APPENDIX III IgG4 Mediated Immune Tolerance/ Suppression

Class Switch toward Noninflammatory, Spike-Specific IgG4 Antibodies after Repeated SARS-CoV-2 MRNA Vaccination [Irrang, et al, 2023]

IgG4 antibodies which are spike-protein tolerant are substantially increased after the booster (3rd) shot.

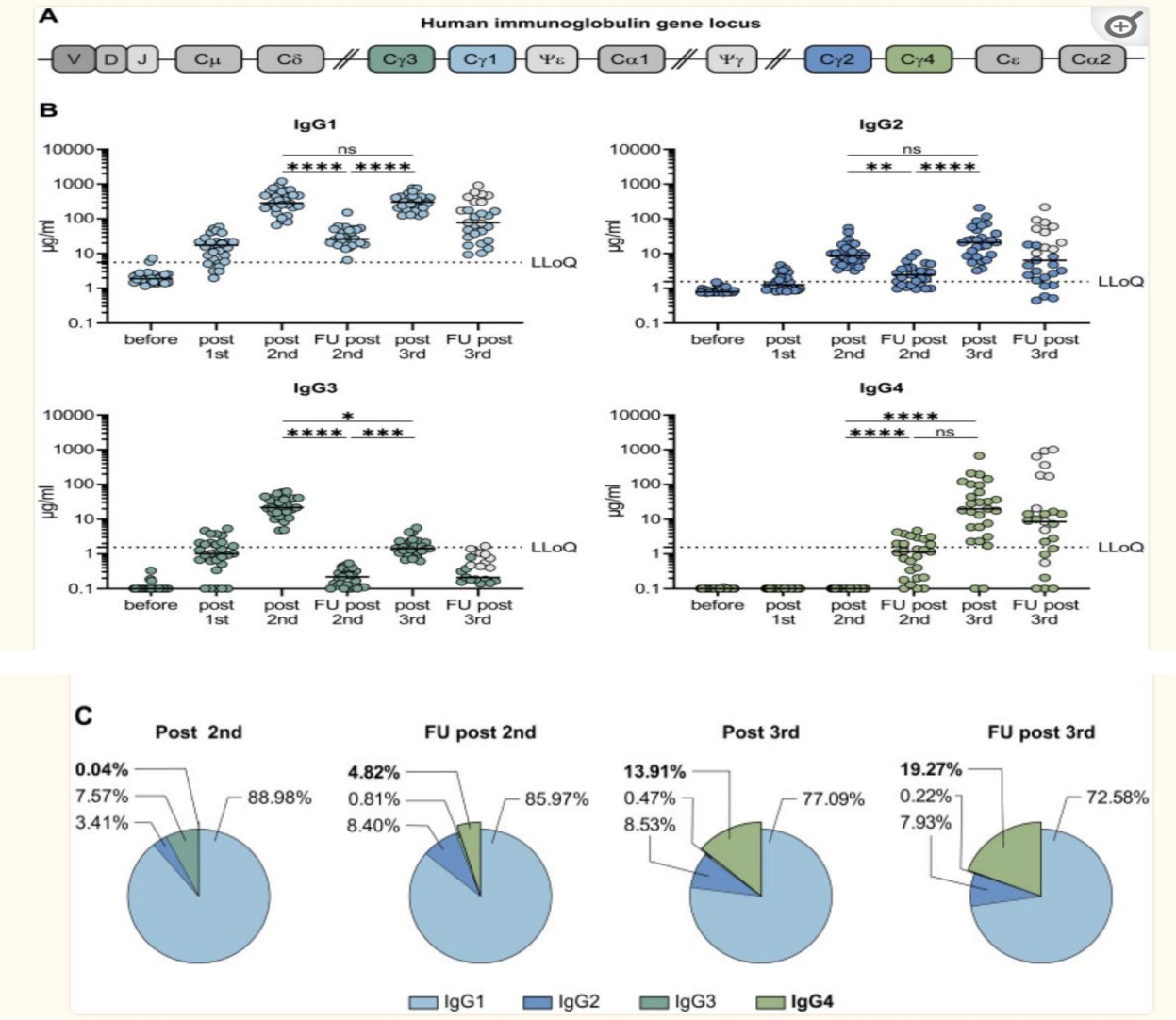

Fig. 1.

Longitudinal analyses of vaccine induced antibody response.

IgG4 Antibodies Induced by Repeated Vaccination May Generate Immune Tolerance to the SARS-CoV-2 Spike Protein [Uversky, et al, 2023]

This paper proposes " a hypothetical immune tolerance mechanism induced by mRNA vaccines, which could have at least six negative unintended consequences:

(1) By ignoring the spike protein synthesized as a consequence of vaccination, the host immune system may become vulnerable to re-infection with the new Omicron subvariants, allowing for free replication of the virus once a re-infection takes place. In this situation, we suggest that even these less pathogenic Omicron subvariants could cause significant harm and even death in individuals with comorbidities and immuno-compromised conditions.

(2) mRNA and inactivated vaccines temporally impair interferon signaling [142,143], possibly causing immune suppression and leaving the individual in a vulnerable situation against any other pathogen. In addition, this immune suppression could allow the re-activation of latent viral, bacterial, or fungal infections and might also allow the uncontrolled growth of cancer cells [144].

(3) A tolerant immune system might allow SARS-CoV-2 persistence in the host and promote the establishment of a chronic infection, similar to that generated by the hepatitis B virus (HBV), the human immune deficiency virus (HIV), and the hepatitis C virus (HCV) [145].

(4) The combined immune suppression (produced by SARS-CoV-2 infection [15,16,17,18,19,20,21,22] and further enhanced by vaccination [142,143,144]) could explain a plethora of autoimmune conditions, such as cancers, re-infections, and deaths temporally associated with both. It is conceivable that the excess deaths reported in several highly COVID-19-vaccinated countries may be explained, in part, by this combined immunosuppressive effect.

(5) Repeated vaccination could also lead to auto-immunity: in 2009, the results of an important study went largely unnoticed. Researchers discovered that in mice that are otherwise not susceptible to spontaneous autoimmune disorders, repeated administration of the antigen promotes systemic autoimmunity. The development of CD4+ T cells that can induce autoantibodies (autoantibody-inducing CD4+ T cells, or aiCD4+ T cells), which had their T cell receptors (TCR) modified, was triggered by excessive stimulation of CD4+ T cells. The aiCD4+ T cell was generated by new genetic TCR modification rather than a cross-reaction. The excessively stimulated CD8+ T cells induced them to develop into cytotoxic T lymphocytes (CTL) that are specific for an antigen. These CTLs were able to mature further by antigen cross-presentation, so in that situation, they induced autoimmune tissue damage resembling systemic lupus erythematosus (SLE) [146]. According to the self-organized criticality theory, when the

immune system of the host is continually overstimulated by antigen exposure at concentrations higher than the immune system's self-organized criticality can tolerate, systemic autoimmunity inevitably occurs [147].

It has been proposed that the amount and duration of the spike protein produced are presumably affected by the higher mRNA concentrations in the mRNA-1273 vaccine (100 µg) compared to the BNT162b2 vaccine (30 µg) [31]. Thus, it is probable that the spike protein produced in response to mRNA vaccination is too high and lasts too long in the body. That could overwhelm the capacity of the immune system, leading to autoimmunity [146,147]. Indeed, several investigations have found that COVID-19 immunization is associated with the development of autoimmune responses [148,149,150,151,152,153,154,155,156,157,158,159,160,161,162,163,164,165,166].

(6) Increased IgG4 levels induced by repeated vaccination could lead to autoimmune myocarditis; it has been suggested that IgG4 antibodies can also cause an autoimmune reaction by impeding the immune system's ability to be suppressed by regulatory T cells [102]. Patients using immune checkpoint inhibitors alone or in combination have been linked to occurrences of acute myocarditis [103,104,105,106,107], sometimes with lethal consequences [102]. As anti-PD-1 antibodies are class IgG4, and these antibodies are also induced by repeated vaccination, it is plausible to suggest that excessive vaccination could be associated with the occurrence of an increased number of myocarditis cases and sudden cardiac deaths.

Finally, these negative outcomes are not expected to affect all people who have received these mRNA vaccines. Individuals with genetic susceptibility, immune deficiencies, and comorbidities are probably the most likely to be affected. However, this gives rise to a disturbing paradox—if people who are the most affected by the COVID-19 disease (the elderly, diabetics, hypertensive, and immunocompromised people like those with HIV) are also more susceptible to suffering the negative effects of repeated mRNA vaccination, is it then justified to booster them? As Omicron subvariants have been demonstrated to be less pathogenic [133,134,135,136,137], and mRNA vaccines do not protect against re-infection [14,138], clinicians should be aware of the possible detrimental effects on the immune system by administering boosters.

Figure 2 describes how IgG4 antibodies are beneficial in muting the effects of allergens:

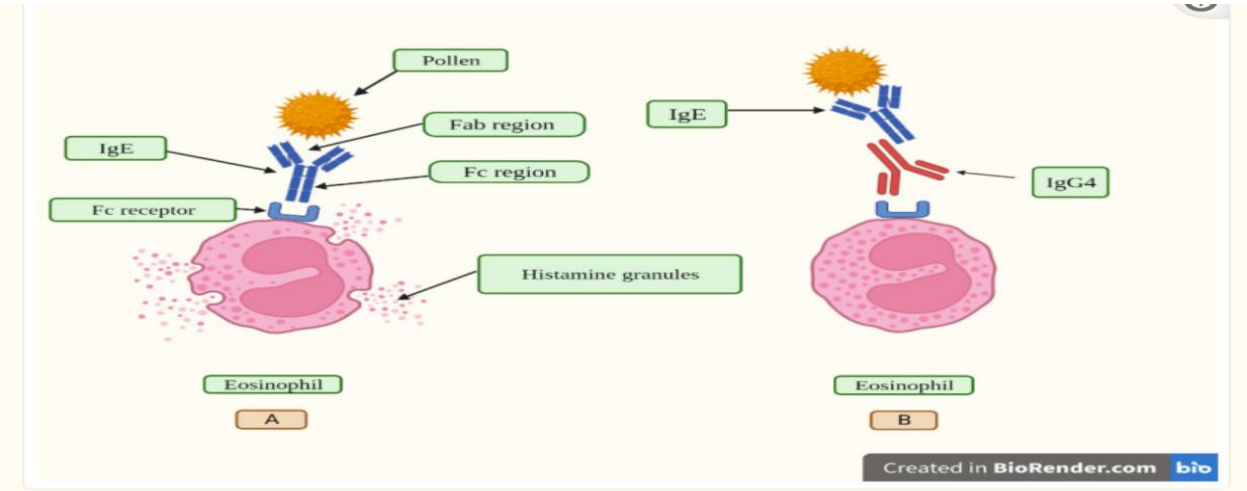

Figure 2

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In (**A**), a pollen grain is recognized through the fragment antigen-binding region (Fab) of an IgE antibody. After that, the IgE attaches to its receptor, called Fc epsilon RI (FcεRI), located on eosinophil leukocytes, and induces histamine release from cytoplasmic granules. Histamine is a vasoactive peptide that causes symptoms such as itching, sneezing, runny nose, itchy throat, eyes, and ears, and trouble breathing during a pollen-induced allergic reaction. In (**B**), the fragment cristalizable (Fc) region of an IgG4 antibody binds to the Fc region of an IgE antibody, inhibiting its binding to the FcεRI receptor and thus blocking IgE-mediated effects. Created with Biorender.

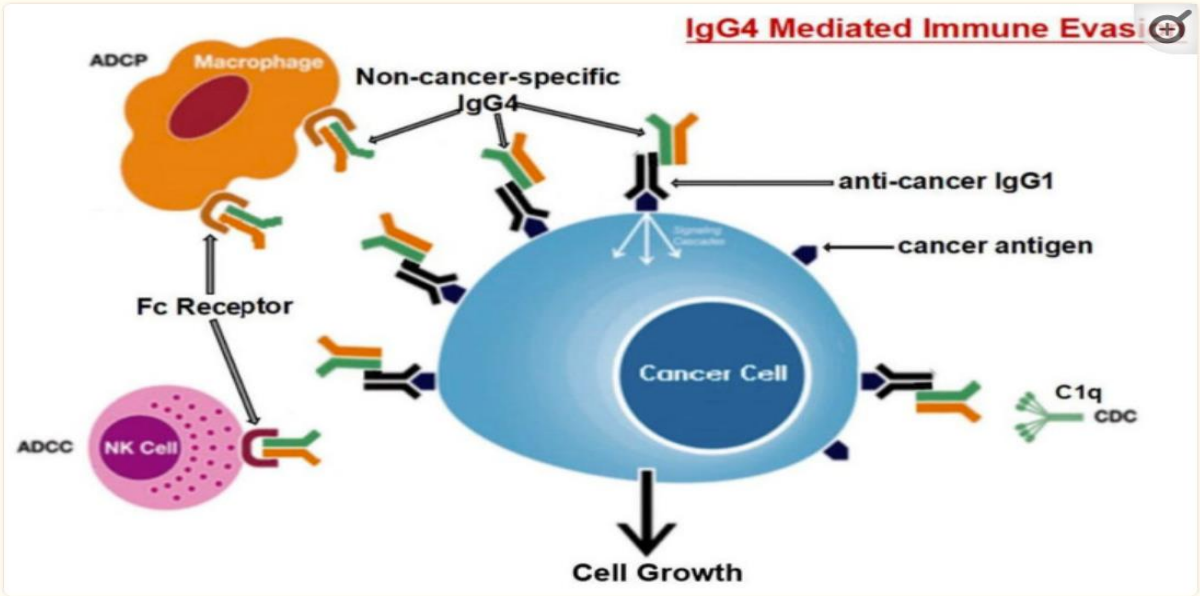

Figure 3

The suggested pathway for immune evasion evolved by cancer cells through IgG4 produced from B lymphocytes is depicted diagrammatically. Prolonged exposure to cancer antigens causes B cells to change their class and generate IgG4. With its Fc-Fc binding characteristic, such enhanced IgG4 can interact with cancer-bound IgG as well as Fc receptors on immune effector cells. Increased IgG4 in the cancer microenvironment promotes an efficient immune evasion mechanism for cancer due to its special structural and biological properties. The acronyms ADCC, ADCP, CDC, and NK stand for antibody-dependent cell-mediated cytotoxicity, antibody-dependent cell phagocytosis, complement-dependent cytotoxicity, and natural killer cells, respectively.

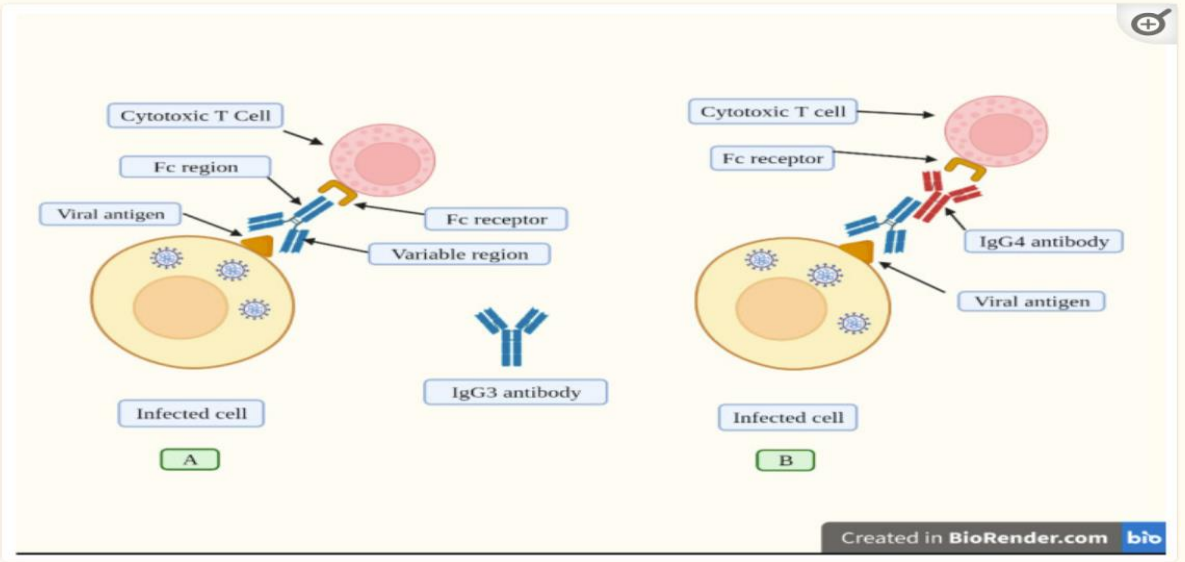

Figure 4

An effective humoral response induced by vaccination consists of the synthesis of high IgG3 concentrations. (**A**). IgG3 antibodies attach to viral antigens exposed on infected cells' membranes through its variable region. This antibody has a constant region (Fc) that is recognized by the corresponding receptor found on cytotoxic T cells and other immune cells. The cytotoxic T cell becomes activated and releases chemical agents that destroy the infected cell. (**B**). Repeated vaccination induces high IgG4 levels (depicted in red). This antibody inhibits the attachment of the Fc region from the IgG3 antibody to its receptor located on cytotoxic T cells, thus blocking its activation, and consequently, the infected cell is not destroyed. In this sense, repeated boosting causes a switch to the production of high IgG4 levels, which impairs immune responses.