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Autoimmune and Neoplastic Outcomes After the mRNA Vaccination: The Role of T Regulatory Cell Responses

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Highlights

- A plethora of autoimmune disease incidences occurred after COVID-19 mRNA injections were rolled out.
- Aggressive cancer cases have occurred in the bodies of recipients at sites where the mRNA was injected and at distant metastatic sites.
- The mRNA vaccines cause thymic involution (shrinking) and dysregulation of the T regulatory (Treg) and T effector (Teff) homeostatic cell balance.
- Activated immune cells deliver spike protein to the thymic epithelial cells, damaging them.
- The Treg/Teff balance may determine the fate of autoimmunity and/or cancer and is different for patients with cancer versus those without any cancerous tissues.
- Repeated mRNA injections lead to empirical evidence of impaired immune functions (elevated IgG4, PD-L1), associated with increased autoimmunity and cancer risks, and decreased resistance to infections.
- In the with-cancer recipients, the mRNA vaccine may be associated with either autoimmunity or further progression of the cancer(s) depending on the immunotherapy treatment the patient receives.

Abstract

When an antigen stimulates the immune system, specific T regulatory (Treg) and T effector (Teff) subpopulations develop from naïve T cells. An imbalance between Treg and Teff cells can lead to either cancer or autoimmunity. Treg cells are beneficial in that they protect from autoimmunity. However, they suppress the immune response to tumors. In this review, we analyze Treg responses after SARS-CoV-2 mRNA vaccination and find distinct pathological

responses under differing conditions. Injection with modified mRNA can lead to a delayed but highly active immune response, resulting in overactivation of the inflammasome. The mRNA "vaccine" induces a very strong IgG antibody response, while suppressing CD8+ T cell activation. Exosomes distribute the recombinant, synthetic spike protein and the mRNA encoding it throughout the organism. In cancer patients, disease progression depends on the starting point of the cancer patient at the time of injection and the type of cancer treatment underway. Migration of circulating dendritic cells and Treg cells back to the thymus, while they are carrying spike protein, damages the medullary thymic epithelial cells and accelerates thymic involution, a direct cause of inflammaging and immunosenescence. In summary, the Treg responses to mRNA injections and subsequent mRNA-encoded SARS-CoV-2 spike protein expression may disrupt immune capacities resulting in accelerated development of autoimmune disease and cancer. The processes discussed here are consistent with both epidemiological findings and case reports.

Keywords: *Treg cells, SARS-CoV-2 mRNA vaccination, immunosenescence, thymic involution, cancer, autoimmunity, TGF-β, IL-6, NF-κB, IgG4*

Abbreviations:

Foxp3: forkhead box P3; **IFN:** interferon; **IgG4-RD:** IgG4-related disease; IgG: immunoglobulin G ; **NF-κB:** nuclear factor kappa-light-chain-enhancer of activated B cells; **PD-1:** programmed cell death 1; **PD-L1:** programmed cell death-ligand 1; **STAT3:** signal transducer and activator of transcription 3; **TGF-β:** transforming growth factor-β; **TLR:** toll-like receptor; **iTregs:** inducible Treg cells; **mTreg:** memory Treg Cell; **nTregs:** naïve Treg cells

Introduction

The immune homeostasis of T regulatory (Treg) cell responses preserves self-tolerance and halts exaggerated T cell immune responses to protect from tissue damage (Smigiel et al., 2014; Kumar et al., 2018). The discovery of Treg cells (either of thymic or peripheral origin) in humans, reveals interesting facts about the regulation of the adaptive immune response (Smigiel et al., 2014). Both CD4+ and CD8+ regulatory T cells offer a homeostatic balance in the immune system to avoid both autoimmunity and cancer (Rocamora-Reverte et al., 2021). Treg cells release cytokines such as interleukin-10 that suppress the activity of T-effector (Teff) cells. When some immune cells stop distinguishing between self- and foreign material, Treg cells can help to prevent an excessive inflammatory response injuring self-tissues. On the other hand, a large population of Treg cells resident in the tumor microenvironment maladaptively protects cancer cells from immune attack, permitting unchecked tumor growth (Shevyrev & Tereschchenka, 2020).

During aging, T cells develop increased affinity to self-antigens, which is concurrent with and offset by a clonal expansion of peripheral (inducible) Tregs (iTregs). In parallel, thymic T cell capacity is reduced, impairing the ability to generate new T cells. The increase in iTregs can help to suppress autoimmunity, but in doing so it increases the risk of tumor growth and of sepsis (Vadasz et al., 2013).

The thymus gland plays a central role in the development of the immune system in mammals. Beginning in utero, stem cells migrate from the bone marrow into the thymus, where they first mature into thymocytes. These thymocytes undergo a transformation involving a complex process of negative and positive selection that ultimately yields a pool of CD4+ and CD8+ T cells, as well as a naïve Treg (nTreg) cell population. The selection process involves exposing the cells to diverse

human proteins, and those thymocytes that bind strongly to human proteins are eliminated via apoptosis. Those that bind weakly are retained and become the dominant source of CD4+ and CD8+ Teff cells. For thymocytes that show intermediate binding, the situation is more complicated. Many of them evolve into nTreg cells, that, when activated, they can suppress clonal expansion and activation of Teff cells. A unique marker for Treg cells is the forkhead box P3 (Foxp3) transcription factor. Some Teff cells still survive in this pool of intermediate-binding cells, and they play a significant role in autoimmune disease, especially in association with immunosenescence and inflammation linked to aging (Kronenberg and Rudensky, 2005; Smigiel et al., 2014; Kumar et al., 2018). Besides nTreg cells that emerge from the thymus, peripheral CD4+ Teff cells can also transform into Treg cells in response to the cytokines IL-2 and transforming growth factor-β (TGF-β), which are overexpressed in association with cellular stress (Horwitz et al., 2008; Yu et al., 2009a).

Ionizable cationic lipids are key components of the lipid nanoparticles used for delivery of mRNA in the mRNA vaccines (Jung et al., 2022). While one important feature of these lipids is that they can release the mRNA by endosomal rupture to support protein synthesis, they can also delay release until the lysosomal stage, which activates the NLRP3 inflammasome (Forster et al., 2022). This can be beneficial as an adjuvant to induce an immune response (Nance & Meier, 2021), but is associated with oxidative stress, mitochondrial damage, necrosis, syncytia formation, and pyroptosis (Lima et al., 2013).

Activation of the NLRP3 inflammasome induces caspase-1 release from mitochondria due to excessive reactive oxygen species and mitochondrial DNA damage (Phulphager et al., 2021). Damage response signaling results in the formation of membrane pores and the initiation of necrosis through pyroptosis. The NLRP3 inflammasome and caspase-1 together lead to secretion of the pro-inflammatory cytokine IL-1β (Phulphager et al., 2021). These activities are essential for launching the immune response to the vaccine antigens that will ultimately lead to a strong antibody response. However, the concept of a weak or a strong antibody response against SARS-CoV-2 spike protein antigens hides dysfunctionalities in the immune system of vaccinees and underlying pathology (Zach & Greslehner, 2023).

There is another lesser known but equally important member of the interleukin-1 family that is also activated by the DNA damage response and caspase-1 signaling, IL-18 (Sansonetti et al., 2000; Cinat et al., 2021). IL-18 plays several roles in immune activation and in autoimmune disease. On the positive side, it promotes the proliferation of cytotoxic CD8+ T cells (Iwai et al., 2008). However, it can also induce self-reactive innate antibody responses that are involved in autoimmune disease (Enoksson et al., 2011). It also promotes inflammation-induced carcinogenesis in squamous cell carcinoma (Huang et al., 2017). The most interesting aspect of IL-18 is its power to induce peripheral activated memory Treg (mTreg) cells to migrate back to the thymus, particularly in younger persons before thymic involution, where they play a powerful role in disrupting innate naïve Treg (nTreg) cell development and release into the periphery (Peligero-Cruz et al., 2020). This effect is likely the means by which IL-18 leads to excessive activation of self-reactive antibodies, through a reduction in the naïve Treg pool in the periphery.

IL-18 signaling upregulates C-C Motif Chemokine Receptor 6 expression in peripheral activated mTreg cells, and this results in their migratory return to and homing in the thymus. These recirculating thymic Tregs then inhibit the production of new nTreg cells in the thymus, by consuming IL-2, resulting in its depletion (Thiault et al., 2015).

The study by Agrati et al. (2021) showed through a whole blood test quantifying the Th1 cytokines interferon-γ (IFN-γ), tumor necrosis factor-α (TNF-α), and IL-2, that spike-specific T cells produced

all these cytokines in abundance at two weeks after the second mRNA injection. However, Prior infection with COVID-19 evidently leads to an increased production of IL-2 in response to the mRNA vaccines (Sedegah et al., 2022), inducing the transformation of peripheral Teff cells into Treg cells. A case study involved a patient who developed severe myocarditis following a single dose of the mRNA vaccine. He had had a mild case of COVID-19 three months earlier, which seemed to prime a powerful NLRP3 inflammasome reaction to the vaccine. This patient's monocytes expressed increased levels of IL-18 compared to others who had been vaccinated for COVID-19, likely leading to the return of the induced memory Tregs to the thymus, increasing the risk for an autoimmune attack on the heart (Won et al., 2022).

The thymus plays a central role in shaping the immune system during childhood. With increasing age, the thymus shrinks over time, a process known as thymic involution. Increasingly, it is becoming clear that thymic involution may be the most important factor in immunosenescence and the associated chronic smoldering inflammatory state known as "inflammaging" (Thomas et al., 2020). The NLRP3 inflammasome has a direct effect on the thymus, accelerating thymic demise (Youm et al., 2012). IL-18 has been shown to suppress regeneration in the thymus, by activating the IL-18 receptor on natural killer cells (Granadier et al., 2022). It is axiomatic that immunosenescence leads to increased risk of infection, autoimmune disease, and impaired cancer immunosurveillance (Liu et al., 2023).

Treg cells play an important role in what is known as "thymic involution" (Thomas et al., 2021). As it progresses, the homing memory Treg cells maintain their numbers, while the counts of all the other cell types in the thymus decrease. The mature memory Tregs, therefore, come to constitute the majority of the Treg pool in the aged thymus (Peligero-Cruz et al., 2020). The elderly population generally has a high Treg/Teff ratio in the periphery, but the Treg population is predominantly composed of long-lived mTregs that have already committed to the specific antigen that they were originally exposed to. These mTregs will suppress the T cell response to new exposures to the same antigen, but they have little power to react to novel threats. Naïve Tregs that can respond to a new insult are in short supply, and this results in poorly controlled autoimmune attack by self-reactive T cells (Thomas et al., 2021).

Those who suffer from conditions associated with immunosenescence, e.g., cancer, cardiovascular disease, rheumatoid arthritis, metabolic diseases, or neurodegenerative diseases, are at increased risk for suffering from severe and sometimes fatal COVID-19 infection (Thomas et al., 2020). When people with high-risk preconditions are vaccinated with the mRNA vaccines, there is an increased production of both TGF-β and IL-2, likely leading to the production of a large mTreg cell population, poor response to the vaccine, and further acceleration of thymic involution (Liu et al., 2021). Moreover, specific alertness that frail, old individuals (>65) who suffer from "inflamm-ageing", should not receive the mRNA vaccinations has been described recently (Kountouras et al., 2023

mRNA Vaccine Responses in Patients with and without Cancer

An analysis by Choueiri et al. (2023), although concluding in support of vaccinating patients with cancer (cancer(+) patients) with mRNA vaccines, reveals important findings for considering immunological disorders of SARS-CoV-2 vaccinees. In this study, it was found that the mRNA vaccinated cancer(+) patients, and especially those who had received 2 or 3 booster doses prior to SARS-CoV-2 infection, develop breakthrough SARS-CoV-2 infections more frequently than the unvaccinated cancer(+) control group, suggesting a Treg-suppressed immune system after repeated

mRNA vaccine exposure. Importantly, within the vaccinated cancer(+) population, the development of hematologic malignancies was encountered more frequently than in the unvaccinated cancer(+) control group. Also, the vaccinated cancer(+) group were given more anti-neoplastic drugs to treat their malignant conditions.

The authors concluded that the use of further mRNA vaccination (Choueiri et al., 2023), in addition to the initial two vaccines in cancer(+) patients, would help to prevent increased mortality rates from COVID-19. However, their findings also imply immunological irregularities in the cancer(+) vaccinees after mRNA exposure. Importantly, they described an ill-defined abnormally enhanced Treg response that suppressed anti-spike-protein Teff cell immunity in the cancer(+) patients, brought about by the mRNA injections. According to the studies on immunosenescence and Treg responses (Liu et al., 2021; Thomas et al., 2021), it is likely that further mRNA vaccinations would lead to even greater immune suppression, and further accelerate cancer progression (Ohue et al., 2019).

Further analysis of the immune system responses developed upon mRNA vaccination in the cancer(+) and cancer(-) populations is needed, with a focus on the Treg cell population. In general, high immunogenicity is associated with more severe side effects, and, depending on the initial state of the immune system, vaccines can, in the extreme cases, either fail to produce an effective immune response or produce such a strong immune response that it induces severe and even life-threatening adverse reactions.

Sophisticated methods have been developed to evaluate vaccine-induced immunity and reactogenicity (Gonzalez-Dias et al., 2020). Tregs behave differently in healthy and in malignant tissues (Luo et al., 2016). A propensity toward autoimmunity is induced by mRNA vaccination in both cancer(+) and cancer(-) individuals. The clinical course in these two scenarios, though, is quite different. Insufficient suppression by an inadequate Treg pool in the cancer(-) scenario creates conditions favoring development of "classical" autoimmunity (autoimmune thyroiditis, rheumatoid arthritis, etc.). In the cancer(+) individual, though, enhanced suppression of the immune response by a resident abundant Treg pool is most relevant for its impairment of anti-cancer immunity and consequent risk of accelerated cancer progression (Dejaco et al., 2006).

Cancer and autoimmunity are in juxtaposition from a deregulated Treg response after mRNA vaccination (Hatzioannou et al., 2021). The autoimmunity occurring in cancer(+) patients under immunotherapy following primary and especially booster mRNA shots is considered to be a downstream effect of a dysregulated T cell response (Alsaab et al., 2017). Moreover, the development of autoimmunity is closely linked to primary immunodeficiency syndromes that manifest with recurrent infections (Schmidt et al., 2018). In this case, the breakthrough infections encountered in the cancer(+) patients after the mRNA vaccination is a sign that the mRNAs in the vaccine exasperate their preexisting immunodeficiency (Choueiri et al., 2023). Breakthrough infections occur also in the cancer(+) patients that have not received immunotherapy treatments (but in lower numbers).

Therefore, with the mRNA vaccinations against COVID-19, important questions arise that concern immune competence in both the cancer(-) and cancer(+) populations. These are: 1) in the cancer(-) population, could the immune system be provoked toward more frequent development of any particular types of malignancy by the mRNA vaccines (Luo et al., 2016)? and 2) what is the absolute increased risk of new cancer (in cancer(-)) or enhanced growth/spread of cancer (in cancer(+)) for individuals receiving one or multiple mRNA vaccines? The Treg responses after the mRNA vaccinations could potentially be of prognostic value (Shang et al., 2015). The functioning Treg cells

have on the one hand a suppressor function that allows malignant cells to survive, but, on the other hand, when the Treg cells are inhibited, this lets autoimmunity develop, as a consequence of the intense inflammatory response induced by the spike protein (Barhoumi et al., 2021). With these questions in mind, we investigate the immune responses after the COVID-19 mRNA vaccinations and examine the similar but distinct implications of the dysregulation of Treg cells in the cancer(-) and cancer(+) populations.

The Criteria for Assessing Treg Dysregulation after mRNA Vaccinations

Autoimmunity involves an impairment of Treg homeostatic balance (Dejaco et al., 2006). Conceptually, when a Treg response is raised upon a specific antigen stimulus, T cells are prevented from becoming activated into functional effector cells. During autoimmunity, the Treg cells lose their suppressive function and Teff cells that have lost self-tolerance cause disease. Concerning the mRNA vaccinations for COVID-19, a review by Diani S et al. (2022) determined that the natural immunity conferred by a previous SARS-CoV-2 infection, both cellular and humoral, is robust and long lasting compared to more rapidly waning protection afforded by vaccines. Vaccination carries greater risk of adverse reactions in previously infected individuals, with a higher risk of inducing autoimmune disease with repeated vaccination (Raw et al., 2022).

Tormo N et al. (2022) have evaluated T cell responses after the mRNA vaccinations according to a) the age (before and after 60 years of age) and b) whether they have been previously infected or not with SARS-CoV-2. The authors noted substantial differences in the immune response to the administered vaccines over time based on both age and previous infection status. Two papers that set the stage are Lourenço EV et al., which provides a review of the role of dysregulated natural Treg cells in autoimmunity (Lourenço et al., 2011), and Sanchez & Yang (2011)., which describes their important role during infection.

We have searched the PubMed and ScienceDirect databases for papers describing the immune response to the mRNA vaccines, as well as a large number of papers that review the complex processes of the immune system and the ways it ages. After depicting the observed Teff and Treg responses, inferred from the Tormo et al. (2022) study, we examine the criteria of autoimmunity development in both cancer(+) and cancer(-) populations. These observations led us to further predict the development of immunosenescence as a consequence of the return of activated dendritic cells and Treg cells to the thymus, accelerating thymic involution. Based in part on the study of Pellerin et al. (2014), which discusses immune loss of regulation due to an altered function of FOXP3+ Treg cells, a subsequent Treg/Teff imbalance in the mRNA vaccinated individuals occurs. The Treg/Teff imbalance involves either an enhancement or a reduction of the Teff cell response in these population groups under differing initial immune states, leading to differing pathological outcomes. Finally, the immune senescence pathogenic processes that are underlying and complicate the final effect of repeated mRNA vaccinations led us to investigate the outcomes from an altered Treg/Teff balance in the immune systems of vaccinees, particularly after repeated mRNA vaccine booster shots (van der Geest et al., 2014; Rocamora-Reverte et al., 2021).

Delayed but Enhanced Immune Response to mRNA Vaccines

RNA viruses induce expression of type I interferons (IFN-α and IFN-β) by infected cells, due to the detection of double-stranded RNA during replication (Baum & Garca-Sastre, 2010). A major distinction between the immune response to the mRNA vaccines and that provoked by a viral infection is that, in the case of the vaccine, the type I IFN response is not induced due to the

absence of replicating viruses. Not only are the enzymes needed for replication lacking, but also the mRNA sequences in the vaccines have been disguised to resemble a human mRNA molecule (Nance & Meier, 2021; Seneff et al., 2022).

Type I IFNs play a major role in the initial immune response to a viral infection. They cause the activation of naïve CD4+ and CD8+ T cells in the early stages of the infection, inducing clonal expansion and differentiation into a pool of Teff cells as well as a pool of iTreg cells (Pellerin et al., 2014). Type I IFNs maintain the Foxp3+ expression that characterizes Treg cells under inflammatory conditions (Lee et al., 2012). However, type I IFNs actually suppress the activity of Treg cells, holding them in check until the viral load has dissipated (Gangaplara et al., 2018). Over time, the level of type I IFNs decreases, due to the fact that cytotoxic immune cells, also induced by the IFN, have cleared the virus-infected cells and halted viral replication. Once the type I IFN expression is sufficiently reduced, the iTreg cells that had been standing by are now free to release the immune-suppressing cytokines, including interleukin-10 (Il-10) and TGF-β, which are effective in alleviating the inflammatory response after the virus has been successfully cleared (Levings et al., 2002).

The SARS-CoV-2 spike protein has been demonstrated experimentally to inhibit and damage the ACE2 receptor protein expression in epithelial cells. This induced a hyperinflammatory signaling cascade that led to activation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-ϰB) and increased release of TNF-α and IL-6 (Patra et al., 2020). A study involving 50 COVID-19 patients revealed that those with severe disease were characterized by a persistent viral load and high levels of TNF-α and IL-6 expression, associated with a seriously impaired type I IFN response, in some cases due to the presence of anti-type-I-IFN autoantibodies. The lack of type I IFN delayed the immune response to the virus, allowing the virus to replicate freely, and inducing severe disease. Furthermore, an insufficient pool of mTreg cells caused sustained immune activation, and the overactive immune response was the major source of severe symptoms (Hadjadj et al., 2020).

A study of the immune response to the mRNA vaccines compared to the response to infection revealed that the vaccine induces a response pattern comparable to that of severe disease (Röltgen et al., 2022). These authors wrote: "We find that BNT162b2 vaccination produces IgG responses to spike and RBD [receptor binding domain] at concentrations as high as those of severely ill COVID-19 patients and follows a similar time course" (Röltgen et al., 2022). This result aligns with the concept that the vaccine simulates an impaired type I IFN response. A detailed study on the mRNA vaccines revealed that there was a refractory period immediately following vaccination prior to the induction of a specific immune response, and the authors proposed that this delay could explain the higher risk of infection during this early period (Gil-Manso et al., 2021). This delay may be a manifestation of a missing or dismantled type I IFN response.

The mRNA vaccines create a mosaic of cells that synthesize spike protein, inducing a response in transfected cells that results in the abundant release of exosomes containing not only the spike protein but also microRNA-148a and microRNA-590, that specifically suppress the response to type I IFN. When these exosomes are taken up by microglia (immune cells in the brain), they induce a potent inflammatory response (Mishra et al., 2021). Exosomes presenting the spike protein on their surface are still present in the circulation four months after vaccination (Bansal et al., 2021). The recombinant expressed spike protein may persist for even longer periods, since it can resist the cell's enzymatic and non-enzymatic degradation systems (Boros et al., 2024). Large-scale single-cell mRNA sequencing technology revealed dramatic alterations in gene expression of almost all immune cells after vaccination. Increased NF-ϰB signaling and a reduced type I IFN response were

most notable, and there was a marked deficiency in CD8+ T cells (Liu et al., 2021). Type I IFNs induce a massive expansion of antigen specific CD8+ T cells, both effector and memory, in response to viral infection (Kolumam et al., 2005). Type I IFNs also protect CD8+ T cells from destruction by natural killer cells (Xu et al., 2014). Treg cells with impaired type I IFN receptors show enhanced suppressor activity during both acute and chronic infection, resulting in CD8+ T cell anergy, defective generation of memory T cells, and viral persistence (Gangaplara et al., 2018).

With mRNA injections, immune cells are responding to an unnatural circumstance in which human cells produce a toxic foreign protein. Spike antigen on the surface of transfected cells activates CD4+ immune cells and launches the cascade that eventually leads to a strong antibody response. This response is heavily skewed towards immunoglobulin G (IgG), with little or no IgM or IgA antibody production (Röltgen et al., 2022). IgG is the primary antibody type that induces autoimmune disease, and this effect is enhanced in the absence of secreted IgM antibodies (Nicolò et al., 2022). The replacement of every uridine in the vaccine mRNA molecules with N1-methylpseudouridine assures that the mRNA will survive for a long time and continue to be translated into spike protein, resulting in sustained immune activation (Morais et al., 2021; Santiago, 2024). A review paper on the effects of N1-methylpseudouridine substitution on the immune response hypothesized that such substitution would facilitate cancer growth and metastasis, in part by suppressing the type I interferon response (Rubio-Casillas et al., 2024).

There is extensive homology between heptapeptides from immunoreactive epitopes in SARS-CoV-2 and human proteins that can lead to autoimmune disease via molecular mimicry. Most of the immunogenic epitopes in the spike protein have similarity to human proteins, and this opens up a serious possibility for pathogenic priming and autoantigenic responses due to molecular mimicry (Lyons-Weiler, 2020). Cross-reactive IgG antibodies could mistakenly attack human proteins with similar peptide sequences, and a constellation of diseases, including neurological disorders, cardiovascular alterations, coagulopathies, pregnancy dysfunctions, multiple cancers, and anosmia, among others, could ensue (Kanduc, 2020). The possibility of molecular mimicry as cause of pathogenesis is supported by the study of LM Yonker et al. (2023). The authors claim that the symptom profile associated with the persistence of recombinant spike protein from the mRNA injection in patients with post-vaccine myocarditis clinically resembles multisystem inflammatory syndrome in children (MIS-C), resulting in hyperinflammation due to the continued presence of SARS-CoV-2 in the gastrointestinal tract (Yonker et al., 2021). The persistence of antigenemia is encountered in other infectious diseases, where lingering viral and microbial pathogens are responsible for the encountered immunopathology (Fujinami et al., 2006).

Recent studies have shown that the spike protein physically interacts with the p53 tumor suppressor, which is known as the "guardian of the genome." The powerful p53 regulatory protein is transported to the nucleus during oxidative stress, where it activates a large number of proteins involved in DNA repair systems. A paper published by Singh and Singh (2020) showed that the S2 subunit of the spike protein strongly interacts with p53, which could interfere with its power to transfer to the nucleus. In support of this, a preprint paper showed that the spike protein suppresses gene activation of p53 targets in the nucleus (Zhang & El_Deiry, 2024). There are recently published case studies that describe *in situ* generation of aggressive cancers that developed shortly after mRNA vaccination, and, in one case, at the vaccination site (Bae et al., 2023; Kyriakopoulos et al., 2023).

The spike protein can induce an intense inflammatory response in endothelial cells via integrin binding. The arginine-glycine-aspartate tripeptide motif exposed on the surface of the receptor binding domain of the spike protein binds to integrin 5β1 expressed by endothelial cells. This

activates the NLRP3 inflammasome through the NF-ϰB signaling pathway. NF-ϰB signaling also induces vascular leakage and leukocyte adhesion. NF-ϰB upregulates proinflammatory cytokines, chemokines, and coagulation factors in endothelial cells (Robles et al., 2022). Treg cells dramatically increase their suppressive function in response to inflammation, releasing high levels of the immunosuppressive cytokines Il-10 and TGF-β (Khantakova et al., 2022).

iTregs interact with endothelial selectins and transmigrate past the endothelial barrier. In response to antigen presentation (e.g., spike), they suppress TNF-α and Il-1β, as well as Teff cell adhesion to the endothelium, which is critical for T cell influx into inflamed tissues (Shimizu et al., 1991). This fast-acting suppression is mediated by TGF-β released by the iTregs (Maganto-Garca et al., 2011). The anti-idiotype antibodies become quite relevant in this regard. They can be structurally identical to the original antigen, i.e., spike proteins, and thus push forward this suppression (Murphy et al., 2022).

Cancer is associated with an imbalance in Teff and Treg cells where the Tregs far outnumber the Teffs in the tumor microenvironment (Gonzalez-Dias et al., 2020; Omland et al., 2016). The NLRP3 inflammasome promotes carcinogenesis. Huang et al. (2017) found that Foxp3 was highly overexpressed in the tumor, and Treg cells comprised 45% of the CD4+ T cells there. Induction of high levels of TNF-α and IL-6 by the spike protein through activation of NLRP3 will lead to increased production of Il-10 and TGF-β by the pre-existing Treg pool. This can be expected to cause excessive immune suppression in the tumor microenvironment, thus facilitating tumor progression. Autoimmune disease expresses the opposite problem (Dejaco et al., 2006; Bednar et al., 2022). The increased activation of Teff cells by the vaccine in the context of an insufficient Treg pool will exacerbate autoimmune disease.

The Treg Response after mRNA Vaccination: The Role for Immune Senescence

Under normal conditions, immunosenescence occurs as the immune system ages (Lee et al., 2022). As aging progresses, the peripheral Treg population increases in number, but most of those Tregs are mTregs already committed to specific antigens, and the power to induce an iTreg response to a novel exposure is lowered (Jagger et al., 2014). As these cell populations continue to shift with time, the cumulative loss of Treg activation in response to self-reactive antibodies results in an increased risk of autoimmune disease with increasing age.

IFN-γ, a Th1 cytokine and the only type II IFN, is produced by hyperactivated CD4+ and CD8+ T cells in response to a virus infection. T cell hyperactivation has been associated with severe cases of COVID-19 (Kalfaoglu et al., 2020). CD25-expressing hyperactivated Teff cells produce the protease furin, which cleaves the spike protein, facilitating viral entry (Kalfaoglu et al., 2020).

Type I IFN induces proliferation of Foxp3+ Treg cells, which, when activated, suppress the expression of IFN-γ (Larkin et al., 2013). A seminal paper comparing the immune response in cases of severe COVID-19 with milder disease revealed many aspects of immune dysfunction that were associated with an impaired type I IFN response. The T cells of severe cases highly expressed CD25 (the IL-2 receptor), but they were deficient in Foxp3. Foxp3-CD25+CD4+ T cells were very effective as Teff cells, producing high, even toxic, levels of IFN-γ, as well as furin. It was concluded that that these cells were very short-lived and died off before being able to transform into Foxp3+ Treg cells. Tissue damage in the lungs associated with severe disease was mainly due to an overactive immune response, leading to excessive and prolonged inflammation. Thus, an impaired Foxp3-mediated negative feedback loop characterized severe disease (Kalfaoglu et al., 2020).

The study of Tormo et al. (2022) provides an opportunity to compare vaccine responses among young and old and to assess the effect of previous exposure to SARS-CoV-2. The authors looked specifically at 50 individuals who were either nursing home residents (old) or nursing home employees (young). Thus, they provided cohorts for both <60 and >60 years of age populations, as well as a distinction between those who had previously recovered and those whose first exposure to the spike protein, and whatever other toxicants the modified mRNA injections may contain (Diblasi, et al., 2024), was through the mRNA vaccine.

Notably, a prior infection resulted in a very modest IgG antibody response to the spike protein. All of the cases had been mild, and this is reflected in the fact that the CoV2+ <60 group had a median IgG level of 5 relative units/ml, (interquartile range) (RU)/ml (IQR)), and the median for the CoV2+ >60 group was only 36 RU/ml, just prior to vaccination. (according to the ELISA test). This is to be contrasted with peak values greater than 800 RU/ml for all four cohorts following vaccination. So, one can conclude that the dramatic response to the vaccine more closely emulates severe disease.

However, prior SARS-CoV-2 infection clearly had a powerful effect on the reaction to the vaccine. The antibody response to the first vaccine was far greater in the CoV2+ cohort than in the CoV2- cohort. This was likely due to memory Teff cells ready to respond immediately to the spike protein being produced by the transfected cells.

The CoV2+ >60 population achieved a median IgG response of 2882 RU/ml in response to the first vaccine. . This was the highest arbitrary value (AV) achieved in this group — the second vaccine added no further benefit. The authors proposed that a single vaccine would be more than adequate for those already infected, and that the second vaccine might even do harm.

The CoV2- cohort showed a slower and lower increase in both humoral (anti-spike IgG antibodies) and cellular (IFN-γ) response markers, compared to the CoV2+ cohort. This was especially true for the CoV2- >60 group. CD4+ IFN-γ responses for this population remained low the entire time, reaching a maximum level of just 0.07 international units (IU/ml four weeks after the second vaccine. (Quanti*FERON*® SARS-CoV-2 RUO (Qiagen) commercial assay). It was not until two weeks after the second vaccine that any of them achieved a level above the proposed cutoff threshold. Since these individuals were all nursing home residents, it is likely that immunosenescence was a cause of their poor response. While the >60 CoV2- group had the poorest immune response, by contrast the >60 CoV2+ group acquired more than twice the serum antibody AV and IFN-γ levels even compared to the <60 CoV2+ group. So, the contrast between CoV2- and CoV2+ was especially dramatic for the 60+ population.

The precipitous fall in IFN-γ during the two-week period following the second vaccine for the CoV2+ population was perhaps the most remarkable result of these experiments, and this was especially pronounced in the >60 group, where CD4+ IFN-γ levels fell from 1.61 IU/ml just before the second vaccine to only 0.89 IU/ml two weeks later. The authors suggested that Treg cells may have suppressed the response to control exacerbated inflammatory damage, but this would also of course limit the effectiveness of the vaccine, and potentially accelerate inflammaging. These Treg cells were likely induced by simultaneous excessive production of IL-2 and TGF-β in response to the first vaccine (Horwitz et al., 2008; Agrati et al., 2021; Liu et al., 2021).

Lozano-Ojalvo et al. compared vaccine reactions in CoV2- and CoV2+ populations with similar findings as those of the Tormo et al. (2022) study. These authors showed that CoV2+ individuals

produced very high levels of both IL-2 and IFN-γ just ten days after the first vaccine. Furthermore, the second vaccine actually set them back by causing a reduction in cellular immunity (Lozano-Ojalvo et al., 2021).

The natural immunity of unvaccinated CoV2+ individuals, both cell-mediated and humoral, is superior to the mRNA vaccine-induced immunity, which decays more rapidly over time (Diani et al., 2022). Natural SARS-CoV-2 antigens are superior to the mRNA-derived spike protein for inducing long-lasting immunity (Antia et al., 2018). A bigger concern is that the vaccine may be inducing immunosenescence, increasing risk to infections with other pathogens. A study based in Israel found a significant increase in non-COVID-19 respiratory infections from April to June 2021, immediately following an aggressive nationwide vaccination campaign (Amar et al., 2022). While the authors suggested easing of social distancing as a likely cause, the induction of immunosenescence by the vaccine might also have contributed to this result.

Potential for Damage to the Thymic Epithelium and Accelerated Thymic Involution

It had long been believed that the thymus is immune privileged (i.e., is insensitive to foreign protein exposure), but more recent research has shown that this is not true. Chronic infection of the thymus by viruses that are highly pathogenic can drive the immune system to immune tolerance towards that pathogen. This could happen through at least three distinct processes: (1) negative selection of pathogen-reactive T cells, (2) excessive generation of pathogen-specific Tregs, or (3) T cell anergy. They may all be at play (Nunes-Alves et al., 2013).

SARS-CoV-2 can infect the thymus, particularly in the youth, and this induces a loss of function that correlates with disease severity (Rosichini et al., 2023). ACE2 is expressed by the thymic epithelium, particularly the medullary thymic epithelial cells, which are mainly responsible for negative selection, and so they should be susceptible to SARS-CoV-2 infection. The SARS-CoV-2 virus can target medullary thymic epithelial cells and downregulate critical genes involved with epithelial cell adhesion and survival. Rosichini et al. (2023) verified that cultured medullary thymic epithelial cells from the thymus of children expressed ACE2 and could be infected with SARS-CoV-2. Spike-positive human medullary thymic epithelial cells were identified at both 24 and 48 hours after infection. There was increased mortality among the medullary thymic epithelial cells compared to cortical medullary thymic epithelial cells, reflecting their higher ACE2 expression. The spike protein induces IL-6 and TNF-α in epithelial cells (Patra et al., 2020). Both of these cytokines have been implicated in acute thymic involution (Ansari et al., 2017). Defects in thymus epithelial cells are associated with the aged thymus (Gui et al., 2007).

An experiment involving mice with a genetic defect interfering with the induction of T-cell tolerance in the thymus resulted in a strong mouse model for autoimmune hepatitis. The mutation led to depletion of medullary thymic epithelial cells that would normally cause autoreactive T-cells to be eliminated before they exit the thymus. This resulted in a reduction in the release of naive Tregs from the thymus and an increase in the release of self-reactive CD4+ and CD8+ T cells (Alexandropoulos et al., 2015). Autoimmune hepatitis is associated with the mRNA vaccines (Zheng et al., 2022). The thymus is easily accessible via the lymphatic system, so this implies that the mRNA vaccines could enable the delivery of the spike protein and even the spike mRNA and the ionizable cationic lipids through the lymph system, beginning with the axillary lymph nodes. Swelling of the axillary and chest lymph nodes is one of the more common side effects of the vaccine, clearly indicating that the dendritic cells (DCs) reacting to the injection in the deltoid muscle are migrating

to the lymph node (Co et al., 2022). The DCs would almost certainly endocytose the mRNA nanoparticles in the muscle tissue.

A case study involved a 64-year-old woman with breast calcification who was assessed for breast cancer via ultrasonography six months before her first SARS-CoV-2 vaccine, and again 7 days after the vaccine due to obvious lymph node enlargement in the vaccinated arm. Six months later, a follow-up examination revealed that the lymph node was still swollen, even though there was no evidence of breast cancer (Yoshimoto et al., 2022).

Dendritic cells play an essential role in controlling the transformation of thymocytes into new antigen-specific T cells in the thymus. As many as half of the DCs in the thymus are of peripheral origin, rather than recently emerging from the bone marrow. Some of the circulating DCs return to the thymus carrying antigens from the periphery to the thymus. Ominously, this implies that DCs could directly deliver vaccine mRNA and synthetic cationic lipids to the thymus. Once in the thymus, these cells proliferate, likely distributing the vaccine mRNA among their offspring. DCs not only present antigen to T cells, but also induce antigen-specific Foxp3+CD25+CD4+ Tregs from Foxp3-CD25-CD4+ thymocytes. By contrast, Tregs are not induced by similar DCs in the spleen (Li et al., 2009).

Thus, these activated antigen-expressing DCs that migrate back to the thymus induce both negative selection of antigen-specific T cells and an antigen-specific Treg pool to further control any self-reactive antibodies that escape selection. These returning DCs are the major hemopoietic cells that serve in this capacity in the thymus. While these activities can serve well to protect from autoimmune disease due to molecular mimicry, they could also induce tolerance to a virus, jeopardizing a memory response.

The S1 segment of the spike protein is cleaved by furin from spike protein exposed on the membrane of DCs and released freely into the external milieu (Colunga Biancatelli et al., 2021). Since S1 contains the receptor binding domain of the spike protein, it could bind to the ACE2 receptors on medullary thymic epithelial cells, inducing a damaging inflammatory effect, as has been demonstrated for endothelial cells (Robles et al., 2022). Even independently of the spike protein, returning DCs have been shown to directly inhibit TEC proliferation and induce their apoptosis by activating the Jagged1/Notch3 signaling pathway (Wu et al., 2021).

An impaired type I IFN response may play a critical role in the pathological overshooting of immune activation associated with the vaccines. In response to a viral infection, type I IFN induces a massive clonal expansion of antigen specific CD8+ T cells. It has been shown that antigen-specific CD8+ T cells expand nearly 10,000-fold during the first week after mice are infected with lymphocytic choriomeningitis virus (Kolumam et al., 2005). The vaccines do not elicit a type I IFN response, due to the lack of double-stranded mRNA associated with viral replication (Gantier et al., 2007; Seneff et al., 2022).

Severe COVID-19 has been linked to a deficiency in the glycoprotein perforin, resulting in a pathogenic auto-inflammatory feedback loop (Cunningham et al., 2021). Perforin, released by cytotoxic CD8+ T cells, generates pores in the target cell membrane, enabling penetration by cytotoxins resulting in cell death. The number of perforin-positive lymphocytes declines precipitously above the age of 70, and this could help explain the increased susceptibility to severe COVID-19 in the elderly (Rukavina et al., 1998). Furthermore, the S1 subunit of the spike protein has been shown experimentally to suppress perforin expression in CD8+ T cells (Huang et al., 2021).

Cytotoxic CD8+ T cells are essential for eliminating hyperactivated antigen presenting DCs in the thymus, a process that critically depends on perforin (Terrell et al., 2013). Hemophagocytic lymphohistiocytosis, also known as macrophage activation syndrome, is a life-threatening, hyperinflammatory disorder, characterized by malignant inflammation and multi-organ failure. A study on perforin-deficient mice provided a compelling demonstration that these mice were susceptible to hemophagocytic lymphohistiocytosis, due to impaired power of CD8+ T cells to prune off hyperactivated antigen-presenting DCs in the thymus (Terrell et al., 2013). Multiple cases of hemophagocytic lymphohistiocytosis as an adverse reaction to the mRNA vaccines have been reported (Zhang et al., 2023).

In summary, the mRNA vaccines would prime the thymus to produce spike-specific autoreactive T cells that fail to be transformed into Treg cells due to a deficiency in activated CD8+ T cells. These T cells can initiate autoimmune disease and hemophagocytic lymphohistiocytosis (a hyperinflammatory attack on the organs). At the same time, transfected DCs in the thymus can continue to produce spike protein for weeks if not several months after vaccination, causing damage to medullary thymic epithelial cells, accelerating thymic involution, and driving the immune system towards anergy. This prominent scenario is illustrated in Figure 1.

The Molecular Reasons for Treg Irregularities in the Cancer(-) Population after the mRNA Doses

Kasper et al. (2016) have detailed the complex molecular networks that control Treg induction and function beyond IL-2 and TGF- β, including transcription factors, kinases, phosphatases, Notch family receptors, mTOR signaling, etc. (Kasper et al., 2016). The Treg cells, when functioning well, have a protective effect against cancer, autoimmune reactivity, and transplant rejection. A key aspect of their protective role is through conferring mTreg immunity; that is, they respond efficiently to re-exposure to an antigen they were primed with earlier (Rosenblum et al., 2016; Khantakova et al., 2022). The main role of Treg CD4+ T cell subpopulations is influenced by a complex cascade of genetic, molecular and T cell interactions, ultimately to provide an efficient mTreg response. Differentiated Treg cells release interleukin-10, TGF-β and other suppressive chemokines which negatively control the pro-inflammatory responses and thus limit prolonged and chronic inflammation. As the Treg cells are subdivided into CD4+ Treg and CD8+ Treg cells, from each Treg subpopulation an antigen-specific mTreg cell subset is created that will keep the immune system in check, preventing an overwhelming future immune stimulation by the same virus re-infection and/or viral antigen vaccine boosters (Lu et al., 2019).

In the case of SARS-CoV-2 spike protein, an extensive robust NF-ϰB activation occurs. This causes an upregulation of genes involved in a) TNF-α signaling, b) the pro-inflammatory response, and c) cytokine-to-cytokine receptor interactions (Liang et al., 2023). Overall, the activation of NF-ϰB signaling upon the stimulation of a specific viral antigen — for a review, see Liu et al. (2017) —, on its own, initiates the formation of a Treg response. The NF-ϰB-mediated Treg response specific to the stimulating antigen thereafter leads to the formation of specific subpopulations of mTreg cells which have the role to become activated upon later stimulations from the specific viral antigen (Daniels et al., 2023). NF-kB has two branches (pathways) that are simultaneously activated by viral antigens, a) the canonical pathway which leads to inflammation, and b) the non-canonical or alternative pathway which is involved in immune cell differentiation, maturation, and organogenesis.

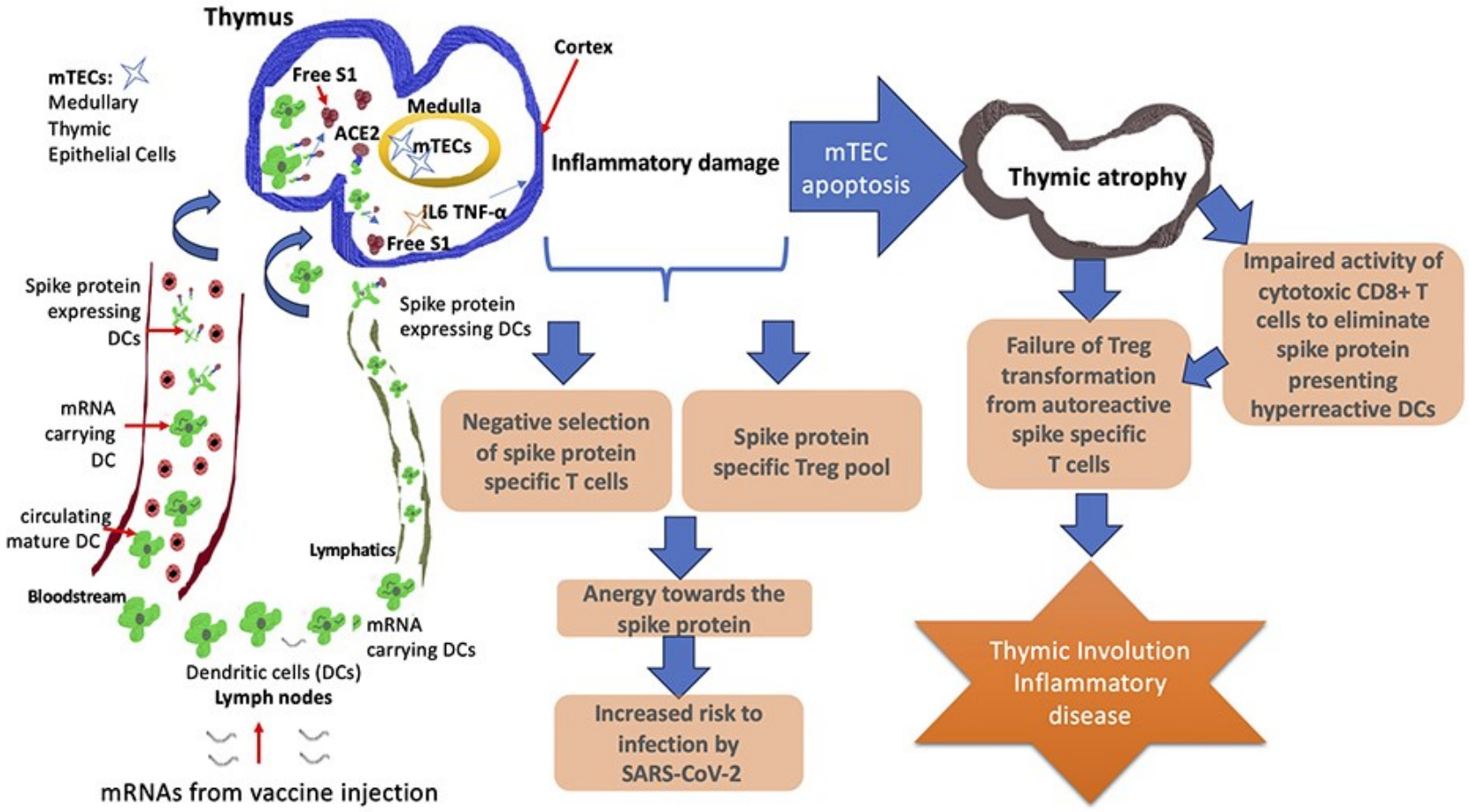

Figure 1. The presentation of SARS-CoV-2 spike protein by DCs to the thymus leads to thymic involution and inflammatory disease. The spike presentation by DCs causes medullary thymic epithelial cell apoptosis that leads to thymic atrophy and failure of Treg transformation, eventually resulting in thymic involution and inflammatory diseases (Rukavina et al., 1998; Gantier et al., 2007; Terrell et al., 2013; Cunningham et al., 2021; Huang et al., 2021; Zhang et al., 2023).

The stimulation of NF-ϰB has been mainly considered as an optimal activator of CD4+ mTreg cells through the activation of the NF-ϰB canonical pathway. The mTreg cells, are needed for the organism to avoid autoimmunity (Oh et al., 2017), but their over-activation promotes cancer progression (Grinberg-Bleyer et al., 2017). The role of the alternative pathway activation in the formation of Treg cells has remained obscure until recently. The SARS-CoV-2 spike protein stimulation of the T lymphocyte toll-like receptor (TLR) system releases excessive TNF cytokines (Keeton et al., 2022). Hence, the stimulation of TNF receptor family members (such as OX40, CD40 and LT-βR) by the spike protein will result also in the activation of the alternative NF-ϰB pathway by the stabilization of NF-ϰB Inducible Kinase (Liu et al., 2017).

Experiments that investigated the role of NIK overexpression in relation to Treg development, showed that the overstimulation and constitutive expression of NIK leads to aggressive and lethal autoimmunity. The Treg cells produced under the overwhelming stimulation of NIK were defective in inducing immune suppression (Murray et al., 2011). In these experiments, the mice were engineered to constitutively overexpress NIK and the phenotype of the T cell response was characterized by OX40+ hyper-reactive T cells and Tregs that were deficient in Foxp3.

The expression of Foxp3 by T cells is catalytic for an optimum Treg suppressive activity. However, under the influence of NIK overstimulation, there is a loss of the capacity to distinguish between self and non-self-antigens by the immune system that leads to a disturbed self-tolerance, a hallmark for autoimmunity initiation and progression (Dejaco et al., 2006). CD4+ T cells at inflammatory sites in rheumatoid arthritis are known to be resistant to suppression by Treg cells (Dejaco et al., 2006). Overall, this leads to a state of hyper-inflammation in the organism.

A study on the immune response to SARS-CoV-2 mRNA vaccines found that IFN-γ and Il-2 were highly expressed following vaccination, with a statistically significant increased expression in those who were vaccinated following infection with COVID-19. The level of these cytokines was strongly correlated with the IgG response (Sedegah et al., 2022). Il-2 plays an important role in Treg induction and persistence. Interestingly, Treg cells accumulate with age, but the reason for this is surprising. It is not through clonal expansion from either the thymic or the peripheral pool, but rather simply because aging Treg cells show reduced expression of the protein Bim, a pro-apoptotic signaling molecule. As a consequence, they survive much longer than Tregs expressing high levels of Bim. Chronic stimulation by Il-2 leads to preferential expansion of Tregs with low expression of Bim, allowing them to accumulate, and increasing the size of the overall Treg pool through lack of attrition (Chougnet et al., 2011). As already discussed, some of these long-lived Treg cells migrate to the thymus and facilitate accelerated thymic involution.

Świerkot, J et al. (2022) investigated the emergence of an autoimmune response after SARS-CoV-2 mRNA vaccination in a cancer(-) population. In this study, the individuals who had completed their mRNA vaccination (2 mRNA injections) and had presented with more severe vaccine adverse reactions, had significantly higher antinuclear antibody (ANA) titers when compared to the individuals with less severe vaccine adverse reactions (Fraiman et al., 2022). The authors did not find a correlation between prior SARS-CoV-2 infection status and severity of vaccine adverse reactions. However, another study found that more severe vaccine adverse reactions were most strongly associated with individuals who had COVID-19 and were subsequently mRNA vaccinated (Kadali et al., 2022). Furthermore, many studies show that autoimmunity can arise after COVID-19 vaccinations. One study describes 27 cases of autoimmune reactions following SARS-CoV-2 vaccination (17 flares and 10 new cases; Watad et al., 2021). In a case report of systemic lupus erythematosus, symptom onset occurred just two days after immunization with the first mRNA

injection (Raviv et al., 2022). A 63-year-old man experienced acute severe autoimmune-like hepatitis just one week after his first dose of an mRNA vaccine (Ghielmetti et al., 2021). A review article described 27 cases of autoimmune hepatitis following COVID-19 vaccines, ranging in age from 27 to 82, 20 of which were due to mRNA vaccines. None of them used any hepatotoxic drugs that could explain their disease (Zheng et al., 2022). Cases of autoimmune hemolytic anemia are described as a serious adverse reaction of mRNA vaccination (Gadi et al., 2021; Fatima et al., 2022). There are many reported cases of autoimmune hepatitis after the mRNA vaccination (Zheng et al., 2022). A study based in Saudi Arabia identified 31 cases of autoimmune disease following mRNA vaccination, including vasculitis, systemic lupus erythematosus and neurological diseases. All but four of them were new-onset disease, where symptoms first appeared on average just seven days after the vaccine (Alqatari et al., 2023). A comprehensive review found considerable evidence of new-onset autoimmune disease following mRNA vaccination, including autoimmune glomerulonephritis, autoimmune rheumatic diseases, and autoimmune hepatitis (Guo et al., 2023). These cases predict a Treg/Teff cell imbalance after the mRNA vaccination, which can influence the course of cancer immunotherapy treatment (Tanaka et al., 2017).

The Immune Response of Cancer(+) Patients after Receiving the mRNA Injections: the Influence of Vaccination on the Treg Responses

The activation of DCs, through the stimulation of Toll like receptors (TLRs), pro-inflammatory cytokines, and CD40, is naturally designed to produce a subpopulation of Treg cells. For review, see Khantakova et al. (2022). The generation of Treg cells exerts immunosuppression in the tumor microenvironment, lowering the natural cellular anti-tumor activity and enhancing the growth of tumors (Shang et al., 2015). The possibilities for tumor enhancement by Treg cells are several, and the generation of Tregs has a prognosis favoring the development of many cancers while at the same time inhibiting the development of autoimmune diseases (Saleh et al., 2020).

Treg cells inhibit anti-tumor immunity, and enhanced Treg responses are associated with cancers of poor prognosis. The elimination of Treg cells in cancer is a hallmark for successful treatment results during immunotherapy (Iglesias-Escudero et al., 2023). When Shimizu et al. (1999) specifically blocked the CD25+ CD4+ suppressive Treg cells, the peripheral CD4+ T cells could eliminate syngeneic tumors in normal naïve mice. The results of Takahashi et al. (1998), showed that the elimination of CD25+ CD4+ Treg cells in naïve mice led to spontaneous development of autoimmune diseases. The CD25+ CD4+ Treg cells are naturally anergic, and when activated exert immune suppression. Moreover, the antigen concentration required to make the Treg cells become suppressive is lower than the antigen concentration required to make the CD25-CD4+ T cells, i.e., Teff cells, become activated and proliferate. The expression of CD25 (also known as the IL-2 receptor α chain) facilitates distinguishing between the true Treg cells, characterized by being responsive to IL-2 and immunosuppressive, and cells that are non-responsive to IL-2 (CD25-), which are not true Treg cells and are non-suppressive.

Only a few subsets of CD25- cells can evolve, regain their CD25 expression, and function as regulatory (suppressive) cells during a specific antigen's repetitive activation of the immune response (Zelenay et al., 2005). A thorough analysis of the T cell responses elicited after the full dose (two injections) of the mRNA vaccination in cancer(+) patients highlights that their T cell responses are very low 6 months after vaccination as compared to their T cell responses that were developed three weeks after their mRNA full (two dose) vaccinations (Lasagna et al., 2022). Although this can be attributed to the overall immunodeficiency caused by cancer in these patients, this can also mean that the immune system of these patients develops a sufficient Treg subclass of cells, specific for

spike protein, which remains responsive in time and eventually suppresses the T cell response against the spike protein.

Cancer(+) patients being treated with immune-suppressing therapy face a difficult situation where they are likely to experience severe disease from a viral infection, but they are also not likely to respond as well as cancer(-) patients, to the vaccine. A careful investigation of the immune response of cancer(+) patients to repeated mRNA vaccination revealed an ominous sign that such patients could reach a point where further vaccination against COVID-19 is counterproductive (Benitez Fuentes et al., 2022). Eleven out of 36 patients being studied showed an optimal response after the second vaccine, but then suffered from T cell exhaustion following the booster shot, due to repeated exposure to the spike antigen and whatever other toxicants may accompany it. A marked fall-off of IFN-γ production was associated with a marked upregulation of programmed cell death 1 (PD-1) on CD4+ and CD8+ T cells (Benitez Fuentes et al., 2022). PD-1 is a known marker for T cell exhaustion (Lee et al., 2015). Several studies have shown that PD-1 is upregulated in CD8+ and CD4+ T cells during COVID-19 disease, and that PD-1 levels are higher in association with severe disease (Al-Mterin et al., 2022 and their references). This suggests that the booster shot may have actually made these patients more susceptible to severe disease from COVID-19. Furthermore, PD-1 expressing exhausted T cells cannot suppress tumor growth efficiently (Simon et al., 2017).

A study on mice clearly demonstrated that repeated booster shots immunizing against the spike receptor binding domain led to increased PD-1 expression in T cells, which was associated with profoundly impaired CD4+ and CD8+ T cell activation and a poor antibody response (Gao et al., 2022).

Severely immunosuppressed cancer patients suffering from multiple myeloma generate a specific memory Teff subpopulation against spike protein which increases after two to five weeks from the second mRNA vaccination dose (Zaleska et al., 2023). A specific mTreg cell subpopulation was also generated after the mRNA vaccines, and was sustained over time, in the immune system of the mRNA vaccinated multiple myeloma patients (Amar et al., 2022). The Treg and mTreg cells are generally CD25+, CD27+, FOXP3+, and CD127+. As a reminder, the general rule is that the CD25+ (true Treg) T cells will become activated with less antigen concentration than the CD25- (not true Treg) T cells (Takahashi et al., 1998).

Furthermore, the immune suppression conferred by the CD25+ Treg cells is independent of the humoral response developed by the B cells encountering the antigen, as this kind of T cell response relies purely on the antigen-presenting cell interactions. Therefore, the increased activation of B cells upon the third booster dose of mRNA in patients with solid cancer shown in the study of Shroff et al. (2021) is not related to the true Treg response developed in these patients. The finding of this study, which illustrates a poor effector T cell response after the third booster mRNA, is alarming and prognoses for further deterioration of the overall health of the solid cancer patients. This is due to the development of the Treg response which suppresses T cell clonal activation.

Because the researchers were unable to detect any presence of antigen presenting cells, specific subtyping of T cells was not performed. Also, after the third (booster) dose of mRNA, the humoral B cell response lacked coordination between various immune aspects which are normally linked, suggesting a diminished T cell effector response. Regarding T cell adaptive immunity, this means that the Treg and subsequently the mTreg responses which have been developed in these cancer(+) patients were feasibly robust, and their suppressive activities outweighed any beneficial Teff cell response against the mRNA coded spike protein after the booster (third) dose of mRNA

vaccination (Rocamora-Reverte et al., 2021). Also, a disorganized B regulatory cell activity leads to a downregulated Teff cell response (Lund & Randall, 2010).

PD-L1 Upregulation Following mRNA Vaccination

Programmed cell death ligand 1 (PD-L1) is a regulatory molecule expressed on many types of immune cells and cancer cells, and, by binding to its receptor PD-1, expressed on the surface of activated T cells, it leads to T cell dysfunction and apoptosis (Qian et al., 2018). Two studies have shown that PD-L1 is overexpressed in circulating immune cells following the second vaccine. Loacker et al. found significant upregulation of PD-L1 expression levels on monocytes and granulocytes two days after the second mRNA vaccine in 62 vaccinated individuals, compared to unvaccinated controls. They suggested that this indicated a regulatory response to avoid autoimmune collateral damage (Loacker et al., 2022). Özbay Kurt et al. (2022) examined expression of PD-L1 in antigen-presenting monocytes at 6 different time points starting before the first mRNA vaccine and ending 12 weeks after the booster shot. They found particularly high expression levels two weeks after the second vaccine. The level subsided somewhat but remained elevated at all the subsequent measurement times, up to 12 weeks after the booster shot. These studies raise concern that sustained upregulation of PD-L1 could accelerate tumor growth, because PD-L1 expressing monocytes in circulation could infiltrate the tumor environment. PD-L1 ligating to PD-1 on activated CD8+ T cells would suppress their activity, preventing them from killing the tumor cells. PD-L1 also causes PD-1-expressing activated CD4+ T cells to transform into Tregs (Ostrand-Rosenberg et al., 2014).

PD-L1 is expressed on many types of cancer cells, and, by binding to its receptor PD-1, expressed on the surface of activated T cells, it leads to T cell dysfunction and apoptosis (Qian et al., 2018). Furthermore, the PD-L1 upregulation depends on sensing IFN-γ secreted by activated CD8+ T cells (Spranger et al., 2013). PD-1/PD-L1 inhibitors are a group of immune checkpoint inhibitors for cancer immunotherapy in multiple types of cancer (Ai et al., 2020). These work by blocking PD-1/PD-L1 signaling, enabling tumor-resident immune cells to kill the tumor cells. However, these drugs often come with severe and even fatal side effects that limit their usefulness. Treatment is associated with increased risk of severe immune-mediated inflammation in the many organs, as well as autoimmune diabetes (Ai et al., 2020). This is due to the fact that the tissue resident immune cells can now launch an inflammatory response.

Impaired PD-1/PD-L1 function plays an important role in many autoimmune diseases (Zamani et al., 2016). The fact that the activation of PD-1 is essential for the prevention of autoimmunity caused by the mRNA vaccines is shown in a study involving cancer(+) patients (Spiliopoulou et al., 2023). These patients were under immunotherapy treatment with checkpoint signaling inhibitors that block PD-L1 expression and therefore PD-1 activation (Alsaab et al., 2017). The patients developed autoimmune antibodies after the mRNA booster doses, likely due to the antigenic over-stimulation of Teff cells by spike protein, in the absence of a protective response normally induced by PD-L1. It is reasonable to assume that the CD25- effector T cells are protagonists in this pathway (Kumar et al., 2018). These T cells permit the development of autoimmunity while offering protection against cancer (Ohue et al., 2019; Rocamora-Reverte et al., 2021). Moreover, the development of autoimmunity in patients under immunotherapy is an unwanted clinical parameter because it can be a life-threatening condition. For a detailed review see Bareke et al. (2021).

A characteristic of the aged immune system is inflexibility and less power to adapt to new challenges. As the system ages, an imbalance sets in between Tregs and Teffs. Age-related loss of Treg function

renders the host susceptible to a syndrome of chronic smoldering inflammation, whereas age-related gain of Treg function leads to increased risk to cancer and infection. It appears that the aged immune system has reached a steady-state condition that often errs in one direction or the other, regarding Treg function, which dictates a trade-off between autoimmune disease and cancer (Jagger et al., 2014). Autoimmunity and cancer, according to Sakowska et al. (2022), are two sides of the same coin.

Impaired mTOR-mediated Treg Cell Function

An important role for type I IFNs is to stimulate the synthesis of a pool of Tregs ready to "spring into action" once the viral load subsides. This process depends on activation of the PI3K/Akt/mTOR pathway (Platanias, 2005). In 2013, Zeng et al. (2013) demonstrated an essential role for mTORC1 as a positive regulator of Treg function, via experiments on mice with a disruption of mTORC1 function through Treg-specific deletion of the essential component raptor. These mice developed a fatal early onset hyperinflammatory disorder due to ineffective Treg suppressor function. Raptor-dependent mammalian target of rapamycin complex 1 (mTORC1) signaling in Tregs coordinates Treg proliferation and upregulation of suppressive molecules to establish Treg functional competency.

The NLRP3 inflammasome recruits macrophages and neutrophils, which in turn cause reactive oxygen species (ROS) production (Dominic et al., 2022). The spike protein has been demonstrated to induce an intense inflammatory response that may be initiated even prior to cellular infection. Even spike pseudovirions and recombinant SARS-CoV-2 spike protein treatment induce apoptosis and phagocytosis in ACE2-expressing cells, as a consequence of ROS inactivation of the PI3K/Akt/mTOR pathway (Li et al., 2021). The authors of this work proposed that this effect could account for multi-organ failure associated with severe cases of COVID-19 (Li et al., 2021).

Interferon regulatory factor 3 (IRF3) is a transcription factor that plays an essential role in detecting double strand viral RNA and then launching the type I IFN response and activating the PI3K/Akt pathway (Tarassishin et al., 2011). It has been argued that the spike protein interacts with IRF3 and mediates its proteasomal degradation, thus terminating IFN-I activation (Freitas et al., 2022). Thus, the vaccines not only fail to induce an early IFN-I response, but also facilitate the production of a large pool of spike proteins that would directly interfere with IFN-I activation by other pathogens, and may be sources of various other problematic concerns (Mead et al. , 2024a; Mead et al., 2024b). The interference with IFN-I is the specific effect of the spike protein that could partially explain the occasional reactivation of latent viruses such as *Herpes* and *Varicella* following mRNA vaccination (Rodríguez-Jiménez et al. 2021).

The sustained hyperinflammatory state induced by the mRNA vaccines may be primarily due to the impaired power of the immune system to provide an adequate pool of activated effector Tregs to suppress the cytokine response by effector T cells. As we will discuss, repeated mRNA vaccination eventually induces a response that suggests the development of immune tolerance to the spike protein, likely to prevent tissue damage due to excessive cytokine production. But this also means that the vaccine will lose its effectiveness to protect from COVID-19 after repeated booster shots. A relevant study on cancer(-) immunosuppressed individuals shows that only after selective drug-induced mTOR inhibition do CD4+ and some CD8+ hyperactive T cells develop after the mRNA vaccination (Netti et al., 2022). When mTOR is active, the differentiation of T effector cells is favored, while the formation of memory T cells is inhibited. Likewise, the inhibition of mTOR promotes the generation of memory immunity (Pollizzi et al., 2015). As already described, the

favoring of memory Teff responses will further promote immunosenescence and inflammaging, and this requires a thorough evaluation of mRNA vaccines safety, especially in the elderly population. Figure 2 shows the processes by which the SARS-CoV-2 spike protein could induce an inflammatory response.

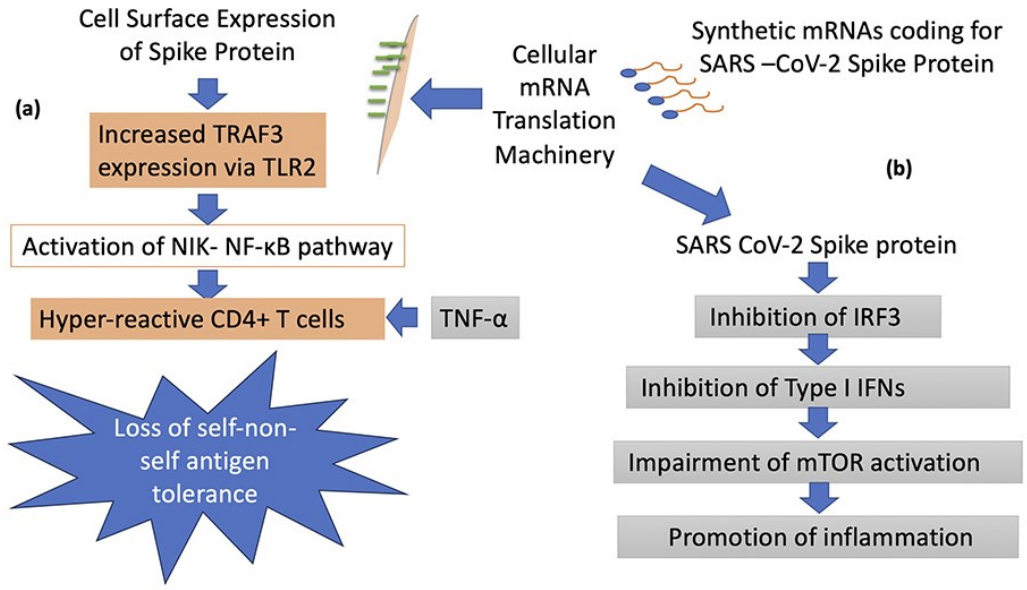

Figure 2. Spike protein induction of an inflammatory response. The events leading to hyper-activation of inflammation can concurrently occur through (a) a CD4+ T cell over-production via the stimulation of NIK and thereby loss of self-antigen tolerance, and (b) a promotion of inflammation through the inhibition of IRF3 and type I IFN, and subsequent impairment of mTOR activity (Murray et al., 2011; Zeng et al., 2013; Pollizzi et al., 2015; Liu et al., 2017; Rodríguez-Jiménez et al. 2021; Freitas et al., 2022).

TGF-β Signaling and the Development of a Th17 Response

The responses of immune cells to the SARS-CoV-2 vaccine cause enhanced TGF-β signaling and an increased NF-kB response, but only in some subtypes of immune cells, as shown in Table 1 (Liu et al., 2021). Specifically, the CD4+ Treg cells, CD4+ T proliferative cells, monocytes and dendritic cells show intense TGF-β signaling. These cells impact the efficient control and development of Treg responses (Rocamora-Reverte et al., 2021). When immune cells encounter huge amounts of SARS-CoV-2 mRNA-encoded spike protein in vaccinated individuals, intense TGF-β signaling and increased IL-6 and TNF-α expression are observed (Biering et al., 2022).

As shown in Table 1, the T cell subsets (including the Treg cells) that show intense TGF-β signaling are also resistant to hypoxia effects. For the T cells to sustain themselves in this environment, they express adequate hypoxia inducible factors (HIFs). HIF has been shown to be protective against uptake of the spike protein through multiple systems (Prieto-Fernández et al., 2021).

Studies show that enhanced TGF-β signaling and HIF expression contribute to the progression of tumors (Mallikarjuna et al., 2019). HIF signaling contributes to the etiopathology of various autoimmune diseases, including multiple sclerosis (MS) (Deng et al., 2016). MS and other severe neurological disorders, including Alzheimer's disease (AD), can emerge as causalities of the anti-SARS-CoV-2 mRNA vaccination and spike protein side effects (Kyriakopoulos et al., 2022; Seneff et

Table 1

The hypoxia effect, and TGF-β signaling responses of progenitor lineages of immune cell subsets to the inactivated SARS-CoV-2 vaccine containing the spike protein mRNA. Adapted from Liu et al. (2021).

Cell Type	Hypoxia	TGF-β signaling
Lymphoid Lineage		
B cells	Moderate	Low
$CD4^+$ T cells	Low	High
$CD4^+$ Treg cells	Low	High
CD4+ T proliferation cells	Low	High
$CD8^+$ T cells	Low	No
$CD8^+$ B T cells*	Moderate	High
CD8+ T proliferation	Moderate	Low
γδ T cells	Slightly moderate	Moderate
MAIT	High moderate	High moderate
NK cells	High	High
Myeloid lineage		
Monocytes/Dendritic Cells	**High**	**High**

*MS4A1, CD79A, CD79B positive CD8+ T cells

al., 2023). A recent large epidemiological study from South Korea reveals a strong potential association between mRNA COVID-19 vaccines and the development of AD (Roh et al, 2024). Moreover, the combination of a) IL-6 and TNF-α overexpression, b) enhanced TGF-β signaling and c) increase of HIF expression by the anti-SARS-CoV-2 vaccine spike protein can be detrimental for the development of the Treg response and lead directly to autoimmunity (Tang et al., 2023).

As illustrated in Figure 3, HIF overexpression increases TGF-β signaling (Xu et al., 2017). At the same time, the abundance of IL-6, when accompanied by the overexpression of TNF-α, results in the decrease of CD4+, CD25+(high), Foxp3+ Treg cells, and the increase of IL-17-producing T helper (Th17) cells (Samson et al., 2012). In addition, the expression of TGF-β, in tandem with IL-6, represses Foxp3 expression and enhances CD17 expression and hence growth of a Th17 cell subpopulation via RORγt nuclear receptor expression and activation of signal transducer and activator of transcription 3 (Manel et al. 2008., Pesce et al., 2013). The spike protein has been shown to activate both TLR2 and TLR4, resulting in JAK/STAT signaling (Khan et al., 2021; Fontes-Dantas et al., 2023).

Moreover, the spike protein potentiates the signaling of the epidermal growth factor receptor (Palakkott et al., 2023). Persistent activation of STAT3 is a common feature of the tumor

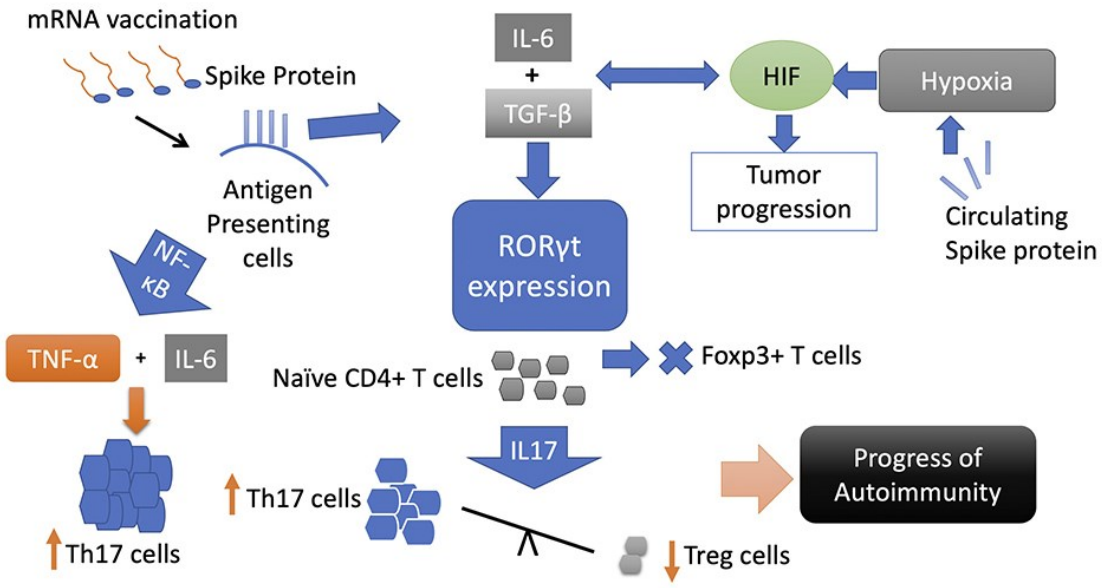

Figure 3. The systems of enhanced Th17 cellular differentiation in cancer(+) patients after mRNA vaccination, facilitated by the SARS-CoV-2 spike protein. The induction of IL-6 and TNF-α, via the NF-κB response to spike, and the TGF-β induced expression of RORγt, enhances the generation of a Th17 population of cells that is responsible for the development of autoimmunity (Manel et al., 2008; Pesce et al., 2013; Deng et al., 2016; Kyriakopoulos et al., 2022; Seneff et al., 2023; Tang et al., 2023; Roh et al, 2024).

microenvironment and a major contributor to the inflammatory state (Yu et al., 2009b). An intense activation of STAT3 can result from 1) an aberrant expression of IL-6 and subsequent stimulation of the IL-6 receptor (Wang et al., 2013), and 2) an intense activation of epidermal growth factor receptor signaling as imposed by the spike protein (Palakkott et al., 2023). These molecular events, illustrated in Figure 4, when they are happening concurrently, have the potential to bypass the inhibitory checkpoint of negative regulator suppressor of cytokine signaling 3. That suppressor would otherwise deactivate JAK in order to diminish STAT3 activation (Wang et al., 2013). Moreover, the abnormally elevated expression of IL-6 and JAK/STAT3 signaling are pro-tumorigenic and enhance the differentiation of Th17 cells (Wang et al., 2013).

Elevated levels of Th17 cells are implicated in the etiopathogenesis of numerous inflammatory and autoimmune diseases (Yasuda et al., 2019). Furthermore, in some instances, Th17 cells can promote cancer (Bailey et al., 2014). Th17 cells are shown to be strongly implicated in spike protein induced immunopathology. In a recent study that concluded that the spike protein aggravates rheumatoid arthritis, the Th17 cell population was markedly increased, whilst the Treg cell population was decreased (Lee et al., 2023).

Th17 cells produce the cytokine IL-17, which promotes inflammation. Th17 cells are believed to be involved in the pathogenesis of myocarditis, which has been identified as a sometimes-fatal complication of mRNA vaccination (Schwab et al., 2023). They recruit other immune cells, such as neutrophils, to the heart, and they release pro-inflammatory molecules such as IL-17. The levels of Th17 cells are elevated in patients with myocarditis. Blocking Th17 cell activity via drugs such as Bazedoxifene ameliorates myocarditis in experimental models (Wang et al., 2021). Furthermore, a connection to macrophage activation syndrome is suggested by a study that confirmed that

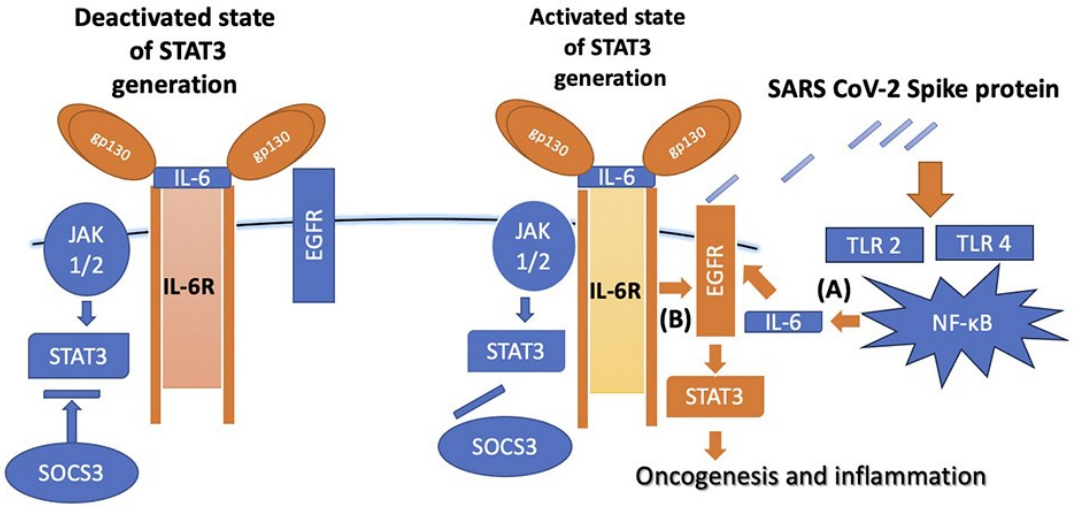

Figure 4. Potential activation of STAT3 by SARS CoV-2 spike protein stimulatory effects. Under normal conditions, IL-6R remains dormant as a) it is inhibited by suppressor of cytokine signaling 3 and b) it does not synergize with unstimulated epidermal growth factor receptors to produce STAT3. This condition can be bypassed and reversed by (A) the activation of NF-ϰB and subsequent expression of IL-6 through TLR2 and TLR4 stimulation by spike protein (Khan et al., 2021; Fontes-Dantas et al., 2023) and (B) the direct stimulation of epidermal growth factor receptor by spike protein and synergy with IL-6R (Palakkott et al., 2023). The final effect between IL-6R, epidermal growth factor receptor and IL-6 would be the continuous production of STAT3, although the suppressor of cytokine signaling 3 will still be present (Wang et al., 2013).

macrophages infiltrated the heart muscle and became activated, releasing toxic cytokines, in association with vaccine-induced myocarditis (Barmada et al., 2023).

Th17 cells also play an important role in autoimmune hemolytic anemia. Xu et al. (2012) found that patients with autoimmune hemolytic anemia had elevated levels of Th17 cells, which were closely correlated not only with disease severity but also with the levels of IL-17 and anti-RBC IgG antibodies. IgG antibodies are the most common class of autoantibodies against RBCs, often acting through molecular mimicry. CD8+ T cells bind to IgG antibodies and become activated to release cytokines that destroy RBCs. Abnormalities of immunoregulatory cytokines associated with autoimmune hemolytic anemia include elevated levels of IL-6, IL-2, and IL-17, and increased secretion of TGF-β (Barcellini et al., 2000). Reduced numbers of circulating CD4+ nTregs are also linked to the disease (Ahmad et al., 2011). As we have seen, all of these are consistent with known effects of the spike protein, and with those of toxicants in general.

Yonker et al. (2023) found that the concentration of free unbound circulating spike protein is elevated in the blood of vaccinated individuals who suffer from post-vaccine myocarditis. Whereas in a control group without myocarditis, circulating spike protein was appropriately bound by antibodies. Some cases of myocarditis due to the mRNA vaccination are considered to be the result of autoimmune activation (Mohiddin et al., 2022). Additionally, it is worrisome that the DCs and monocytes increase their TGF-β and IL-2 signaling upon engagement with the spike protein synthesized by human cells from the vaccine mRNA (see Table 1; also Liu et al., 2021). The spike protein on its own has been shown to activate TGF-β signaling (Biering et al., 2022). The monocytes and macrophages are mainly dendritic-cell-derived antigen presenting cells (Sung, 2008). The intense TGF-β signaling can also be attributed to spike protein induced inflammation via TLR2-mediated NF-ϰB hyperactivation (Khan et al., 2021).

Proper Treg-dendritic cell interactions are crucial for the well-controlled suppression of the effector CD4+ T lymphocytes (Kumar et al., 2018). Impairments during Treg-dendritic cell interactions will produce autoimmune disease (Kumar et al., 2018). TGF-β signaling inhibits dendritic cell functions in general, and latent TGF-β signaling by the DCs will contribute in favour of Th17 cell differentiation, and to the development of autoimmune disease.

This immune impairment seems to be tightly connected with the mRNA vaccines. An autoimmune origin of disease is sufficiently described in a relevant case of encephalomyelitis due to mRNA vaccination (Sanjabi et al., 2017). Moreover, the several pathological neurological outcomes that follow COVID-19 mRNA vaccines, including Guillain Barré syndrome, transverse myelitis, and acute disseminated encephalomyelitis (amongst several others), also have an autoimmune origin (Sriwastava et al., 2022). Again, in relation to the spike protein expressed by the mRNAs, autoimmune encephalitis was the diagnosis of disease after three doses of mRNA (Pfizer) vaccination in a case study, and the mRNA vaccines were found to be the only factor causing the disease in this patient (Abu-Abaa et al., 2022).

Th17, PD-L1 and IgG4

The mRNA vaccines induce a strong IgG antibody response to the spike protein. There are four subtypes of IgG antibodies, labelled as IgG1, IgG2, IgG3, and IgG4. IgG3 is very effective at protecting from infection, whereas IgG4 is uniquely powerless to protect from infection, and actively blocks access to the spike protein by effector antibodies (Vidarsson et al., 2014). IgG4 is normally the least common variant in human serum. Elevated levels of IgG4 are triggered by repeated exposure to inflammation-inducing antigens. A seminal paper tracked the evolution of IgG variant distribution over time following the initial two shots and subsequent booster shots of mRNA SARS-CoV-2 vaccines (Irrgang et al., 2023). Remarkably, they found that class switching towards IgG4 increased over time in the months following vaccination. IgG4, which normally represents no more than 5% of the total pool, was sharply elevated upon administration of the booster shot. Furthermore, the level continued to rise after the booster, reaching nearly 20% of the IgG pool five months after the booster shot. A subsequent article proposed that IgG4 induced by the booster shot constitutes an immune tolerance factor that would suppress the natural antiviral responses to the SARS-CoV-2 virus (Uversky et al., 2023). Another publication confirmed that IgG4 is highly expressed several months after mRNA vaccination, and that this phenomenon does not occur for the DNA vector-based vaccines (Kiszel et al., 2023).

IgG4-related disease (IgG4-RD) is a fibro-inflammatory condition that is characterized by elevated serum levels of IgG4 and excess fibrosis in multiple organs (Zhang et al., 2022). PD-L1 plays a role in IgG4-RD. Concentrations of soluble PD-1 and PD-L1 are significantly elevated in patients with IgG4-RD, and the expression of PD-1 on Treg cells is upregulated. Furthermore, stimulation of naïve T cells from IgG4-RD patients with PD-L1 caused them to transform into CD4+CD25+ iTreg cells. The authors concluded that the PD-1/PD-L1 pathway could promote Treg cell differentiation into iTregs, and that this may play an important role in the observed elevation of Treg cells in IgG4-RD patients (Zhang et al., 2022). Most target organs of IgG4-RD have Treg cell infiltration, and Treg cells are also abundant in the blood (Akiyama et al., 2016).

Type I autoimmune pancreatitis is commonly found in association with IgG4-RD. An increased number of circulating iTregs, particularly those releasing Il-10, was found in association with IgG4-RD-related pancreatitis (Kusuda et al., 2011). On the other hand, circulating nTreg levels are low, a pattern consistent with immunosenescence. These abundant iTregs appear to be ineffective at controlling the inflammation, and a likely explanation for this is a decreased expression of

Mammalian Sterile 20-like Kinase 1, which is essential for allowing the cell-to-cell contact needed for iTregs to act on Teff cells (Uchida & Okazaki, 2022). Patients with IgG4-RD are at increased risk to both pancreatic cancer and lymphoma (Yu et al., 2022). Several case reports of acute pancreatitis have been reported in association with mRNA vaccines (Hussain et al., 2024). A case study described a patient who experienced rapid progression of lymphoma following an mRNA booster vaccine (Goldman et al., 2021). Several other case reports involving lymphoma following mRNA vaccination have been published (Mizutani et al., 2022; Cavanna et al., 2023; Tachita et al., 2023).

Conclusion

In this paper, we have extensively reviewed the possible role of Treg cells dysregulation in the immune system, caused by the mRNA vaccines. The vaccines typically induce an intense IgG antibody response due to the toxicity of the spike protein, along with an extreme inflammatory response through cytokine release by T cells, and, ultimately, the potential for autoantibodies to attack the tissues through recognition of non-self spike protein on the cell surface. Because a natural infection is replaced by an abnormal situation in which human cells are producing large quantities of spike protein, the type I IFN response is suppressed. Normally, this response to double-stranded viral RNA induces the clonal expansion of a pool of Treg cells, but also keeps them suppressed until the viral load has sufficiently subsided. The mRNA in the vaccines is resistant to breakdown and concealed from the immune system due to its N1-methylpseudouridine replacements and humanized code. This causes an unnatural and often inappropriate immune response, where the consequences are highly dependent on the prior immune state of the vaccinated individual, particularly with respect to their Treg cell population. Some of the activated DCs return to the thymus and induce a response that damages the thymic epithelium and accelerates thymic involution, leading to inflammaging and immunosenescence. This can also induce a life-threatening macrophage activation syndrome (hemophagocytic lymphohistiocytosis), as was observed in several case studies on the mRNA vaccines. Repeated booster vaccination can lead to the development of self-tolerance to the spike protein, which may make the person less resistant to the virus than a fully unvaccinated person. Moreover, iatrogenic autoimmunity raised though molecular mimicry across recombinant SARS-CoV-2 spike protein epitopes from mRNA vaccines that attack the host immune system can be responsible for immune defects in the mRNA vaccinated individuals. This is a sound clinical explanation for the post-mRNA myocarditis cases that have been investigated and is readily occurring in other infectious diseases. A simplified schematic of the complex response to the mRNA injections is presented in Figure 5.

We have analyzed the response to the mRNA vaccines against COVID-19 differentially depending on a distinction between cancer(-) and cancer(+) populations. The mRNA vaccines cause Treg dysregulation in both populations. The Treg dysregulation in the cancer(-) population predominantly causes immune senescence and promotes autoimmunity, in part, we suppose, due to homing of mTreg cells to the thymus and accelerated thymic involution. In cancer(+) cases, depending in part upon whether they receive PD-1/PD-L1 inhibitors, and this is crucial for cancer patients under immunotherapy, patients develop a hyperimmune response and also have a tendency to develop autoimmunity. This threatens their health and negatively influences the progress of whatever immunotherapy treatments may be used. Moreover, the cancer(+) patients who do not receive PD-1/PD-L1 blockers are prone to cancer progression by the mRNA vaccines. Furthermore, the development of a high Th17 response may also result in tumorigenesis. Further studies are needed to evaluate the potential of the mRNA vaccines to induce cancer. The inhibition of mTOR may

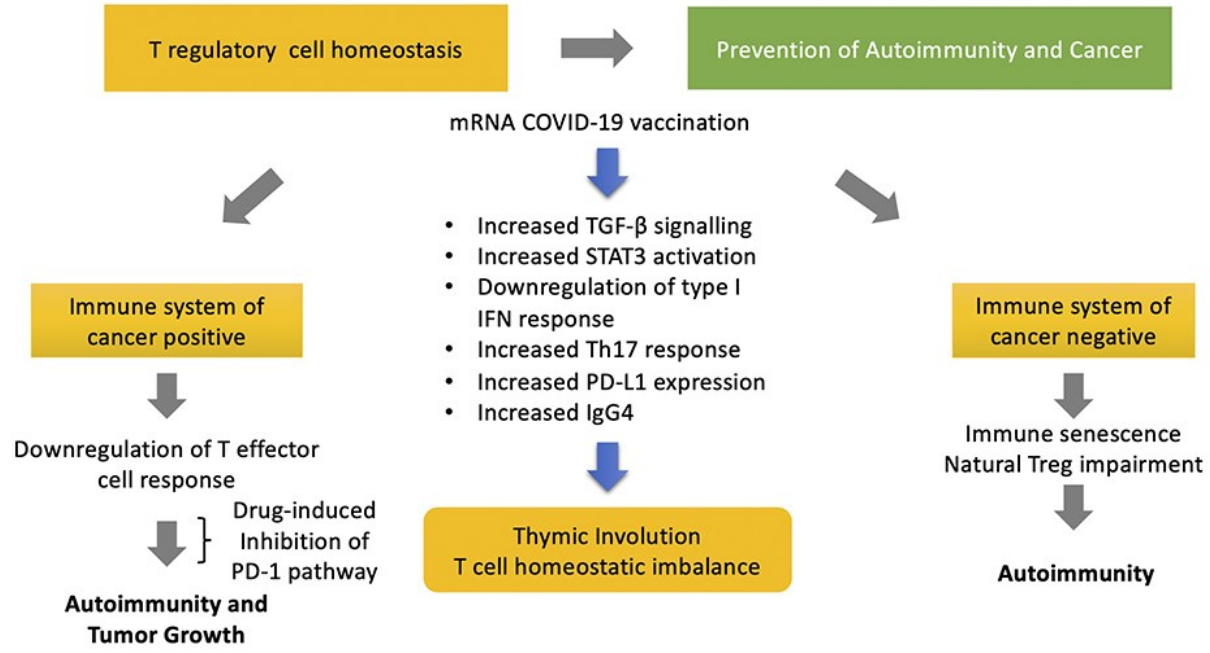

Figure 5: Schematic of the cascade of events leading to immune dysfunction due to SARS-CoV-2 mRNA vaccination. Repeated vaccination can potentially lead to thymic involution and accelerated immune senescence, increasing risk to autoimmune disease and cancer, through impaired Treg cell signaling.

accelerate immunosenescence due to enhancement of the memory Teff response. This is especially concerning for the elderly population receiving the mRNA vaccines, who are at risk for both autoimmune and neoplastic disease.

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