Gag from gene sequences optimized for efficient expression in mammalian cells (codon-optimized). BALB/c mice vaccinated in separate hind legs with each plasmid individually elicited high titer immune responses, however, when HIV-1 Env(gp120) and HIV-1 Gag(p55) DNA plasmids were co-inoculated, there was a reduction in the immune responses elicited to HIV-1 Gag(p55).....Therefore, anti-HIV-1 Gag immune interference appears specific to co-immunizations with HIV-1 Env(gp120) and may involve a yet undefined immunological mechanism(s)." [83].

"We compared the effect of order of administration of investigational alphavirus vaccines on neutralizing antibody response. Volunteers who received the inactivated eastern and western equine encephalitis (EEE and WEE) vaccines before live attenuated Venezuelan (VEE) vaccine had significantly lower rates of antibody response than those receiving VEE vaccine before EEE and WEE vaccines (66.7% vs. 80.6%; p=0.026). The odds of having a VEE antibody non-response among those initially receiving EEE and WEE vaccines, adjusted for gender, were significant (odds ratio [OR]=2.20; 95% CI=1.2-4.1 [p=0.0145]) as were the odds of non-response among females adjusted for group (OR=1.81; 95% CI=1.2-2.7 [p=0.0037]). Antibody interference and gender effect have major implications for vaccine strategy among those receiving multiple alphavirus vaccines and those developing next generation vaccines for these threats." [84].

3C2l1. Original antigenic sin (propensity of the body's immune system to preferentially utilize immunological memory based on a previous infection when a second slightly different version of that foreign entity (e.g. a virus or bacterium) is encountered.)

"Since all flaviviruses are antigenically related, they are prone to phenomena of immunological memory ('original antigenic sin'), which can modulate immune responses in the course of sequential infections and/or vaccinations. In our study, we analyzed the influence of pre-existing YF vaccine-derived immunity on the antibody response to TBE vaccination. By comparing samples from YF pre-vaccinated

and flavivirus-naive individuals, we show that YF immunity not only caused a significant impairment of the neutralizing antibody response to TBE vaccination but also a reduction of the specific TBE virus neutralizing activities (NT/ELISA-titer ratios). Our results point to a possible negative effect of pre-existing cross-reactive immunity on the outcome of flavivirus vaccination that may also pertain to other combinations of sequential flavivirus infections and/or vaccinations." [85].

"We found that single-clade A, B and C vaccines applied alone induced only limited cross-clade reactivity and that the epitope hierarchy varied according to the immunizing clade. However, combining single-clade HIV-1 vaccines into multi-clade formulations resulted in multiple forms of in vivo immune interference such as original antigenic sin and antagonism, which dampened or even abrogated induction of responses to epitope variants and reduced the breadth of induced T cell responses. Simultaneous administration of individual clade-specific vaccines into anatomically separated sites on the body alleviated antagonism and increased the number of detectable epitope responses." [86].

"it seems clear that OAS can also be detrimental when the boosting of memory responses to conserved, but nonprotective, epitopes comes at the expense of generating new responses against protective, but antigenically drifted, epitopes." [87].

"Antigenic sin has been demonstrated to occur in several infectious diseases in both animals and humans, including human influenza infection and dengue fever…..In the context of viral infections, it is expected that if we are exposed to a native strain of a pathogen, we should be able to mount a secondary immune response on subsequent exposure to the same pathogen. "Original antigenic sin" will not contradict this well-established immunological process, as long as the subsequent infectious antigen is identical to the original one. But "original antigenic sin" implies that when the epitope varies slightly, then the immune system relies on memory of the earlier infection, rather than mount another primary or secondary response to the new epitope which would allow faster and stronger responses. The result is that the immunological response may be inadequate against the new strain, because the immune system does not adapt and instead relies on its memory to mount a response. In the case of vaccines, if we only immunize to a single strain or epitope, and if that strain/epitope changes over time, then the immune system is unable to mount an accurate secondary response. In addition, depending of the first viral exposure the secondary immune response can result in an antibody-dependent enhancement of the disease or at the opposite, it could induce anergy. Both of them triggering loss of pathogen control and inducing aberrant clinical consequences." [88].

3C2m. Prior Influenza Infection (effects of prior influenza infection on severity of future disease symptoms)

"Seventy patients met selection criteria. Mean age was 66 years. Sixty-four (91%) patients had at least one underlying co-morbid condition; these conditions included COPD, congestive heart failure, diabetes, and cancer. 60/70 (85%) tested positive for Influenza A, and 43 tested positive for H1N1. Oseltamivir was initiated in 55 (78%) patients. Forty-four percent of the patients had been vaccinated. When separated by vaccination status, those who had been vaccinated had higher rates of ICU admission, need for mechanical or non-invasive ventilation, and mortality. All but mortality reached statistical significance…..The data suggest that there was no protective effect from prior vaccination in preventing hospital admission, respiratory failure, and mortality in this population of older men admitted to the hospital with influenza." [89].

"mice were first primed by either infection or immunization with A/Puerto Rico/8/34 (PR8) virus, then immunized with whole-inactivated A/Fort Monmouth/1/47 (FM1) virus. The ensuing vaccine responses and the protective efficacy of FM1 were superior in PR8 infection-primed mice compared to PR8 immunization-primed or unprimed mice. Increased FM1-specific Ab responses of PR8 infection-primed mice also broadened cross-reactivity against contemporary as well as antigenically more drifted strains. Further, prior infection heightened the protective efficacy of antigenically distant strains, such as A/Brisbane/59/2006 infection followed by immunization with split pandemic H1N1 vaccine (A/California/07/2009). Therefore, influenza infection is a significant priming event that intensifies future vaccine responses against drift strains." [90].

"During the three influenza seasons, 5838 ILI [influenza-like illness] episodes (4127 subjects) were analysed. Subjects who had an episode of MA-fluA [medically attended influenza A] in the prior season were at a significantly lower risk of MA-fluA in the current season (adjusted odds ratio: 0.38, 95% CI: 0.30-0.50). The overall adjusted VE was 28% (95% CI, 14-40). VE was substantially lower in subjects vaccinated in the prior season compared to those who had not been vaccinated in prior season (19%; 95% CI: 0-35 vs 46%; 95% CI: 26-60, test for interaction, P value <0.05). In subjects who did not have MA-fluA in the prior season showed the attenuation of VE due to repeated vaccination (13%; 95% CI: -7 to 30 vs 44%; 95% CI: 24-59, test for interaction, P < 0.05). However this effect was not detected in subjects who had contracted MA-fluA in the prior season…..Negative effects of repeated vaccination were significant among those without history of MA-fluA in the prior season." [91].

"Reduced seasonal influenza vaccine effectiveness (VE) was observed in individuals who received repeated annual vaccinations. Preexisting influenza antibody levels were also found inversely correlated with postvaccination titers. These reports suggest that preexisting immunity may affect contemporary seasonal vaccine performance." [92].

"Current- and previous-season vaccination generated similar levels of protection, and vaccine-induced protection was greatest for individuals not vaccinated during the prior 5 years." [93].

"Participants who reported receipt of vaccination during either of the previous 2 years had a lower mean fold rise against all strains than with those who did not. Mean fold rises for A(H3N2) and B/Yamagata were particularly weak after repeated vaccination with the same vaccine strain, but we did not generally find significant differences in the proportions of participants with postvaccination titers >= 40 and >= 160…..Overall, we found that reduced antibody responses in repeat vaccinees were particularly reduced among older adults who had received vaccination against the same strains in preceding years." [94].

"long-term effects on mortality have never been supported by direct evidence. In this study we assessed the long-term outcome of influenza vaccination on mortality in the elderly by conducting a 25-year follow-up study of a RCT on the efficacy of influenza vaccination as baseline…..outcomes included all-cause mortality, influenza-related mortality and seasonal mortality…..Single influenza vaccination did not reduce all-cause mortality when compared to placebo (adjusted HR 0.95, 95% CI 0.85-1.05). Also, no differences between vaccination and placebo group were shown for underlying causes of death or seasonal mortality…..In conclusion, this study did not demonstrate a statistically significant effect following single influenza vaccination on long-term mortality in community-dwelling elderly in general." [95].

"Influenza was identified in 78 (24%) households and 125 (9%) individuals; the infection risk was 8.5% in the vaccinated and 8.9% in the unvaccinated (P = .83). Adjusted vaccine effectiveness in preventing community-acquired influenza was 31% (95% confidence interval [CI], -7% to 55%). In vaccinated subjects with no evidence of prior season vaccination, significant protection (62% [95% CI, 17%-82%]) against community-acquired influenza was demonstrated. Substantially lower effectiveness was noted among subjects who were vaccinated in both the current and prior season. There was no evidence that vaccination prevented household transmission once influenza was introduced; adults were at particular risk despite vaccination…..Vaccine effectiveness estimates were lower than those demonstrated in other observational studies carried out during the same season. The unexpected findings of lower effectiveness with repeated vaccination and no protection given household exposure require further study." [96].

3C2n. Timing between Viral Exposures (elapsed time between viral exposures)

"Comprehending the mechanisms behind the impact of vaccine regimens on immunity is critical for improving vaccines. Indeed, the time-interval between immunizations may influence B and T cells, as well as innate responses. We compared two vaccine schedules using cynomolgus macaques immunized with an attenuated vaccinia virus. Two subcutaneous injections 2 weeks apart led to an impaired secondary antibody response and similar innate myeloid responses to both immunizations. In contrast, a delayed boost (2 months) improved the quality of the antibody response and involved more activated/mature innate cells, induced late after the prime and responding to the recall. The magnitude and quality of the secondary antibody response correlated with the abundance of these neutrophils, monocytes, and dendritic cells that were modified phenotypically and enriched prior to revaccination at 2 months, but not 2 weeks. These late phenotypic modifications were associated with an enhanced ex vivo cytokine production (including IL-12/23 and IL-1beta) by PBMCs short after the second immunization, linking phenotype and functions. This integrated analysis reveals a deep impact of the timing between immunizations, and highlights the importance of early but also late innate responses involving phenotypical changes, in shaping humoral immunity." [97].

"This study evaluates the influenza vaccine effectiveness (VE) in preventing laboratory-confirmed cases in Navarre, Spain, in the 2011/12 season in which the peak was delayed until week 7 of 2012…..These results suggest a low preventive effect of the 2011/12 seasonal influenza vaccine, and a decline in VE with time since vaccination." [98].

"During 3 influenza seasons, 1668 influenza-like illness episodes were analyzed, including 421 and 358 episodes of MA-fluA and MA-fluB, respectively……Repeated previous vaccinations over multiple seasons had significant dose-dependent negative impacts on VE against both MA-fluA and MA-fluB." [99].

"Six studies assessed efficacy against MAI in children, yielding the risk ratios (RR) of 2.04 (95% CI 1.29-3.22) when circulating strains mismatched vaccine strains, and 0.64 (0.33-1.22) when circulating strains matched vaccine strains. When stratified by vaccine types, the reduced efficacy was significant for live attenuated influenza vaccine only…..Influenza vaccine efficacy against mismatch strains was lower in repeatedly vaccinated children as compared with those vaccinated for the current season only." [100].

3C2o. Vaccine associated enhanced respiratory disease (where vaccination enhances respiratory disease)

"Vaccine-induced disease enhancement has been described in connection with several viral vaccines in animal models and in humans. We investigated a swine model to evaluate mismatched influenza vaccine-associated enhanced respiratory disease (VAERD) after pH1N1 infection. Vaccinating pigs with whole inactivated H1N2 (human-like) virus vaccine (WIV-H1N2) resulted in enhanced pneumonia and disease after pH1N1 infection…..These cross-reactive anti-HA2 antibodies enhanced pH1N1 infection of Madin-Darby canine kidney cells by promoting virus membrane fusion activity. The enhanced fusion activity correlated with lung pathology in pigs." [101].

"Vaccine-associated enhanced respiratory disease (VAERD) can occur when pigs are challenged with heterologous virus in the presence of non-neutralizing but cross-reactive antibodies elicited by whole inactivated virus (WIV) vaccine. The aim of this study was to compare the effects of heterologous delta1-H1N2 influenza A virus (IAV) challenge of pigs after vaccination with 2009 pandemic H1N1 virus (H1N1pdm09) recombinant hemagglutinin (HA) subunit vaccine (HA-SV) or temperature-sensitive live attenuated influenza virus (LAIV) vaccine, and to assess the role of immunity to HA in the development of VAERD. Both HA-SV and LAIV vaccines induced high neutralizing antibodies to virus with homologous HA (H1N1pdm09), but not heterologous challenge virus (delta1-H1N2). LAIV partially protected pigs, resulting in reduced virus shedding and faster viral clearance, as no virus was detected in the lungs by 5 days post infection (dpi). HA-SV vaccinated pigs developed more severe lung and tracheal lesions consistent with VAERD following challenge. These results demonstrate that the immune response against the HA protein alone is sufficient to cause VAERD following heterologous challenge." [102].

"Control of influenza A virus (IAV) in pigs is done by vaccination of females to provide maternally-derived antibodies (MDA) through colostrum. Our aim was to evaluate if MDA interfere with IAV infection, clinical disease, and transmission in non-vaccinated piglets. In the first study, naive sows were vaccinated with H1N2-delta1 whole inactivated virus (WIV) vaccine. In a follow-up study seropositive sows to 2009 pandemic H1N1 (H1N1pdm09) were boosted with H1N1pdm09 WIV or secondary experimental infection (EXP). MDA-positive pigs were challenged with homologous or heterologous virus, and MDA-negative control groups were included. WIV-MDA piglets were protected from homologous infection. However, piglets with WIV-derived MDA subsequently challenged with heterologous virus developed vaccine associated enhanced respiratory disease (VAERD), regardless of history of natural exposure in the sows. Our data indicates that although high titers of vaccine-derived MDA reduced homologous virus infection, transmission, and disease, MDA alone was sufficient to induce VAERD upon heterologous infection." [103].

3C2p. Chronic Immune Activation

"Dr. Sharilyn Stanley and colleagues inoculated 13 asymptomatic HIV-infected people and 10 uninfected volunteers with tetanus booster shots to stimulate their immune system. Blood samples were drawn on the day of the injection and 3, 7, 14, 21, 28, and 42 days later. Findings showed that the amount of HIV in the blood streams of HIV-infected subjects increased 2- to 36-fold following immunization. Notably, the virus was much more readily grown from the blood cells of 9 of the HIV-infected patients after immunization. In addition, examinations of the immune system cells of the uninfected volunteers found that cells from 7 of these 10 people were more easily infected with HIV in the test tube after immunization than before immunization. Overall, data suggest that ongoing immune

activation may play a part in HIV pathogenesis and may also enhance susceptibility of uninfected people to HIV." [104].

"HIV co-infection is the most critical risk factor for the reactivation of latent tuberculosis (TB) infection (LTBI). While CD4(+) T cell depletion has been considered the major cause of HIV-induced reactivation of LTBI, recent work in macaques co-infected with Mycobacterium tuberculosis (Mtb)/simian immunodeficiency virus (SIV) suggests that cytopathic effects of SIV resulting in chronic immune activation and dysregulation of T cell homeostasis correlate with reactivation of LTBI. This review builds on compelling data that the reactivation of LTBI during HIV co-infection is likely to be driven by the events of HIV replication and therefore highlights the need to have optimum translational interventions directed at reactivation due to co-infection." [105].

"Systemic chronic immune activation is considered today as the driving force of CD4+ T-cell depletion and acquired immunodeficiency syndrome (AIDS). A residual chronic immune activation persists even in HIV-infected patients in which viral replication is successfully inhibited by anti-retroviral therapy, with the extent of this residual immune activation being associated with CD4+ T-cell loss." [106].

3C3. Potential Short- and Long-Term Diseases Resulting from Vaccines

3C3a. Tracking Deficiencies for Vaccine Adverse Effects

While the efficacy issues for a COVID-19 vaccine have been enumerated extensively in recent reviews [29, 34], more emphasis needs to be placed on ensuring mid- and long-term safety are achieved. Vaccines do not appear to have the same safety requirements as many drugs. For example, consider the following excerpts from selected vaccine inserts relative to safety [107]:

-MMR Vaccine "M-M-R II has not been evaluated for carcinogenic or mutagenic potential, or potential to impair fertility ... Animal reproduction studies have not been conducted with M-M-R II.";

-Influenza Vaccine "FLUARIX QUADRIVALENT has not been evaluated for carcinogenic or mutagenic potential or male infertility in animals.";

-DTAP Vaccine "INFANRIX has not been evaluated for carcinogenic or mutagenic potential or for impairment of fertility."; and

-HPV Vaccine "GARDASIL 9 has not been evaluated for the potential to cause carcinogenicity, genotoxicity or impairment of male fertility."

Long-term safety studies of vaccines are rare. The typical vaccine study is aimed at efficacy. Such studies tend to be a few months long, and the main evaluation criterion is titers of antibody in the serum.

Vaccines, especially childhood vaccines, are administered according to a schedule, which now comprises about seventy+ doses covering about sixteen vaccines. The schedule-based combination effects of these seventy+ vaccine doses have not been tested, and, therefore, adverse effects due to real-life vaccine synergies are unknown. Such vaccine combination experiments cannot be limited to the pristine environment of the laboratory, but require testing in humans who are exposed to myriad toxic stimuli that could impact vaccine combination synergies.

Much of the published data for vaccine adverse events (at least in the USA) originates from the Vaccine Adverse Event Reporting System (VAERS) database. VAERS is a passive monitoring system, and, like all similar systems, suffers from substantial under-reporting of adverse events [108]. A groundbreaking study [109], performed by Harvard Pilgrim Healthcare, Inc, reported that "fewer than 1% of vaccine adverse events are reported". In other words, the actual numbers of adverse reactions to vaccines are one to two orders of magnitude higher than those reported in VAERS!

The methodology used by Harvard Pilgrim Healthcare. Inc, for obtaining this result was as follows: "Every patient receiving a vaccine was automatically identified, and for the next 30 days, their health care diagnostic codes, laboratory tests, and medication prescriptions are evaluated for values suggestive of an adverse vaccine event. When a possible adverse event was detected, it was recorded, and the appropriate clinician was to be notified electronically."

Thus, these adverse events that were identified are single-visit short-term adverse events (within thirty days of the vaccination). They do not reflect the results of vaccination combinations administered over a longer period than thirty days, and they do not reflect results of vaccinations of any type in the mid-or long-term [109].

If fewer than 1% of vaccine adverse events are reported, how well does this sample reflect the total number of adverse events actually experienced? This is not a randomly-selected sample, as would be required for a statistically-valid result. Thus, even analyses of short-term adverse effects based on VAERS data are severely flawed. And, if fewer than 1% of these short-term adverse events are reported, what fraction of longer-term adverse events (where the connection between the adverse event and the vaccination becomes more tenuous as time proceeds) would be reported? One can only conclude that a negligible fraction of long-term adverse events is reported in a passive monitoring system like VAERS.

3C3b. Diseases Triggered by Vaccines

A brief analysis was performed of the vaccine biomedical literature to identify diseases potentially triggered by vaccination, especially in the long-term. It should be noted the biomedical literature is very sparse with studies on long-term vaccine effects, especially long-term adverse effects. Large numbers of people and long periods of time are required to identify such adverse events, and draw statistically-valid connections between vaccinations and disease. These efforts would be very resource-intensive, and there appears to be little motivation among the vaccine producers and regulators to make these resources available for such studies. Thus, the following examples reflect the extremely small tip of an extremely large iceberg of long-term adverse vaccine effects.

The two main categories of diseases reported in the biomedical literature triggered by vaccinations are Autoimmune (e.g., Systemic Lupus Erythematosus, Psoriasis, Arthritis, Multiple Sclerosis, Hepatitis, Uveitis, Pseudolymphoma, Guillain-Barre Syndrome, Thrombocytopenic Purpura, etc.) and Neurological (e.g., Autism, Central Demyelinating Diseases, Developmental Disability, Febrile seizures, Narcolepsy, Encephalomyelitis, Autonomic Dysfunction, etc.). Others include Diabetes, Gastrointestinal, Joint-related, Necrobiotic Granuloma, Neutropenia, Pulmonary Fibrosis, etc.

A few specific examples are presented in the following, and a larger bibliography of vaccine adverse effects is presented in Appendix 1.

"Main syndromes associated with systemic toxicity of adjuvanted vaccines…..Acute phase response (APR). . . . .Hypersensitivity reactions. . . . .Induction or worsening of autoimmune diseases. . . . . Modification of drug hepatic metabolism . . . . .Vascular leak syndrome (VLS) . . . . . Oral immunosuppression or tolerance post vaccination." [110].

"vaccinations may also contribute to the mosaic of autoimmunity. Evidence for the association of vaccinations and the development of these diseases is presented in this review. Infrequently reported post-vaccination autoimmune diseases include systemic lupus erythematosus, rheumatoid arthritis, inflammatory myopathies, multiple sclerosis, Guillain-Barre syndrome, and vasculitis." [111].

"Toplak et al…..reported the production of autoantibodies (such as antinuclear and antiphospholipid antibodies) in 92 healthy medical workers up to 6 months after influenza vaccination. Other studies have demonstrated a latency period of years between HiB vaccination and diabetes mellitus, and between HBV vaccination and demyelinating events…..In conclusion, latency periods can range from days to years for postinfection and postvaccination autoimmunity." [112].

"Adults receiving HBV had significantly increased odds ratios (OR) for multiple sclerosis (OR = 5.2, $p < 0.0003$, 95% Confidence Interval (CI) = 1.9 - 20), optic neuritis (OR = 14, $p < 0.0002$, 95% CI = 2.3 - 560), vasculitis (OR = 2.6, $p < 0.04$, 95% CI = 1.03 - 8.7), arthritis (OR = 2.01, $p < 0.0003$, 95% CI = 1.3 - 3.1), alopecia (OR = 7.2, $p < 0.0001$, 95% CI = 3.2 - 20), lupus erythematosus (OR = 9.1, $p < 0.0001$, 95% CI = 2.3 - 76), rheumatoid arthritis (OR = 18, $p < 0.0001$, 95% CI = 3.1 - 740), and thrombocytopenia (OR = 2.3, $p < 0.04$, 95% CI = 1.02 - 6.2) in comparison to the TCV group. Minimal confounding or systematic error was observed." [113].

"The difference in cumulative incidence between those receiving 4 doses and those receiving 0 doses is 54 cases of IDDM/100,000 (P =0.026) at 7 years, (relative risk=1.26). Most of the extra cases of IDDM appeared in statistically significant clusters that occurred in periods ***starting approximately 38 months after immunization*** and lasting approximately 6-8 months. Immunization with pediatric vaccines increased the risk of insulin diabetes in NOD mice…..Exposure to HiB immunization is associated with an increased risk of IDDM. NOD mice can be used as an animal model of vaccine induced diabetes." [114].

3C4. Time Required for Credible COVID-19 Vaccine Safety Studies

As the above results have shown, vaccines can have long-term impacts on the immune system (positive and negative), and short and long-term effects on other diseases. The effects of vaccines can vary according to route of infection, prior history of vaccinations, and, as stated by Benn et al above, administration "with other vaccines, drugs, or micronutrients and in different sequences." [58]. To accelerate the time required to demonstrate long-term safety, laboratory experiments are usually done using animals with relatively short lifespans whose responses to myriad toxic stimuli are similar to that of human beings.

One major difference between these animal experiments and the human model is that the laboratory experiments are usually performed with the administration of a single toxic stimulant, or maybe two, while the human model lives in a sea of toxic stimuli. Also, it is not always clear which animal model simulates the human model best for response to vaccination.

There are many examples in the biomedical literature where combined exposures to toxic stimuli showed adverse effects whereas exposures to the same stimuli in isolation (at the same dosages)

showed no adverse effects [63-64]. Thus, unless these laboratory experiments are performed with a range of combinations of associated immunomodulators, they would not be credible for safety assessment purposes. Such experiments would require enormous amounts of financial and time resources.

The other alternative is to perform these safety studies with human beings. For long-term safety studies (e.g., potential vaccine effects on initiating cancer or Alzheimer's Disease), decades could be required for credible results. Thus, there is a major disconnect between the time required for credible safety studies of a COVID-19 vaccine and the one-year or less vaccine commercialization being propounded by decision-makers and the media today.

3C5. Molecular mimicry and the invalid genetic basis of vaccine pre-clinical tests: the new vaccinology scenario for designing safe and effective vaccines.

The above analyzed Covid-19 vaccine safety considerations become even more cogent in light of the fact that cross-reactivity might represent the mechanism underlying the immunopathology and the disease multitude associated with the coronavirus infection [115]. The rationale is that the sharing of peptides between SARS-CoV-2 and human proteins might trigger immune responses hitting not only the virus but also the human proteins, with consequent autoimmune pathologies in the human host [116]. Hence, the massive viral vs. human peptide commonalities described since 2000 [117-118] clearly explain how the protective anti-viral antibody immune response can become a pathogenic autoimmune attack against the human organism, thereby addressing the issue of why SARS-CoV-2 so heavily attacks the respiratory system [119]. The scientific cross-reactivity context and the clinical data showing that immunization with SARS-CoV antigens causes severe pneumonia [120] suggest a prominent pathogenic role of anti-SARS-CoV antibodies in Covid. In fact, emerging reports show that severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection precedes the appearance of various autoimmune and autoinflammatory diseases, including paediatric inflammatory multisystemic syndrome or multisystem inflammatory syndrome in children [121-122]. Simply put, the current race for obtaining a highly immunogenic anti-SARS-CoV-2 vaccine might actually equate to a race for producing a highly lethal **vaccine also in light of the fact that adjuvanted anti-SARS-CoV-2 would have a higher immunogenicity and autoimmune pathogenicity when compared to SARS-CoV-2 infection.**

This risk of cross-reactivity further increases when considering that it cannot be estimated with the current vaccine pre-clinical tests [123-124]. Indeed, the level of peptide sharing is highest between pathogens and human, murine, and rat proteomes, while is minimal (or absent) with proteomes from nonhuman primates such as gorilla, chimpanzee, and rhesus macaque. That is, from the genetic point of view, primates are unreliable animal models for revealing potential autoimmune cross-reactions in preclinical testing of immunotherapies since, obviously, no cross-reactions can occur in primates in absentia of shared sequences.

On the whole, the above-exposed data open new scenarios in vaccinology by confirming the basic concept first stated in 2000 [117] and then repeatedly illustrated [125-128], according to which only peptide sequences derived from pathogens and absent in the human proteome, i.e., 'non-self' peptides', can lead to safe and efficacious immunotherapies.

Chapter 4 – References

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Appendix 1 - Vaccine Bibliography - Short and Long-Term Vaccine Impacts

The following is a short sampling of records showing potential adverse short and long-term vaccine effects.

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