Oncogenesis and autoimmunity as a result of mRNA COVID-19 vaccination

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Abstract

When an antigen stimulates the immune system, specific T regulatory (Treg) and T effector (Teff) subpopulations develop from naïve T cells. The Treg cell population will produce the memory Treg (mTreg) cells against that specific antigen. An inappropriate homeostatic balance among Teff, Treg and mTreg cells can direct the immune system toward either cancer or autoimmunity. When cancer is present, Treg cells suppress anti-tumor immunity, and, when cancer is absent, Treg cells play the beneficial role of preventing the development of autoimmunity. In this review, we analyze Treg responses after SARS-CoV-2 mRNA vaccination and find distinct pathological responses under differing conditions. In cancer patients, the degree of disease progression depends on the cancer status at the time of vaccination and the type of cancer treatment they receive concurrently. We hypothesize that migration of circulating dendritic cells and mTreg cells back to the thymus accelerates thymic involution, a direct cause of immunosenescence. In summary, the Treg responses produced after mRNA vaccination and the subsequent mRNA-encoded SARS-CoV-2 spike protein expression may lead to a harmful influence on the immune system of vaccinees, and subsequent accelerated development of cancer and autoimmune disease. These mechanisms are consistent with both epidemiological findings and case reports.

Abbreviations: ADEM: acute disseminated encephalomyelitis; AIHA: autoimmune hemolytic anemia; AIP: autoimmune pancreatitis; ANA: antinuclear antibody; CCR6: C-C Motif Chemokine Receptor 6; DCs: dendritic cells; EGFR: epidermal growth factor receptor; Foxp3: forkhead box P3 ; GBS: Guillain Barré syndrome; HLH: Hemophagocytic lymphohistiocytosis; IFN: interferon; IL-10: interleukin-10; IgG4-RD: IgG4-related disease; IgG: immunoglobulin G ; NF-ϰB: nuclear factor kappa-light-chain-enhancer of activated B cells; NIK: NF-ϰB Inducible Kinase; PD-1: programmed cell death 1; PD-L1: programmed cell death-ligand 1; RBD: receptor binding domain; RGD: arginine-glycine-aspartate tripeptide motif; SOCS3: suppressor of cytokine signalling 3; STAT3: signal transducer and activator of transcription 3; TB-IRIS: tuberculosis-immune reconstitution inflammatory syndrome; TGF-β: transforming growth factor-β· TLR: toll-like receptor; TME: tumor microenvironment; Teff: T effector cell ; Treg: T regulatory cell; VARs: vaccine adverse reactions; iTregs: inducible Treg cells; MST1: Mammalian Sterile 20-like Kinase 1; mTECs: medullary thymic epithelial cells; mTreg: memory Treg Cell; miR: microRNA; nTregs: naïve Treg cells; scRNA-seq: single-cell mRNA sequencing.

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

1. Introduction

Upon stimulation from a specific antigen, the immune homeostasis of T regulatory cell responses preserves self-tolerance and halts exaggerated T cell immune responses to protect from tissue damage [1,2] The discovery of Treg cells (either of thymic or peripheral origin) in mammals, including humans, has offered considerable insights into the regulation of the adaptive immune response [1]. Both CD4+ and CD8+ regulatory T cells offer a homeostatic balance in the immune system to avoid both autoimmunity and cancer [3]. Treg cells release cytokines such as interleukin-10 (IL-10) that suppress the activity of Teff cells. When the immune cells lose self-tolerance, Treg cells play a role in preventing an excessive inflammatory response that could injure tissues. On the other hand, a large population of Treg cells resident in the tumor microenvironment maladaptively protects cancer cells from immune attack, leading to accelerated tumor growth [4].

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 shrinks, impairing the ability to generate new T cells. The increase in iTregs can help to suppress autoimmunity, but it comes with a high cost of increased risk to cancer and sepsis [5].

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 cells that show intermediate binding, the situation is more complicated. Many of them evolve into nTreg cells, that, when activated, are able to 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 [6,7]. 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 [8,9].

Ionizable cationic lipids are key components of the lipid nanoparticles used for delivery of mRNA in the mRNA vaccines [10]. 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 [11,12]. This can be beneficial as an adjuvant to induce an immune response, but it may cause unintended negative consequences through oxidative stress leading to mitochondrial damage and inducing necrosis, syncytia formation, and pyroptosis [13].

Activation of the NLRP3 inflammasome induces caspase-1 release from mitochondria due to excessive reactive oxygen species and mitochondrial DNA damage [14]. Damage response signalling results in the formation of membrane pores and the initiation of a necrotic form of cell death called pyroptosis. The NLRP3 inflammasome and caspase-1 together lead to secretion of the pro-inflammatory cytokine IL-1β [14]. These activities are essential for launching the immune response to the vaccine antigens that will ultimately lead to a strong antibody response, the desired outcome.

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 signalling, IL-18 [15,16]. IL-18 plays several roles both in immune activation and in autoimmune disease. On the positive side, it promotes the proliferation of cytotoxic CD8+ T cells [17]. However, through an unusual mechanism that we will describe in detail later on, it induces self-reactive innate antibody responses that play an essential role in autoimmune disease [18]. It also promotes inflammation-induced carcinogenesis in squamous cell carcinoma [19]. For our purposes, the most interesting aspect of IL-18 is its ability to induce peripheral activated mTreg cells to migrate back to

the thymus, particularly in younger persons before thymic involution, where they play a powerful role in disrupting innate nTreg development and release into the periphery [20]. We hypothesize that this effect is the primary mechanism 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 signalling upregulates C-C Motif Chemokine Receptor 6 (CCR6) expression in peripheral activated mTreg cells, and this results in their migration 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 [21].

A multi-author study has shown 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 dose [22]. Prior infection with COVID-19 leads to an increased production of IL-2 in response to the mRNA vaccines [23], inducing the transformation of peripheral Teff cells into Treg cells. A published 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 primed 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 homing of induced mTregs to the thymus and increasing the risk for an autoimmune attack on the heart [24].

The thymus plays a central role in shaping the immune system during childhood. With in- creasing age, the thymus shrinks over time, a process known as thymic involution, a property common to all vertebrates. 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" [25,26]. The NLRP3 inflammasome has a direct effect on the thymus, accelerating thymic demise [27]. IL-18 has been shown to suppress regeneration in the thymus, by activating the IL-18 receptor on natural killer cells [28]. It is widely accepted that immunosenescence leads to increased risk to infection, autoimmune disease, and impaired cancer immunosurvelliance [29].

Treg cells play an important role in thymic involution [30]. Remarkably, as thymic involution progresses, the homing mTreg cells maintain their numbers, while the counts of all the other cell types in the thymus decrease. In fact, these mature Tregs constitute the majority of the Treg pool in the aged thymus [20]. 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 ability to react to novel threats. Naïve Tregs able to respond to a new insult are in short supply, and this results in poorly controlled autoimmune attack by self-reactive T cells [31].

Those who suffer from conditions associated with immunosenescence, e.g., cancer, cardiovascular disease, rheumatoid arthritis, metabolic diseases, neurodegenerative diseases, etc., are at increased risk for suffering from severe and sometimes fatal COVID-19 infection [32]. 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 [33].

2. mRNA Vaccine Responses in Patients with and without Cancer

A recent analysis by Chouerini TK et al. [34], 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 exposure. Importantly, within the vaccinated cancer(+) population in this study, the development of hematologic malignancies was encountered more frequently than in the unvaccinated cancer(+) control group. Also, the vaccinated cancer(+) group

required more anti-neoplastic drugs to treat their malignant conditions.

The authors of this study concluded that the use of further mRNA vaccination [34], in addition to the initial two vaccines in cancer(+) patients, would help to prevent increased mortality rates from COVID-19 in this population group. 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 aforementioned studies on immunosenescence and Treg responses, [31-33], it is therefore likely that further injections would lead to even greater immune suppression, and further accelerate cancer progression [35].

These findings led us to review the literature and provide further analysis of the immune system responses developed upon mRNA vaccination in the cancer(+) and cancer(-) populations, 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 machine learning methods have been developed to evaluate vaccine-induced immunity and reactogenicity [36].

Tregs behave differently in healthy and in malignant tissues [37]. 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 [38,39].

Cancer and autoimmunity are in juxtaposition from a deregulated Treg response [40], which we argue is happening after mRNA vaccination. 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 [41]. Moreover, the development of autoimmunity is closely linked to primary immunodeficiency syndromes that manifest with recurrent infections [42]. In this case, the breakthrough infections encountered in the cancer(+) patients after the mRNA vaccination is a sign that the mRNAs produce an exacerbation of their preexisting immunodeficiency [34]. Breakthrough infections occur also in the cancer(+) patients that have not received immunotherapy treatments (although 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 [39]? 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 injections? In this regard, the Treg responses after the mRNA vaccinations could potentially be of prognostic value [43]. 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 [44]. With these questions in mind, we review the available literature on immune responses after the mRNA vaccinations. We then examine the similar but distinct implications of the dysregulation of Treg cells in the cancer(-) and cancer(+) populations. In doing so, we offer clear and concerning answers to the questions posed.

3. The Criteria for Assessing Treg Dysregulation after mRNA Vaccinations

Autoimmunity involves an impairment of Treg homeostatic balance [45]. 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 thorough review by

Diani S et al. 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 [46].

A study of Tormo N et al. 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. They noted substantial differences in the immune response to the administered vaccines over time based on both age and previous infection status [47]. We will describe their results in more detail later in this paper, as they nicely illustrate the concepts we are proposing. Two papers that set the stage for our arguments are Lourenço EV et al., which provides a review of the role of dysregulated natural Treg cells in autoimmunity [48], and Sanchez et al., which describes their important role during infection [49].

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 mechanisms of the immune system and the processes by which it ages. In the below, we begin with a section specifically focusing on the unique aspects of the immune response to the vaccines compared to SARS-CoV-2 infection. After depicting the observed Teff and Treg responses, inferred from the Tormo et al. study [47], 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. [50], which discusses immune loss of regulation due to an altered function of FOXP3+ Treg cells, we predict a subsequent Treg/Teff imbalance in the mRNA vaccinated individuals. 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 mechanisms that are underlying and complicate the final effect of repeated mRNA vaccinations led us to investigate the deleterious outcomes from an altered Treg/Teff balance in the immune systems of vaccinees, particularly after repeated booster shots [3,51].

4. Delayed but Enhanced Immune Response to mRNA Vaccines

mRNA 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 [52]. 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 has been disguised to resemble a human mRNA molecule [53].

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 [50]. Type I IFNs maintain the Foxp3+ expression that characterizes Treg cells under inflammatory conditions [54]. However, type I IFNs actually suppress the activity of Treg cells, holding them in check until the viral load has dissipated [55]. 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 shutting down the inflammatory response after the virus has been successfully cleared [56].

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 signalling 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 [57]. 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 highly 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 [58].

A study of the immune response to the vaccines compared to the response to infection revealed that the vaccine induces a response pattern comparable to that of severe disease [59]. 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." [59] 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 [60]. This delay may be a manifestation of a missing 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 microRNAs (miR-148a and miR-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 [61]. Exosomes presenting the spike protein on their surface are still present in the circulation four months after vaccination [62]. Large-scale single-cell mRNA sequencing (scRNA-seq) technology revealed dramatic alterations in gene expression of almost all immune cells after vaccination. Increased NF-ϰB signalling and a reduced type I IFN response were most notable, and there was a marked deficiency in CD8+ T cells [33]. Type I IFNs induce a massive expansion of antigen specific CD8+ T cells, both effector and memory, in response to viral infection [63]. Type I IFNs also protect CD8+ T cells from destruction by natural killer cells [64]. A study on mice with impaired type I IFN receptors in Tregs found that these Tregs had enhanced suppressor activity during both acute and chronic infection, resulting in CD8+ T cell anergy, defective generation of memory T cells, and viral persistence [55].

With mRNA vaccines, immune cells would be expected to respond to the situation as an unnatural circumstance in which human cells are producing a toxic foreign protein. The detection of antigen on the surface of transfected cells activates CD4+ immune cells and launches the cascade that eventually leads to a strong antibody response to the spike protein. This response is heavily skewed towards immunoglobulin G (IgG), with little or no IgM or IgA antibody production [59]. IgG is the primary antibody type that induces autoimmune disease, and this effect is enhanced in the absence of secreted IgM antibodies [65,66]. The replacement of every uridine in the vaccine mRNA molecules with methylpseudouridine assures that the mRNA will survive for a long time and continue to be translated into spike protein, resulting in sustained immune activation [67].

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. 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 [68].

The spike protein can induce an intense inflammatory response in endothelial cells via integrin binding. The arginine-glycine-aspartate (RGD) tripeptide motif exposed on the surface of the receptor binding domain (RBD) of the spike protein binds to integrin 51 expressed by endothelial cells. This activates the NLRP3 inflammasome through the NF-ϰB signalling pathway. NF-ϰB signalling also induces vascular leakage and leukocyte adhesion. As a result of NF-ϰB activation, proinflammatory cytokines, chemokines, and coagulation factors are upregulated in endothelial cells [69]. Treg cells dramatically increase their suppressive function in response to inflammation, releasing high levels of the immunosuppressive cytokines Il-10 and TGF-β [70].

iTregs, but not nTregs, 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 [71]. This fast-acting suppression is mediated by TGF-β released by the iTregs [72]. 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 [73].

Cancer is associated with an imbalance in Teff and Treg cells where the Tregs far outnumber the Teffs in the tumor microenvironment [35,74]. The NLRP3 inflammasome promotes carcinogenesis in squamous cell carcinoma. Huang et al. found that Foxp3 was highly overexpressed in the tumor, and Treg cells comprised 45% of the CD4+ T cells there [19]. 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, leading to accelerated tumor progression. Autoimmune disease has the opposite problem [45,75]. The increased activation of Teff cells by the vaccine in the context of an insufficient Treg pool will exacerbate autoimmune disease.

5. The Treg Response after mRNA Vaccination: Potential Role for Immune Senescence

Under normal conditions, immunosenescence occurs as the immune system ages [76]. As aging progresses, the peripheral Treg population increases in number, but most of those Tregs are mTregs already committed to specific antigens, and the ability to induce an iTreg response to a novel exposure is lowered [77]. 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 developing 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 [78]. During Mycobacterium tuberculosis infection in the lungs, IFN-γ-producing CD4+ T cells are essential for controlling the pathogen, but overproduction of this cytokine causes lung injury, leading to tuberculosis-immune reconstitution inflammatory syndrome (TB-IRIS) [79]. CD25-expressing hyperactivated Teff cells produce the protease furin, which cleaves the spike protein, facilitating viral entry [78].

Type I IFN induces proliferation of Foxp3+ Treg cells, which, when activated, suppress the expression of IFN-γ [80]. 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. The authors hypothesized that these cells were very short-lived and died off before being able to transform into Foxp3+ Treg cells. They concluded that 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 [78].

The aforementioned study by Tormo et al. provides an opportunity to compare vaccine responses among young and old and to assess the effect of previous exposure to SARS-CoV-2 [47]. 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 a <60 and a >60 population, as well as a distinction between those who had previously recovered and those whose first exposure to the spike protein was through the vaccine.

The first thing to note from this study is that 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 RU/ml, and the median for the CoV2+ >60 group was only 36 RU/ml, just prior to vaccination. 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 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 titer 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 IU/ml four weeks after the second vaccine. 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 titers 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 just before the second vaccine to only 0.89 two weeks later. The authors hypothesized 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 [9,22,33]. This sharp downregulation appeared to be transient, however, as a level comparable to that following the first vaccine was restored one month after the second dose, perhaps due to persistent production of the spike protein by transfected cells showing a continued Treg/Teff cell imbalance.

Lozano-Ojalvo et al. compared vaccine reactions in CoV2- and CoV2+ populations with similar findings as those of the Tormo et al. 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 [81].

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 [82]. Natural SARS-CoV-2 antigens are superior to the mRNA-derived spike protein for inducing long-lasting immunity [83]. 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 respiratory infections from April to June 2021, immediately following an aggressive nationwide vaccination campaign [84]. 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.

6. 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. In fact, 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 mechanisms: (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 [85].

SARS-CoV-2 can infect the thymus, particularly in the youth, and this induces a loss of function that correlates with disease severity [86]. ACE2 is expressed by the thymic epithelium, particularly the medullary thymic epithelial cells (mTECs), 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 TECs and downregulate critical

genes involved with epithelial cell adhesion and survival [86]. Rosichini et al. verified in an in vitro study that cultured TECs from the thymus of children expressed ACE2 and were able to be infected with SARS-CoV-2. Spike-positive human TECs were identified at both 24 and 48 hours after infection. There was increased mortality among the mTECs compared to cortical TECs, reflecting their higher ACE2 expression [86]. The spike protein induces IL-6 and TNF-α in epithelial cells [57]. Both of these cytokines have been implicated in acute thymic involution [87]. Defects in thymus epithelial cells are associated with the aged thymus [88].

An experiment involving mice with a genetic defect that interfered 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 mTECs 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 [89]. Autoimmune hepatitis has been associated with the mRNA vaccines [90].

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 [91]. The DCs would almost certainly endocytose the mRNA nanoparticles while resident 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, although somewhat reduced, even though there was no evidence of breast cancer [92].

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 home to the thymus and carry 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. They not only present antigen to T cells, but also induce antigen-specific Foxp3+CD25+CD4+ Tregs from Foxp3-CD25-CD4+ thymocytes. By contrast, Tregs were not induced by similar DCs in the spleen [93].

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 could be cleaved by furin from spike protein exposed on the membrane of DCs and released freely into the external milieu [94]. Since S1 contains the receptor binding domain of the spike protein, it could bind to the ACE2 receptors on mTECs, inducing a damaging inflammatory effect, as has been demonstrated for endothelial cells [69]. 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 signalling pathway [95].

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 [63]. As we have stated, the vaccines do not elicit a type I IFN response, due to the lack of double-stranded mRNA associated with viral replication [53,96].

Severe COVID-19 has been linked to a deficiency in the glycoprotein perforin, resulting in a pathogenic auto-inflammatory feedback loop [97]. Perforin, released by cytotoxic CD8+ T cells, generates pores in the target cell membrane, 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 [98]. Furthermore, the S1 subunit of the spike protein has been shown experimentally to suppress perforin expression in CD8+ T cells [99].

Cytotoxic CD8+ T cells are essential for eliminating hyperactivated antigen presenting DCs in the thymus, a process that critically depends on perforin [100]. Hemophagocytic lymphohistiocytosis (HLH), 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 HLH, due to an impaired ability of CD8+ T cells to prune off hyperactivated antigen-presenting DCs in the thymus [100]. HLH has been reported as an adverse reaction to the mRNA vaccines in multiple publications [101-104].

In summary, the mRNA vaccines 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 be a source of autoimmune disease and HLH, a hyperinflammatory attack on the organs. At the same time, transfected DCs in the thymus may continue to produce spike protein for weeks if not months after vaccination, causing damage to mTECs, accelerating thymic involution, and driving the immune system towards anergy. These ideas are schematized graphically in Figure 1.

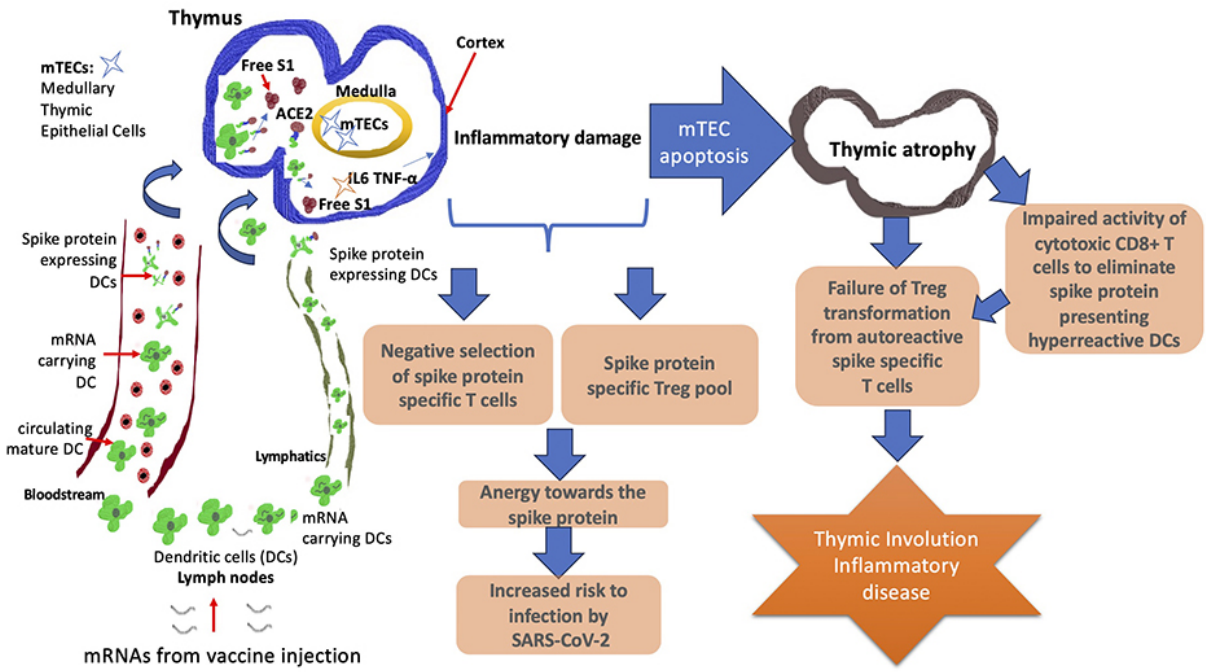

Figure 1. The presentation of SARS-CoV-2 spike protein by DCs to the thymus leads to thymic involution and inflammatory disease. The spike presentation by DCs causes mTEC apoptosis that leads to thymic atrophy and failure of Treg transformation, eventually resulting in thymic involution and inflammatory diseases [96-104].

7. 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 signalling, etc. [105]. 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 [70,106]. In general, the main role of Treg CD4+ T cell subpopulations is influenced by a complex cascade of genetic, molecular and T cell interactions (for more details, as this description is outside the scope of this review, see [70]) ultimately to provide an efficient mTreg response. The final outcome of Treg cellular interactions is for differentiated Treg cells to release IL-10, TGF-β and other suppressive chemokines that will 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 [107,108], 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 [109].

However, 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 signalling, b) the pro-inflammatory response, and c) cytokine-to-cytokine receptor interactions [110]. Overall, the activation of NF-ϰB signalling upon the stimulation of a specific viral antigen (for a detailed review see [111]), 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 [112]. 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.

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, as we have stated previously, are needed for the organism to avoid autoimmunity [113], but their activation promotes cancer progression [114]. 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 [115]. 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 stabilisation and energisation of NF-ϰB Inducible Kinase (NIK) [111].

In experiments that investigated the role of NIK overexpression in relation to Treg development, it has been shown that the overstimulation and constitutive expression of NIK leads to aggressive and lethal autoimmunity. The Treg cells produced under the overwhelming stimulation of NIK in these experiments were defective in inducing immune suppression [116]. In these experiments, the tested 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. 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 [45]. CD4+ T cells at inflammatory sites in rheumatoid arthritis are known to be resistant to suppression by Treg cells [45]. 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 highly correlated with the IgG response [23]. Il-2 plays an important role in Treg induction and persistence [30]. 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 signalling 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 [117]. As we have already discussed, some of these long-lived Treg cells migrate to the thymus and facilitate accelerated thymic involution.

The extensive study of Świerkot, J et al., investigated the emergence of an autoimmune response after SARS-CoV-2 mRNA vaccination in a cancer(-) population [118]. In this study, the individuals who had completed their mRNA vaccination (2 mRNA injections) and had presented with more severe vaccine adverse reactions (VARs,), had significantly higher antinuclear antibody (ANA) titers when compared to the individuals with less severe VARs [119]. The authors did not find a correlation between prior SARS-CoV-2 infection status and severity of VARs. However, another study found that more severe VARs was most strongly associated with individuals who had COVID-19 and were subsequently mRNA vaccinated [120]. 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) [121]. In a case report of systemic lupus erythematosus, symptom onset occurred just two days after immunization with the first mRNA injection [122]. A 63-year-old man experienced acute severe autoimmune-like hepatitis just one week after his first dose of an mRNA vaccine [123]. 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 [90]. Cases of autoimmune hemolytic anemia are described as a serious adverse reaction of mRNA vaccination [124,125]. A single-center 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 [126]. A comprehensive review article found considerable evidence of new-onset autoimmune disease following mRNA vaccination, including autoimmune glomerulonephritis, autoimmune rheumatic diseases, and autoimmune hepatitis [127].

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

In general, the activation of dendritic cells, 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 [70]). The generation of Treg cells promotes cancer development and exerts immunosuppression in the tumor microenvironment (TME), lowering the natural cellular anti-tumor activity and enhancing the growth of tumors [43]. The mechanisms of tumor enhancement by Treg cells are several, and the generation of Tregs has a prognosis favouring the development of many cancers while at the same time inhibiting the development of autoimmune diseases [128,129].

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 [130]. Basic research on Treg inhibition in the past has provided fundamental insights on tumor regression and, moreover, has revealed correlations between the inhibition of a Treg cell response and the development of autoimmunity. When the research group of Shimizu et al. [131] specifically blocked the CD25+ CD4+ suppressive Treg cells, the peripheral CD4+ T cells were able to eliminate syngeneic tumors in normal naïve mice. The results of another research group, that of Takahashi et al. [132], 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 that is 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 ∝ 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 [133]. 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 [134]. 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 [135]. 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. 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 [135]. PD-1 is a known marker for T cell exhaustion [136]. 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 [137] (and references therein). 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 are less able to suppress tumor growth [138].

A study on mice clearly demonstrated that repeated booster shots immunizing against the spike RBD 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 [139].

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 [140]. 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 [84]. 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 [132].

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 Scroff FT et al. [141] 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 [3]. Also, a disorganised B regulatory cell activity leads to a downregulated Teff cell response [142].

9. 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 [143]. At least two studies have shown that PD-L1 is overexpressed in circulating immune cells following the second vaccine. Loacker et al. (2022) 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 [144]. Özbay et al. (2022) examined expression of PD-L1 in antigen-presenting monocytes at 6 different time points starting before the first 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 [145]. They too suggested that this reaction could be a protective mechanism suppressing overactivated T cells induced by vaccines. However, there is 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 in the tumor microenvironment 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 [146].

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 [143]. Furthermore, the PD-L1 upregulation depends on sensing IFN-γ secreted by activated CD8+ T cells [147]. PD-1/PD-L1 inhibitors are a group of immune checkpoint inhibitors that are becoming an attractive choice for cancer immunotherapy in multiple types of cancer [148]. These work by blocking PD-1/PD-L1 signalling, 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 lungs, the colon, the liver and the kidneys, as well as autoimmune diabetes [148]. This is likely due to the fact that the tissue resident immune cells are now able to launch an inflammatory response.

Impaired PD-1/PD-L1 function plays an important role in many autoimmune diseases [149]. 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 [150]. These patients were under immunotherapy treatment with checkpoint signalling inhibitors that block PD-L1 expression and therefore PD-1 activation [41]. 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 [1]. These T cells permit the development of autoimmunity while offering protection against cancer [3,35].

A characteristic of the aged immune system is inflexibility and an inability 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 [77]. Thus, autoimmunity and cancer are two sides of the same coin [151].

10. Impaired mTOR-mediated Treg Cell Function

As we mentioned earlier, 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 [152]. In 2013, Zeng et al. 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 [153]. These mice developed a fatal early onset hyperinflammatory disorder due to ineffective Treg suppressor function. Mechanistically, Raptor-dependent mammalian target of rapamycin complex 1 (mTORC1) signalling 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 [28] [154]. Excessive production of ROS is known to inhibit the phosphoinositide 3 kinase (PI3K)/Akt pathway [155]. 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 [156]. The authors of this work proposed that this effect could account for multi-organ failure associated with severe cases of COVID-19 [156].

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 [157]. It has been demonstrated experimentally that the spike protein interacts with IRF3 and mediates its proteasomal degradation, thus terminating IFN-I activation [158]. Thus, the vaccines not only do not induce an 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. This specific effect of the spike protein could partially explain the occasional reactivation of latent viruses such as Herpes and Varicella following mRNA vaccination [159].

The sustained hyperinflammatory state induced by the mRNA vaccines may be primarily due to the impaired ability 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 see in subsequent sections, 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 [160]. 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 [161]. 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 [162]. Figure 2 shows the mechanisms by which the SARS-CoV-2 spike protein could induce an inflammatory response.

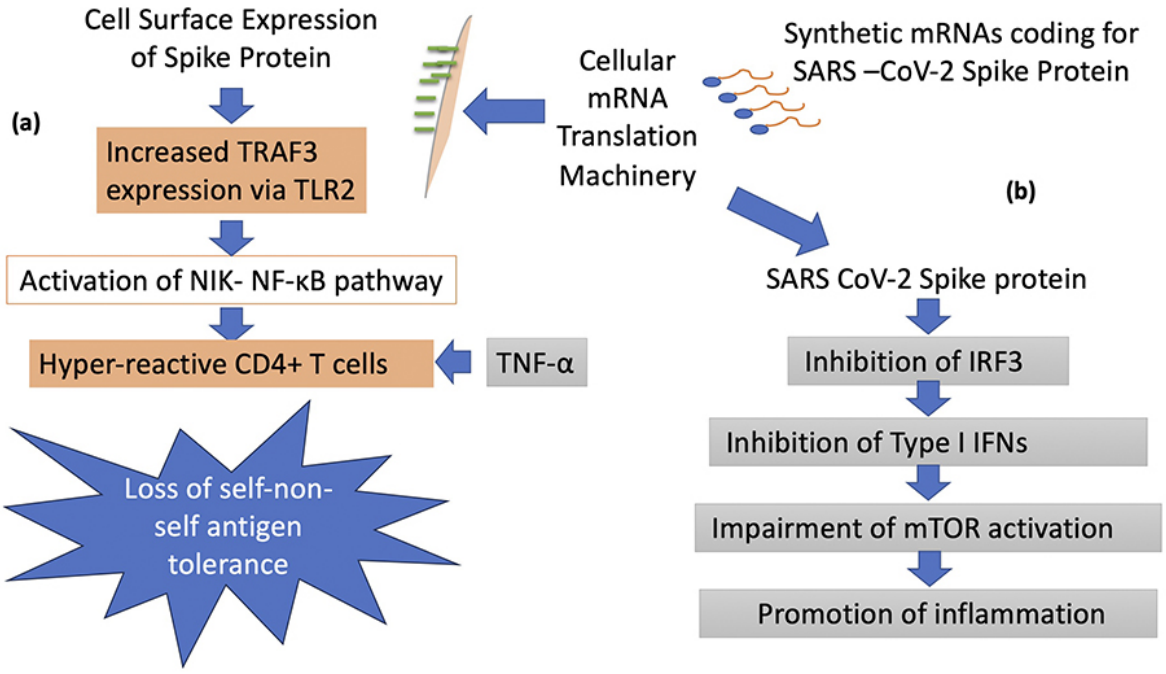

Figure 2. Spike protein induction of an inflammatory response. The events leading to hyper-activation of inflammation can concurrently occur through (a) a CD4+ T cell over-production via the stimulation of NIK (the alternative NF-κB pathway) and thereby loss of self-antigen tolerance, and (b) a promotion of inflammation through the inhibition of IRF3 and type I IFN, and subsequent impairment of mTOR activity. [111,116,153,158,159,161,162].

11. ΤΓΦ-β Σιγναλλινγ ανδ τηε Δεελοπμεντ οφ α Τη17 Ρεσπονσε

In the remarkable work of Liu L et al. [33], the responses of immune cells to the inactivated SARS-CoV-2 vaccine, as shown in Table 1, cause enhanced TGF-β signalling. This is in addition to the increased NF-kB response. This is enhanced only in some subtypes of immune cells. Specifically, the CD4+ Treg cells, CD4+ T proliferative cells, monocytes and dendritic cells show intense TGF-β signalling. These immune cells have roles that impact the efficient control and development of Treg responses [3]. Furthermore, intense TGF-β signalling and increased IL-6 and TNF-α expression are observed when the immune cells encounter huge amounts of the SARS-CoV-2 mRNA coded spike protein in vaccinated individuals [163].

As shown in Table 1, the T cell subsets (including the Treg cells) that show intense TGF-β signalling are also resistant to hypoxia effects. For the T cells to sustain themselves in this environment, they likely express adequate hypoxia inducible factors (HIFs). Moreover, HIF has been shown to be protective against uptake of the spike protein through multiple mechanisms [164].

Table 1. The hypoxia effect, and TGF-β signalling 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 [33].*

Cell Type	Hypoxia	ΤΓΦ-β σιγναλλινγ
Lymphoid Lineage	**Lymphoid Lineage**	**Lymphoid Lineage**
B cells	Moderate	Low
$CD4^+$ T cells	Low	High
$CD4^+$ Treg cells	Low	High

Cell Type	Hypoxia	ΤΓΦ-β σιγναλλινγ
$CD4^+$ T prolif cells	Low	High
$CD8^+$ T cells	Low	No
$CD8^+$ B T cells*	Moderate	High
$CD8^+$ T prolif	Moderate	Low
γδ T cells	Slightly moderate	Moderate
MAIT	High moderate	High moderate
NK cells	High	High
Myeloid lineage	**Myeloid lineage**	**Myeloid lineage**
Monocytes/ Dendritic Cells	High	High

*MS4A1, CD79A, CD79B positive $CD8^+$ T cells.

Experimental studies show that enhanced TGF-β signalling and HIF expression contribute to the progression of tumors [165]. HIF signalling contributes to the etiopathology of various autoimmune diseases, including multiple sclerosis (MS) [166]. MS and other severe neurological disorders, including Alzheimer's, can emerge as causalities of the anti-SARS-CoV-2 mRNA vaccination and spike protein side effects [167,168]. Moreover, the combination of a) IL-6 and TNF-α overexpression, b) enhanced TGF-β signalling 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 [169].

As illustrated in Figure 3, HIF overexpression increases TGF-β signalling [170]. 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 [171]. 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 (STAT3) [172,173]. The spike protein has been shown to activate both TLR2 and TLR4, resulting in JAK/STAT signalling [174-176].

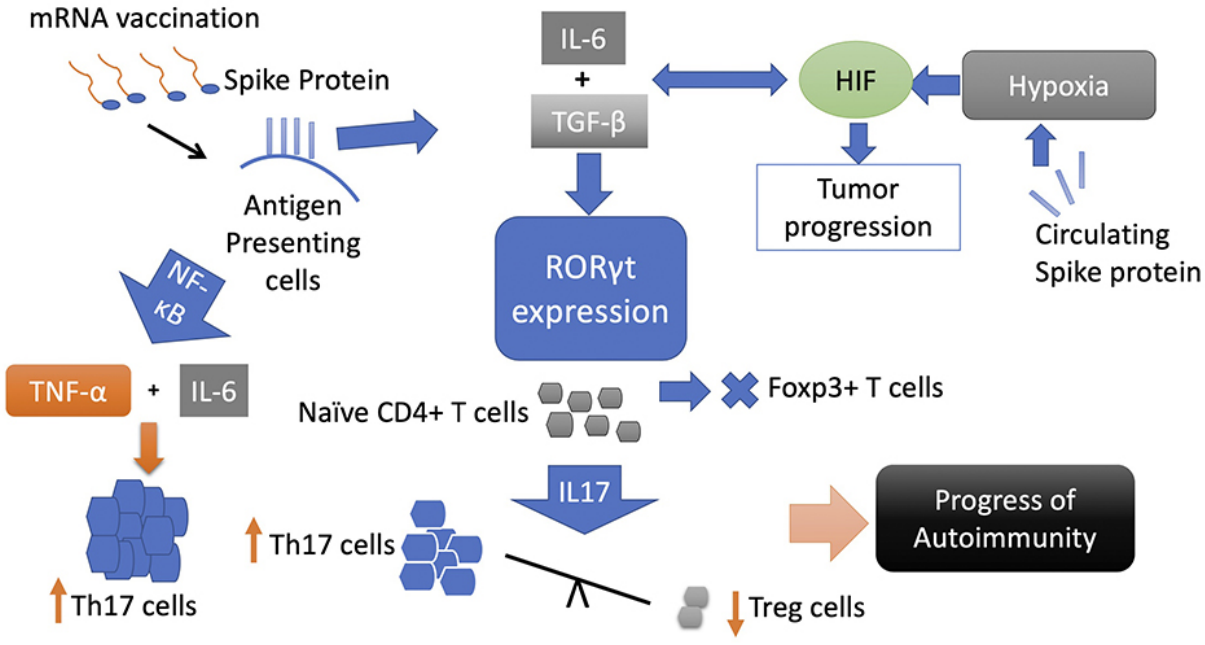

Figure 3. The mechanisms of enhanced Th17 cellular differentiation in cancer(+) patients after mRNA vaccination, facilitated by the SARS-CoV-2 spike protein. The induction of IL-6 and TNF-α, via the NF-ϰB response to spike, and the TGF-β induced expression of RORγt, enhances the generation of a Th17 population of cells that is responsible for the development of autoimmunity [166-169,172,173].

On top of this, the spike protein potentiates the signalling of the epidermal growth factor receptor (EGFR) [177]. Persistent activation of STAT3 is a common feature of the tumor microenvironment and a major contributor to the inflammatory state [178]. An intense activation of STAT3 can result from 1) an aberrant expression of IL-6 and subsequent stimulation of the IL-6 receptor [179], and 2) an intense activation of EGFR signalling as imposed by the spike protein [177]. 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 signalling 3 (SOCS3). SOCS3 would otherwise deactivate JAK in order to diminish STAT3 activation [179]. Moreover, the abnormally elevated expression of IL-6 and JAK/STAT3 signalling are pro-tumorigenic and enhance the differentiation of Th17 cells [179,180].

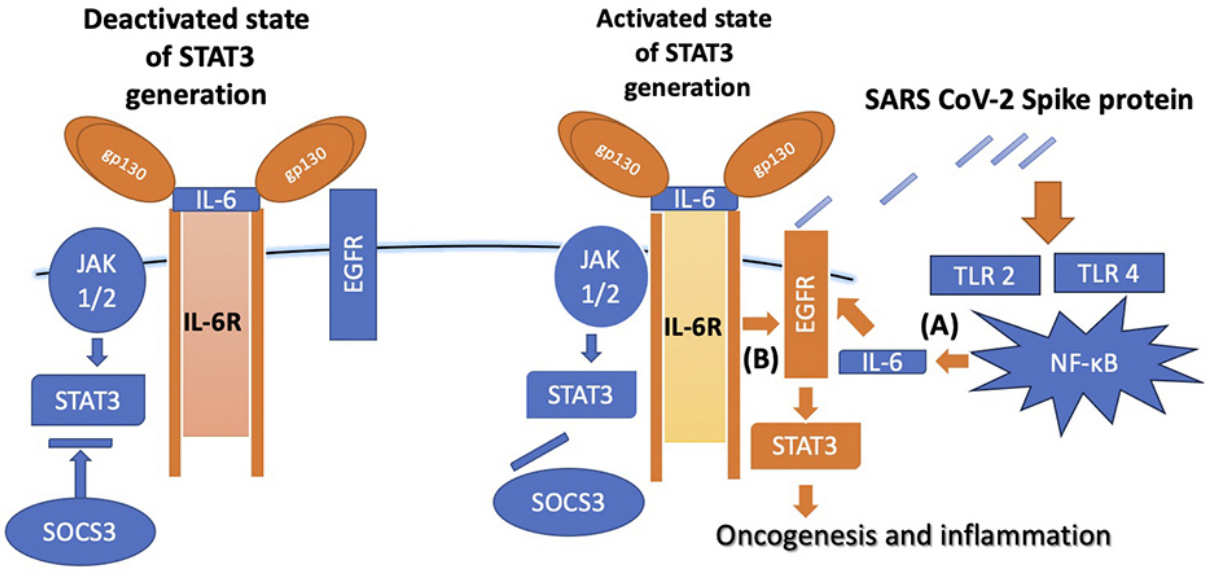

Figure 4. Potential activation of STAT3 by SARS CoV-2 spike protein stimulatory effects. Under normal conditions, IL-6R remains dormant as a) it is inhibited by SOCS3 and b) it does not synergise with unstimulated EGFRs to produce STAT3. This condition can be bypassed and reversed by (A) the activation of NF-ϰB and subsequent expression of IL-6 through TLR2 and TLR4 stimulation by spike protein [174,176] and (B) the direct stimulation of EGFR by spike protein and synergy with IL-6R [177]. The final effect between IL-6R, EGFR and IL-6 would be the continuous production of STAT3, although the SOCS3 will still be present [179].

Elevated levels of Th17 cells are implicated in the etiopathogenesis of numerous inflammatory and autoimmune diseases [181]. Furthermore, in some instances, Th17 cells can promote cancer [182]. 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 [183].

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 [184,185]. 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 [186]. Furthermore, a connection to macrophage activation syndrome is suggested by a study that confirmed that macrophages infiltrated the heart muscle and became activated, releasing toxic cytokines, in association with vaccine-induced myocarditis [187].

Th17 cells also play an important role in autoimmune hemolytic anemia (AIHA). A study by Xu et al. found that patients with AIHA 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 [188]. 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 AIHA include elevated levels of IL-6, IL-2, and IL-17, and increased secretion of TGF-β [189]. Reduced numbers of circulating CD4+ nTregs are also linked to the disease [190]. As we have seen, all of these are consistent with known effects of the spike protein.

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 [191]. 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 [192]. Additionally, it is worrisome that the DCs and monocytes increase their TGF-β and IL-2 signalling upon engagement with the spike protein synthesized by human cells from the vaccine mRNA (Table 1) [33]. The spike protein on its own has been shown to activate TGF-β signalling [163]. The monocytes and macrophages are mainly DC-derived antigen presenting cells [193]. The intense TGF-β signalling can also be attributed to spike protein induced inflammation via TLR2-mediated NF-ϰB hyperactivation [174].

Proper Treg-DC cellular interactions are crucial for the well-controlled suppression of the effector CD4+ T lymphocytes [1]. Impairments during Treg-DC cellular interactions will produce autoimmune disease [1]. TGF-β signalling inhibits DC functions in general, and latent TGF-β signalling by the DCs will contribute in favour of Th17 cell differentiation, and, hence, 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 [194]. Moreover, the several pathological neurological outcomes that follow COVID-19 mRNA vaccines, including Guillain Barré syndrome (GBS), transverse myelitis, and acute disseminated encephalomyelitis (ADEM) (amongst several others), also have an autoimmune origin [195]. 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 [196].

12. Th17, PD-L1 and IgG4

As we have said, 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 unable to protect from infection, and, in fact, actively blocks access to the spike protein by effector antibodies [197]. IgG4 is normally the least common variant in human serum. However, 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 [198]. 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 mechanism that would suppress the natural antiviral responses to the SARS-CoV-2 virus [199].

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 [200].

IgG4-related disease (IgG4-RD) is a newly recognized disease that is characterized by elevated serum levels of IgG4 and excess fibrosis in multiple organs [201]. 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 [202]. Most target organs of IgG4-RD have Treg cell infiltration, and Treg cells are also abundant in the blood [203].

Type I autoimmune pancreatitis (AIP) 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 [204]. 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 (MST1), which is essential for allowing the cell-to-cell contact needed for iTregs to act on Teff cells [205]. Patients with IgG4-RD are at increased risk to both pancreatic cancer and lymphoma [206]. Several case reports of acute pancreatitis have been reported in association with mRNA vaccines [207-209]. A case study described a patient who experienced rapid progression of lymphoma following an mRNA booster vaccine [210]. Several other case reports involving lymphoma following mRNA vaccination have been published [211-214]. One of 14 mice involved in a study of the BNT162b2 mRNA vaccine developed a fatal aggressive multi-organ malignant B-cell lymphoblastic lymphoma shortly after the second shot [215].

13. Conclusion

In this paper, we have provided an extensive review of the role of Treg cells in the immune system, with a particular focus on the apparent disruption of their behavior caused by the mRNA vaccines. It appears that 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 a toxic viral 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 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 (HLH), 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.

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 a Treg dysregulation in both populations. The Treg dysregulation in the cancer(-) population predominantly causes immune senescence and promotes autoimmunity, in part 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, the patients develop a hyperimmune response and also have a tendency to develop autoimmunity. 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, and, therefore, further studies are needed to evaluate the potential of the mRNA vaccines to induce cancer.

The inhibition of mTOR may accelerate immunosenescence due to enhancement of the memory Teff response, and this is especially dangerous for the elderly population receiving the mRNA vaccines, who are at risk for both autoimmune and neoplastic disease.

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References

1. Smigiel KS, Srivastava S, Stolley JM, et al. Regulatory T-cell homeostasis: steady-state maintenance and modulation during inflammation. Immunol Rev 2014; 259(1): 40-59. doi: 10.1111/imr.12170.
2. Kumar P, Bhattacharya P and Prabhakar BS. A comprehensive review on the role of co-signaling receptors and Treg homeostasis in autoimmunity and tumor immunity. J Autoimmun 2018; 95: 77-99. doi: 10.1016/j.jaut.2018.08.007
3. Rocamora-Reverte L, Melzer FL, Würzner R, et al. The complex role of regulatory T cells in immunity and aging. Front Immunol 2021; 11: 616949. doi: 10.3389/fimmu.2020.616949.
4. Shevyrev D and Tereshchenko V. Treg Heterogeneity, function, and homeostasis. Front Immunol 2020; 10:3100. doi: 10.3389/fimmu.2019.03100.
5. Vadasz Z, Haj T, Kessel A, Toubi E. Age-related autoimmunity. BMC Med 2013; 11: 94. doi: 10.1186/1741-7015-11-94.
6. Kronenberg M and Rudensky A. Regulation of immunity by self-reactive T cells. Nature 2005; 435: 598604. doi: 10.1038/nature03725.
7. Workman CJ, Szymczak-Workman AL, et al. The development and function of regulatory T cells. Cell Mol Life Sci 2009; 66(16): 2603-22. doi: 10.1007/s00018-009-0026-2.
8. Yu A, Zhu L, Altman NH, Malek TR. A low interleukin-2 receptor signaling threshold supports the development and homeostasis of T regulatory cells. Immunity 2009; 30: 20417. doi: 10.1016/j.immuni.2008.11.014.
9. Horwitz DA, Zheng SG, Wang J, et al. Critical role of IL-2 and TGF-β in generation, function and stabilization of Foxp3+CD4+ Treg. Eur J Immunol 2008; 38(4): 912-5. doi: 10.1002/eji.200738109.
10. Jung HN, Lee SY, Lee S, et al. Lipid nanoparticles for delivery of RNA therapeutics: Current status and the role of in vivo imaging. Theranostics 2022; 12(17): 7509-7531. doi: 10.7150/thno.77259.
11. Qiu Y, Shi YN, Zhu N, et al. A lipid perspective on regulated pyroptosis. Int J Biol Sci 2023; 19(8): 2333-2348. doi: 10.7150/ijbs.81017.
12. Forster J. III, Nandi D, Kulkarni A. mRNA-carrying lipid nanoparticles that induce lysosomal rupture activate NLRP3 inflammasome and reduce mRNA transfection efficiency. Biomater Sci 2022; 10: 5566-82. doi: 10.1039/d2bm00883a.
13. Lima H Jr, Jacobson LS, Goldberg MF, et al. Role of lysosome rupture in controlling Nlrp3 signaling and necrotic cell death. Cell Cycle 2013; 12(12): 1868-78. doi: 10.4161/cc.24903.
14. Phulphagar K, Khn LI, Ebner S, Frauenstein A, et al. Proteomics reveals distinct mechanisms regulating the release of cytokines and alarmins during pyroptosis. Cell Rep 2021; 34(10): 108826. doi: 10.1016/j.celrep.2021.108826.
15. Cinat D, Coppes RP, Barazzuol L. DNA Damage-induced inflammatory microenvironment and adult stem cell response. Front Cell Dev Biol 2021; 9: 729136. doi: 10.3389/fcell.2021.729136.
16. Sansonetti PJ, Phalipon A, Arondel J, et al. Caspase-1 activation of IL-1β and IL-18 are essential for Shigella flexneri-induced inflammation. Immunity 2000; 12(5): 581-90. doi: 10.1016/s1074-

7613(00)80209-5.
17. Iwai Y, Hemmi H, Mizenina O, et al. An IFN-gamma-IL-18 signaling loop accelerates memory CD8+ T cell proliferation. PLoS One 2008; 3(6): e2404. doi: 10.1371/journal.pone.0002404.
18. Enoksson SL, Grasset EK, Hägglöf T, et al. The inflammatory cytokine IL-18 induces self-reactive innate antibody responses regulated by natural killer T cells. Proc Natl Acad Sci U S A 2011; 108(51): E1399-407. doi: 10.1073/pnas.1107830108.
19. Huang C-F, Chen L, Li Y-C, et al. NLRP3 inflammasome activation promotes inflammation-induced carcinogenesis in head and neck squamous cell carcinoma. J. Exp. Clin. Cancer Res 2017; 36: 113. doi: 10.1186/S13046-017-0589-Y.
20. Peligero-Cruz C, Givony T, Seb-Pedrs A, et al. IL18 signaling promotes homing of mature Tregs into the thymus. Elife 2020; 9: e58213. doi: 10.7554/eLife.58213.
21. Thiault N, Darrigues J, Adoue V, et al. Peripheral regulatory T lymphocytes recirculating to the Thymus suppress the development of their precursors. Nature Immunology 2015; 16: 628634. doi: 10.1038/ni.3150.
22. Agrati C, Castilletti C, Goletti D, et al. Coordinate induction of humoral and spike specific T-cell response in a cohort of Italian health care workers receiving BNT162b2 mRNA vaccine. Microorganisms 2021; 9(6): 1315. doi: 10.3390/microorganisms9061315.
23. Sedegah M, Porter C, Goguet E, et al. Cellular interferon-γ and interleukin-2 responses to SARS-CoV-2 structural proteins are broader and higher in those vaccinated after SARS-CoV-2 infection compared to vaccinees without prior SARS-CoV-2 infection. PLoS One 2022; 17(10): e0276241. doi: 10.1371/journal.pone.0276241.
24. Won T, Gilotra NA, Wood MK, et al. Increased interleukin 18-dependent immune responses are associated with myopericarditis after COVID-19 mRNA vaccination. Front Immunol 2022; 13: 851620. doi: 10.3389/fimmu.2022.851620.
25. Thomas R, Wang W, Su DM. Contributions of age-related thymic involution to immunosenescence and inflammaging. Immun Ageing 2020; 17: 2. doi: 10.1186/s12979-020-0173-8.
26. Palmer S, Albergante L, Blackburn C, et al. Thymic involution and rising disease incidence with age. PNAS 2018; 115(8): 18831888. doi: 10.1073/pnas.1714478115.
27. Youm YH, Kanneganti TD, Vandanmagsar B, et al. The NLRP3 inflammasome promotes age-related thymic demise and immunosenescence. Cell Rep 2012; 1(1):56-68. doi: 10.1016/j.celrep.2011.11.005.
28. Granadier D, Cooper K, Kinsella S, et al. Interleukin 18 suppresses regeneration of the thymus. Blood 2022; 140(Suppl 1): 116901170. doi: 10.1182/blood-2022-168432.
29. Liu Z, Liang Q, Ren Y, et al. Immunosenescence: molecular mechanisms and diseases. Sig Transduct Target Ther 2023; 8: 200. doi: 10.1038/s41392-023-01451-2.
30. Churov AV, Mamashov KY, Novitskaia AV. Homeostasis and the functional roles of CD4+ Treg cells in aging. Immunol Lett 2020; 226: 83-89. doi: 10.1016/j.imlet.2020.07.004.
31. Thomas R, Oh J, Wang W, et al. Thymic atrophy creates holes in Treg-mediated immuno-regulation via impairment of an antigen-specific clone. Immunology 2021; 163(4): 478-492. doi: 10.1111/imm.13333
32. Barbé-Tuana F, Funchal G, Schmitz CRR, et al. The interplay between immunosenescence and age-related diseases. Semin Immunopathol 2020; 42: 545-557. doi: 10.1007/s00281-020-00806-z.
33. Liu J, Wang J, Xu J, et al. Comprehensive investigations revealed consistent pathophysiological alterations after vaccination with COVID-19 vaccines. Cell Discov 2021; 7(1): 99. doi: 10.1038/s41421-021-00329-3.
34. Choueiri TK, Labaki C, Bakouny Z, et al. Breakthrough SARS-CoV-2 infections among patients with cancer following two and three doses of COVID-19 mRNA vaccines: a retrospective observational study from the COVID-19 and Cancer Consortium. The Lancet Regional Health – Americas 2023; 19: 100445. doi: 10.1016/j.lana.2023.100445.
35. Ohue Y, Nishikawa H. Regulatory T (Treg) cells in cancer: Can Treg cells be a new therapeutic target? Cancer Sci 2019; 110(7): 2080-2089. doi: 10.1111/cas.14069.
36. Gonzalez-Dias P, Lee EK, Sorgi S, et al. Methods for predicting vaccine immunogenicity and reactogenicity. Hum Vaccin Immunother 2020; 16(2): 269-276. doi: 10.1080/21645515.2019.1697110.

37. Luo CT, Liao W, Dadi S, et al. Graded Foxo1 activity in Treg cells differentiates tumour immunity from spontaneous autoimmunity. Nature 2016; 529(7587): 532-6. doi: 10.1038/nature16486.
38. Goswami TK, Singh M, Dhawan M, et al. Regulatory T cells (Tregs) and their therapeutic potential against autoimmune disorders – Advances and challenges. Hum Vaccin Immunother 2022; 8(1): 2035117. doi: 10.1080/21645515.2022.2035117.
39. Hanahan D, Weinberg RA. Hallmarks of cancer: The next generation. Cell 2011; 144(5): 646-74. doi: 10.1016/j.cell.2011.02.013.
40. Hatzioannou A, Boumpas A, Papadopoulou M, et al. Regulatory T cells in autoimmunity and cancer: A duplicitous lifestyle. Front Immunol 2021; 12: 731947. doi: 10.3389/fimmu.2021.731947.
41. Alsaab HO, Sau S, Alzhrani R, Tatiparti K, et al. PD-1 and PD-L1 checkpoint signaling inhibition for cancer immunotherapy: Mechanism, combinations, and clinical outcome. Front Pharmacol 2017; 8: 561. doi: 10.3389/fphar.2017.00561.
42. Schmidt R, Grimbacher B, Witte T. Autoimmunity and primary immunodeficiency: two sides of the same coin? Nat Rev Rheumatol 2018; 14: 7-18. doi: 10.1038/nrrheum.2017.198
43. Shang B, Liu Y, Jiang SJ, et al. Prognostic value of tumor-infiltrating FoxP3+ regulatory T cells in cancers: a systematic review and meta-analysis. Sci Rep 2015; 5: 15179. doi: 10.1038/srep15179.
44. Barhoumi T, Alghanem B, Shaibah H, et al. SARS-CoV-2 coronavirus spike protein-induced apoptosis, inflammatory, and oxidative Stress responses in THP-1-like-macrophages: Potential role of angiotensin-converting enzyme inhibitor (Perindopril). Front Immunol 2021; 12: 728896. doi: 10.3389/fimmu.2021.728896.
45. Dejaco C, Duftner C, Grubeck-Loebenstein B, et al. Imbalance of regulatory T cells in human autoimmune diseases. Immunology 2006; 117(3): 289-300. doi: 10.1111/j.1365-2567.2005.02317.x.
46. Raw RK, Rees J, Kelly CA, et al. Prior COVID-19 infection is associated with increased Adverse Events (AEs) after the first, but not the second, dose of the BNT162b2/Pfizer vaccine. Vaccine 2022; 40(3): 418-423. doi: 10.1016/j.vaccine.2021.11.090.
47. Tormo N, Navalpotro D, Martínez-Serrano M, et al. Commercial interferon-gamma release assay to assess the immune response to first and second doses of mRNA vaccine in previously COVID-19 infected versus uninfected individuals. Diagn Microbiol Infect Dis 2022; 102(4): 115573. doi: 10.1016/j.diagmicrobio.2021.115573.
48. Lourenço EV, La Cava A. Natural Regulatory T cells in autoimmunity. Autoimmunity 2011; 44(1): 33-42. doi: 10.3109/08916931003782155.
49. Sanchez AM, Yang Y. The role of natural regulatory T cells in infection. Immunol Res 2011; 49(1-3): 124-34. doi: 10.1007/s12026-010-8176-8.
50. Pellerin L, Jenks JA, Bégin P, et al. Regulatory T cells and their roles in immune dysregulation and allergy. Immunol Res 2014; 58(2-3): 358-68. doi: 10.1007/s12026-014-8512-5.
51. van der Geest KS, Abdulahad WH, Tete SM, et al. Aging disturbs the balance between effector and regulatory CD4+ T cells. Exp Gerontol 2014; 60: 190-6. doi: 10.1016/j.exger.2014.11.005.
52. Baum A, Garca-Sastre A. Induction of type I interferon by RNA viruses: cellular receptors and their substrates. Amino Acids 2010; 38(5): 1283-99. doi: 10.1007/s00726-009-0374-0.
53. Seneff S, Nigh G, Kyriakopoulos AM, et al. Innate immune suppression by SARS-CoV-2 mRNA vaccinations: The role of G-quadruplexes, exosomes, and microRNAs. Food Chem Toxicol 2022; 164: 113008. doi: 10.1016/j.fct.2022.113008.
54. Lee SE, Li X, Kim JC, Lee J, et al. Type I interferons maintain Foxp3 expression and T-regulatory cell functions under inflammatory conditions in mice. Gastroenterology 2012; 143(1): 145-54. doi: 10.1053/j.gastro.2012.03.042.
55. Gangaplara A, Martens C, Dahlstrom E, et al. Type I interferon signaling attenuates regulatory T cell function in viral infection and in the tumor microenvironment. PloS Pathog 2018; 14(4): e1006985. doi: 10.1371/journal.ppat.1006985.
56. Levings MK, Bacchetta R, Schulz U, et al. The role of IL-10 and TGF-beta in the differentiation and effector function of T regulatory cells. Int Arch Allergy Immunol 2002; 129(4): 263-76. doi: 10.1159/000067596.

57. Patra T, Meyer K, Geerling L, et al. SARS-CoV-2 spike protein promotes IL-6 trans-signaling by activation of angiotensin II receptor signaling in epithelial cells. PLoS Pathog 2020; 16(12): e1009128. doi: 10.1371/journal.ppat.1009128.
58. Hadjadj J, Yatim N, Barnabei L, et al. Impaired type I interferon activity and inflammatory responses in severe COVID-19 patients. Science 2020; 369(6504): 718724. doi: 10.1016/j.cell.2020.04.026.
59. Röltgen K, Nielsen SCA, Silva O, et al. Immune imprinting, breadth of variant recognition, and germinal center response in human SARS-CoV-2 infection and vaccination. Cell 2022; 185(6): 1025-1040.e14. doi: 10.1016/j.cell.2022.01.018.
60. Gil-Manso S, Carbonell D, López-Fernández L, et al. Induction of high levels of specific humoral and cellular responses to SARS-CoV-2 after the administration of COVID-19 mRNA vaccines requires several days. Front. Immunol 2021; 12: 726960. doi: 10.3389/fimmu.2021.726960.
61. Mishra R, Banerjea AC. SARS-CoV-2 spike targets USP33-IRF9 axis via exosomal miR-148a to activate human microglia. Front. Immunol 2021; 12: 656700. doi: 10.3389/fimmu.2021.656700.
62. Bansal S, Perincheri S, Fleming T, et al. Cutting edge: Circulating exosomes with COVID spike protein are induced by BNT162b2 (Pfizer-BioNTech) vaccination prior to development of antibodies: A novel mechanism for immune activation by mRNA vaccines. J Immunol 2021; 207(10): 2405-2410. doi: 10.4049/jimmunol.2100637.
63. Kolumam GA, Thomas S, Thompson LJ, et al. Type I interferons act directly on CD8 T cells to allow clonal expansion and memory formation in response to viral infection. J Exp Med 2005; 202(5): 637-50. doi: 10.1084/jem.20050821. E
64. Xu HC, Grusdat M, Pandyra AA, et al. Type I interferon protects antiviral CD8+ T cells from NK cell cytotoxicity. Immunity 2014; 40: 949-960. doi: 10.1016/j.immuni.2014.05.004.
65. Nicol A, Amendt T, El Ayoubi O, et al. Rheumatoid factor IgM autoantibodies control IgG homeostasis. Front Immunol 2022; 13: 1016263. doi: 10.3389/fimmu.2022.1016263.
66. Boes M, Schmidt T, Linkemann K, et al. Accelerated development of IgG autoantibodies and autoimmune disease in the absence of secreted IgM. Proc Natl Acad Sci U S A 2000; 97(3): 1184-9. doi: 10.1073/pnas.97.3.1184.
67. Morais P, Adachi H, Yu YT. The critical contribution of pseudouridine to mRNA COVID-19 vaccines. Front Cell Dev Biol 2021; 9: 789427. doi: 10.3389/fcell.2021.789427.
68. Kanduc D. From anti-SARS-CoV-2 immune responses to COVID-19 via molecular mimicry. Antibodies 2020; 9: 33. doi: 10.3390/antib9030033.
69. Robles JP, Zamora M, Adan-Castro E, et al. The spike protein of SARS-CoV-2 induces endothelial inflammation through integrin A5β1 and NF-kB signalling. J Biol Chem 2022; 298(3): 101695. doi: 10.1016/j.jbc.2022.101695.
70. Khantakova JN, Bulygin AS, Sennikov SV. The regulatory-T-cell memory phenotype: What we know. Cells 2022; 11(10): 1687. Doi: 10.3390/cells11101687.
71. Shimizu Y, Newman W, Gopal TV, et al. Four molecular pathways of T cell adhesion to endothelial cells: roles of LFA-1, VCAM-1, and ELAM-1 and changes in pathway hierarchy under different activation conditions. J Cell Biol 1991; 113(5): 1203-12. doi: 10.1083/jcb.113.5.1203.
72. Maganto-Garca E, Bu DX, Tarrio ML, et al. Foxp3+-inducible regulatory T cells suppress endothelial activation and leukocyte recruitment. J Immunol 2011; 187(7): 3521-9. doi: 10.4049/jimmunol.1003947.
73. Murphy WJ, Longo DL. A possible role for anti-idiotype antibodies in SARS-CoV-2 infection and vaccination. N Engl J Med 2022; 386(4): 394-396. doi: 10.1056/NEJMcibr2113694.
74. Omland SH, Nielsen PS, Gjerdrum LM, et al. Immunosuppressive environment in basal cell carcinoma: The role of regulatory T cells. Acta Derm Venereol 2016; 96(7): 917-921. doi: 10.2340/00015555-2440.
75. Bednar KJ, Lee JH, Ort T. Tregs in autoimmunity: Insights into intrinsic brake mechanism driving pathogenesis and immune homeostasis. Front Immunol 2022; 13: 932485. doi: 10.3389/fimmu.2022.932485.
76. Lee KA, Flores RR, Jang IH, et al. Immune senescence, immunosenescence and aging. Front Aging 2022; 3: 900028. doi: 10.3389/fragi.2022.900028.

77. Jagger A, Shimojima Y, Goronzy JJ, et al. Regulatory T cells and the immune aging process: a mini-review. Gerontology 2014; 60(2): 130-7. doi: 10.1159/000355303.
78. Kalfaoglu B, Almeida-Santos J, Tye CA, et al. T-cell hyperactivation and paralysis in severe COVID-19 infection revealed by single-cell analysis. Front Immunol 2020; 1: 589380. doi:10.3389/fimmu.2020.589380.
79. Kumar P. IFNγ-producing CD4+ T lymphocytes: the double-edged swords in tuberculosis. Clin Transl Med 2017; 6(1): 21. doi: 10.1186/s40169-017-0151-8.
80. Larkin J 3rd, Ahmed CM, Wilson TD, Johnson HM. Regulation of interferon gamma signaling by suppressors of cytokine signaling and regulatory T cells. Front Immunol 2013; 4: 469. doi: 10.3389/fimmu.2013.00469.
81. Lozano-Ojalvo D, Camara C, Lopez-Granados E, et al. Differential effects of the second vaccine on T cell immunity in naïve and COVID-19 recovered individuals. Cell Rep 2021; 36(8): 109570. doi: 10.1016/j.celrep.2021.109570.
82. Diani S, Leonardi E, Cavezzi A, et al. SARS-CoV-2 – The role of natural immunity: A narrative review. J Clin Med 2022; 11(21): 6272. doi: 10.3390/jcm11216272.
83. Antia A, Ahmed H, Handel A, et al. Heterogeneity and longevity of antibody memory to viruses and vaccines. PloS Biol 2018; 16(8): e2006601. doi: 10.1371/journal.pbio.2006601.
84. Amar S, Avni YS, O'Rourke N, et al. Prevalence of common infectious diseases After COVID-19 vaccination and easing of pandemic restrictions in Israel. JAMA Netw Open 2022; 5(2): e2146175. doi:10.1001/jamanetworkopen.2021.46175.
85. Nunes-Alves C, Nobrega C, Behar SM, et al. Tolerance has its limits: how the thymus copes with infection. Trends Immunol 2013; 34(10): 502-10. doi: 10.1016/j.it.2013.06.004.
86. Rosichini M, Bordoni V, Silvestris DA, et al. SARS-CoV-2 infection of thymus induces loss of function that correlates with disease severity. J Allergy Clin Immunol 2023; 151(4): 911-921. doi: 10.1016/j.jaci.2023.01.022.
87. Ansari AR, Liu H. Acute thymic involution and mechanisms for recovery. Arch Immunol Ther Exp 2017; 65: 401-20. doi: 10.1007/s00005-017-0462-x.
88. Gui J, Zhu X, Dohkan J, et al. The aged thymus shows normal recruitment of lymphohematopoietic progenitors but has defects in thymic epithelial cells. Int Immunol 2007; 19(10): 1201-11. doi: 10.1093/intimm/dxm095.
89. Alexandropoulos K, Bonito AJ, Weinstein EG, et al. Medullary thymic epithelial cells and central tolerance in autoimmune hepatitis development: novel perspective from a new mouse model. Int J Mol Sci 2015; 16(1): 1980-2000. doi: 10.3390/ijms16011980.
90. Zheng H, Zhang T, Xu Y, et al. Autoimmune hepatitis after COVID-19 vaccination. Front Immunol 2022; 13: 1035073. doi: 10.3389/fimmu.2022.1035073.
91. Co M, Wong PCP, Kwong A. COVID-19 vaccine associated axillary lymphadenopathy - A systematic review. Cancer Treat Res Commun 2022; 31: 100546. doi: 10.1016/j.ctarc.2022.100546.
92. Yoshimoto N, Yanagi A, Takayama S, et al. Timing and duration of axillary lymph node swelling after COVID-19 vaccination: Japanese Case Report and Literature Review. In Vivo 2022; 36(3): 1333-1336. doi: 10.21873/in-vivo.
93. Li J, Park J, Foss D, Goldschneider I. Thymus-homing peripheral dendritic cells constitute two of the three major subsets of dendritic cells in the steady-state thymus. J Exp Med 2009; 206(3): 607-22. doi: 10.1084/jem.20082232.
94. Colunga Biancatelli RML, Solopov PA, Sharlow ER, et al. The SARS-CoV-2 spike protein subunit S1 induces COVID-19-like acute lung injury in 18-hACE2 transgenic mice and barrier dysfunction in human endothelial cells. Am J Physiol Lung Cell Mol Physiol 2021; 321(2): L477-L484. doi: 10.1152/ajplung.00223.2021.
95. Wu H, Li X, Zhou C, et al. Circulating mature dendritic cells homing to the thymus promote thymic epithelial cells involution via the Jagged1/Notch3 axis. Cell Death Discov 2021; 7: 225. doi: 10.1038/s41420-021-00619-5.
96. Gantier MP, Williams BR. The response of mammalian cells to double-stranded RNA. Cytokine Growth

Factor Rev. 2007; 18(5-6): 363-71. doi: 10.1016/j.cytogfr.2007.06.016.
97. Cunningham L, Kimber I, Basketter D, Simmonds P, et al. Perforin, COVID-19 and a possible pathogenic auto-inflammatory feedback loop. Scand J Immunol 2021; 94(5): e13102. doi: 10.1111/sji.13102.
98. Rukavina D, Laskarin G, Rubesa G, et al. Age-related decline of perforin expression in human cytotoxic T lymphocytes and natural killer cells. Blood 1998; 92(7): 2410-20.
99. Huang CF, Hsieh SM, Pan SC, et al. Dose-related aberrant inhibition of intracellular perforin expression by S1 subunit of spike glycoprotein that contains receptor-binding domain from SARS-CoV-2. Microorganisms 2021; 9(6): 1303. doi: 10.3390/microorganisms9061303.
100. Terrell CE, Jordan MB. Perforin deficiency impairs a critical immunoregulatory loop involving murine CD8(+) T cells and dendritic cells. Blood 2013; 121(26): 5184-91. doi: 10.1182/blood-2013-04-495309.
101. Hieber ML, Sprute R, Eichenauer DA, et al. Hemophagocytic lymphohistiocytosis after SARS-CoV-2 vaccination. Infection 2022; 50(5): 1399-1404. doi: 10.1007/s15010-022-01786-y.
102. Lin TY, Yeh YH, Chen LW, et al. Hemophagocytic lymphohistiocytosis Following BNT162b2 mRNA COVID-19 Vaccination. Vaccines (Basel) 2022; 10(4): 573. doi: 10.3390/vaccines10040573.
103. Ashizawa N, Takazono T, Umeda M, et al. Macrophage activation syndrome after BNT162b2 mRNA vaccination successfully treated with corticosteroids. Clin Exp Rheumatol 2022; 40(5): 1060. doi: 10.55563/clinexprheumatol/a9hrmo.
104. Franzblau LE, Mauskar M, Wysocki CA. Macrophage activation syndrome complicated by toxic epidermal necrolysis following SARS-CoV-2 mRNA vaccination. J Clin Immunol 2023; 43: 521-524. doi: 10.1007/s10875-022-01408-0.
105. Kasper IR, Apostolidis SA, Sharabi A, et al. Empowering regulatory T cells in autoimmunity. Trends Mol Med 2016; 22(9): 784-797. doi: 10.1016/j.molmed.2016.07.003.
106. Rosenblum MD, Way SS, Abbas AK. Regulatory T cell memory. Nat Rev Immunol. 2016; 16(2): 90-101. doi: 10.1038/nri.2015.1.
107. Niederlova V, Tsyklauri O, Chadimova T, et al. CD8+ Tregs revisited: A heterogeneous population with different phenotypes and properties. Eur J Immunol 2021; 51(3): 512-530. doi: 10.1002/eji.202048614.
108. Hall BM, Verma ND, Tran GT, et al. Distinct regulatory CD4+T cell subsets; differences between naïve and antigen specific T regulatory cells. Curr Opin Immunol 2011; 23(5): 641-7. doi: 10.1016/j.coi.2011.07.012.
109. Lu C, Zanker D, Lock P, et al. Memory regulatory T cells home to the lung and control influenza A virus infection. Immunol Cell Biol 2019; 97(9): 774-786. doi: 10.1111/imcb.12271.
110. Liang S, Bao C, Yang Z, et al. SARS-CoV-2 spike protein induces IL-18-mediated cardiopulmonary inflammation via reduced mitophagy. Sig Transduct Target Ther 2023; 8: 108. doi: 10.1038/s41392-023-01368-w.
111. Liu T, Zhang L, Joo D, et al. NF-κB signalling in inflammation. Sig Transduct Target Ther 2017; 2: 17023. doi: 10.1038/sigtrans.2017.23
112. Daniels MA, Luera D, Teixeiro E. NFκB signaling in T cell memory. Front Immunol 2023; 14: 1129191. doi: 10.3389/fimmu.2023.1129191.
113. Oh H, Grinberg-Bleyer Y, Liao W, et al. An NF-κB Transcription-factor-dependent lineage-specific transcriptional program promotes regulatory T cell identity and function. Immunity 2017; 47(3): 450-465.e5. doi: 10.1016/j.immuni.2017.08.010.
114. Grinberg-Bleyer Y, Oh H, Desrichard A, et al. NF-κB c-Rel Is Crucial for the Regulatory T cell immune checkpoint in cancer. Cell 2017; 170(6): 1096-1108.e13. doi: 10.1016/j.cell.2017.08.004.
115. Keeton R, Tincho MB, Ngomti A, et al. T cell responses to SARS-CoV-2 spike cross-recognize Omicron. Nature 2022; 603(7901): 488-492. doi: 10.1038/s41586-022-04460-3. Erratum in: Nature 604(7907): E25, 2022.
116. Murray SE, Polesso F, Rowe AM, et al. NF-κB-inducing kinase plays an essential T cell-intrinsic role in graft-versus-host disease and lethal autoimmunity in mice. J Clin Invest 2011; 121(12): 4775-86. doi: 10.1172/JCI44943.
117. Chougnet CA, Tripathi P, Lages CS, et al. A major role for Bim in regulatory T cell homeostasis. J Immunol 2011; 186: 156163. doi: 10.4049/jimmunol.1001505.

118. Świerkot J, Madej M, Szmyrka M, et al. The risk of autoimmunity development following mRNA COVID-19 vaccination. Viruses 2022; 14(12): 2655. doi: 10.3390/v14122655.
119. Fraiman J, Erviti J, Jones M, et al. Serious adverse events of special interest following mRNA COVID-19 vaccination in randomized trials in adults. Vaccine 2022; 40(40): 5798-5805. doi: 10.1016/j.vaccine.2022.08.036
120. Kadali RA, Janagama R, Yedlapati SH, et al. *S* ide effects of messenger RNA vaccines and prior history of COVID-19, a cross-sectional study, American Journal of Infection Control 2022; 50(1): 8-14. doi: 10.1016/j.ajic.2021.10.017.
121. Watad A, De Marco G, Mahajna H, et al. Immune-mediated disease flares or new-onset disease in 27 subjects following mRNA/DNA SARS-CoV-2 vaccination. Vaccines (Basel) 2021; 9(5): 435. doi: 10.3390/vaccines9050435.
122. Raviv Y, Betesh-Abay B, Valdman-Grinshpoun Y, et al. First presentation of systemic lupus erythematosus in a 24-year-old male following mRNA COVID-19 vaccine. Case Rep Rheumatol 2022; 2022: 9698138. doi: 10.1155/2022/9698138.
123. Ghielmetti M, Schaufelberger HD, Mieli-Vergani G, et al. Acute autoimmune-like hepatitis with atypical anti-mitochondrial antibody after mRNA COVID-19 vaccination: a novel clinical entity? J. Autoimmun 2021; 123: 102706. doi: 10.1016/j.jaut.2021.102706.
124. Fatima Z, Reece BRA, Moore JS, et al. Autoimmune hemolytic anemia after mRNA COVID vaccine. J Investig Med High Impact Case Rep 2022; 10: 23247096211073258. doi: 10.1177/23247096211073258.
125. Gadi SRV, Brunker PAR, Al-Samkari H, et al. Severe autoimmune hemolytic anemia following receipt of SARS-CoV-2 mRNA vaccine. Transfusion 2021; 61(11): 3267-3271. doi: 10.1111/trf.16672.
126. Alqatari S, Ismail M, Hasan M, et al. Emergence of post COVID-19 vaccine autoimmune diseases: A single center study. Infect Drug Resist 2023; 16: 1263-1278. doi: 10.2147/IDR.S394602.
127. Guo M, Liu X, Chen X, et al. Insights into new-onset autoimmune diseases after COVID-19 vaccination. Autoimmun Rev 2023; 22(7): 103340. doi: 10.1016/j.autrev.2023.103340.
128. Saleh R, Elkord E. FoxP3+ T regulatory cells in cancer: Prognostic biomarkers and therapeutic targets. Cancer Lett. 2020; 490: 174-185. doi: 10.1016/j.canlet.2020.07.022.
129. Sharabi A, Tsokos MG, Ding Y, et al. Regulatory T cells in the treatment of disease. Nat Rev Drug Discov 2018; 17: 823-844. doi: 10.1038/nrd.2018.148.
130. Iglesias-Escudero M, Arias-González N, Martínez-Cáceres E. Regulatory cells and the effect of cancer immunotherapy. Mol Cancer 2023; 22: 26. doi: 10.1186/s12943-023-01714-0.
131. Shimizu J, Yamazaki S, Sakaguchi S. Induction of tumor immunity by removing CD25+CD4+ T cells: a common basis between tumor immunity and autoimmunity. J Immunol 1999; 163(10): 5211-8. doi: 10.4049/jimmunol.163.10.5211.
132. Takahashi T, Kuniyasu Y, Toda M, et al. Immunologic self-tolerance maintained by CD25+CD4+ naturally anergic and suppressive T cells: induction of autoimmune disease by breaking their anergic/suppressive state. Int Immunol 1998; 10(12): 1969-80. doi: 10.1093/intimm/10.12.1969.
133. Zelenay S, Lopes-Carvalho T, Caramalho I, et al. Foxp3+ CD25-CD4 T cells constitute a reservoir of committed regulatory cells that regain CD25 expression upon homeostatic expansion. Proc Natl Acad Sci U S A 2005; 102(11): 4091-6. doi: 10.1073/pnas.0408679102.
134. Lasagna A, Lilleri D, Agustoni F, et al. Analysis of the humoral and cellular immune response after a full course of BNT162b2 anti-SARS-CoV-2 vaccine in cancer patients treated with PD-1/PD-L1 inhibitors with or without chemotherapy: an update after 6 months of follow-up. ESMO Open 2022; 7(1): 100359. doi: 10.1016/j.esmoop.2021.100359.
135. Benitez Fuentes JD, Mohamed KM, de Luna Aguilar A, et al. Evidence of exhausted lymphocytes after the third anti-SARS-CoV-2 vaccine dose in cancer patients. Front Oncol 2022; 12: 975980. doi: 10.3389/fonc.2022.975980.
136. Lee J, Ahn E, Kissick HT, Ahmed R. Reinvigorating exhausted T cells by blockade of the PD-1 pathway. For Immunopathol Dis Therap 2015; 6(1-2): 7-17. doi: 10.1615/ForumImmunDisTher.2015014188.
137. Al-Mterin MA, Alsalman A, Elkord E. Inhibitory immune checkpoint receptors and ligands as prognostic biomarkers in COVID-19 patients. Front Immunol 2022; 13: 870283. doi: 10.3389/fim-

mu.2022.870283.
138. Simon S, Labarriere N. PD-1 expression on tumor-specific T cells: Friend or foe for immunotherapy? Oncoimmunology 2017; 7(1): e1364828. doi: 10.1080/2162402X.2017.1364828.
139. Gao FX, Wu RX, Shen MY, et al. Extended SARS-CoV-2 RBD booster vaccination induces humoral and cellular immune tolerance in mice. iScience 2022; 25(12): 105479. doi: 10.1016/j.isci.2022.105479.
140. Zaleska J, Kwasnik P, Paziewska M, et al. Response to anti-SARS-CoV-2 mRNA vaccines in multiple myeloma and chronic lymphocytic leukemia patients. Int J Cancer 2023; 152(4): 705-712. doi: 10.1002/ijc.34209.
141. Shroff RT, Chalasani P, Wei R, et al. Immune responses to two and three doses of the BNT162b2 mRNA vaccine in adults with solid tumors. Nat Med 2021; 27(11): 2002-2011. doi: 10.1038/s41591-021-01542-z.
142. Lund FE, Randall TD. Effector and regulatory B cells: modulators of CD4+ T cell immunity. Nat Rev Immunol 2010; 10(4): 236-47. doi: 10.1038/nri2729.
143. Qian J, Wang C, Wang B, et al. The IFN-γ/PD-L1 axis between T cells and tumor microenvironment: hints for glioma anti-PD-1/PD-L1 therapy. J Neuroinflammation 2018; 15: 290. doi: 10.1186/s12974-018-1330-2.
144. Loacker L, Kimpel J, Bánki Z, et al. Increased PD-L1 surface expression on peripheral blood granulocytes and monocytes after vaccination with SARS-CoV2 mRNA or vector vaccine. Clin Chem Lab Med 2022; 61(1): e17-e19. doi: 10.1515/cclm-2022-0787.
145. Özbay Kurt FG, Lepper A, et al. Booster dose of mRNA vaccine augments waning T cell and antibody responses against SARS-CoV-2. Front Immunol 2022; 13: 1012526. doi: 10.3389/fimmu.2022.1012526.
146. Ostrand-Rosenberg S, Horn LA, Haile ST. The programmed death-1 immune-suppressive pathway: barrier to antitumor immunity. J Immunol 2014; 193(8): 3835-41. doi: 10.4049/jimmunol.1401572.
147. Spranger S, Spaapen RM, Zha Y, et al. Up-regulation of PD-L1, IDO, and T(regs) in the melanoma tumor microenvironment is driven by CD8(+) T cells. Sci Transl Med 2013; 5(200): 200ra116. doi: 10.1126/scitranslmed.3006504.
148. Ai L, Chen J, Yan H, et al. Research status and outlook of PD-1/PD-L1 inhibitors for cancer therapy. Drug Des Devel Ther 2020; 14: 3625-3649. doi: 10.2147/DDDT.S267433.
149. Zamani MR, Aslani S, Salmaninejad A, et al. A growing relationship. Cell Immunol 2016; 310: 27-41. doi: 10.1016/j.cellimm.2016.09.009.
150. Spiliopoulou P, Janse van Rensburg HJ, Avery L, et al. Longitudinal efficacy and toxicity of SARS-CoV-2 vaccination in cancer patients treated with immunotherapy. Cell Death Dis 2023; 14: 49. doi: 10.1038/s41419-022-05548-4.
151. Sakowska J, Arcimowicz Ł, Jankowiak M, et al. Autoimmunity and cancer-two sides of the same coin. Front Immunol 2022; 13: 793234. doi: 10.3389/fimmu.2022.793234.
152. Platanias L: Mechanisms of type-I- and type-II-interferon-mediated signalling. Nat Rev Immunol 2005; 5: 375-386. doi: 10.1038/nri1604.
153. Zeng H, Yang K, Cloer C, et al. mTORC1 couples immune signals and metabolic programming to establish T(reg)-cell function. Nature 2013; 499(7459): 485-490. doi: 10.1038/nature12297.
154. Dominic A, Le NT, Takahashi M. Loop between NLRP3 inflammasome and reactive oxygen species. Antioxid Redox Signal 2022; 36(10-12): 784-796. doi: 10.1089/ars.2020.8257.
155. Wen C, Wang H, Wu X, et al. ROS-mediated inactivation of the PI3K/AKT pathway is involved in the antigastric cancer effects of thioredoxin reductase-1 inhibitor chaetocin. Cell Death Dis 2019; 10(11): 809. doi: 10.1038/s41419-019-2035-x.
156. Li F, Li J, Wang PH, et al. SARS-CoV-2 spike promotes inflammation and apoptosis through autophagy by ROS-suppressed PI3K/AKT/mTOR signaling. Biochim Biophys Acta Mol Basis Dis 2021; 1867(12): 166260. doi: 10.1016/j.bbadis.2021.166260
157. Tarassishin L, Suh HS, Lee SC. Interferon regulatory factor 3 plays an anti-inflammatory role in microglia by activating the PI3K/Akt pathway. J Neuroinflammation 2011; 8:187. doi: 10.1186/1742-2094-8-187.
158. Freitas RS, Crum TF, Parvatiyar K. SARS-CoV-2 spike antagonizes innate antiviral immunity by targeting interferon regulatory factor 3. Front Cell Infect Microbiol 2022; 11: 789462. doi:

10.3389/fcimb.2021.789462.
159. Rodríguez-Jiménez P, Chicharro P, Cabrera LM, et al. Varicella-zoster virus reactivation after SARS-CoV-2 BNT162b2 mRNA vaccination: Report of 5 cases. JAAD Case Rep 2021; 12: 58-59. doi: 10.1016/j.jdcr.2021.04.014.
160. Netti GS, Infante B, Troise D, et al. mTOR inhibitors improve both humoral and cellular response to SARS-CoV-2 messenger RNA BNT16b2 vaccine in kidney transplant recipients. Am J Transplant 2022; 22(5): 1475-1482. doi: 10.1111/ajt.16958.
161. Pollizzi KN, Patel CH, Sun IH, et al. mTORC1 and mTORC2 selectively regulate CD8+ T cell differentiation. J Clin Invest 2015; 125(5): 2090-108. doi: 10.1172/JCI77746.
162. Tu W, Rao S. Mechanisms underlying T cell immunosenescence: Aging and Cytomegalovirus infection. Front Microbiol 2016; 7: 2111. doi: 10.3389/fmicb.2016.02111.
163. Biering SB, Gomes de Sousa FT, Tjang LV, et al. SARS-CoV-2 spike triggers barrier dysfunction and vascular leak via integrins and TGF-β signaling. Nat Commun 2022; 13(1): 7630. doi: 10.1038/s41467-022-34910-5.
164. Prieto-Fernández E, Egia-Mendikute L, Vila-Vecilla L, et al. Hypoxia reduces cell attachment of SARS-CoV-2 spike protein by modulating the expression of ACE2, neuropilin-1, syndecan-1 and cellular heparan sulfate. Emerg Microbes Infect 2021; 10(1): 1065-1076. doi: 10.1080/22221751.2021.1932607.
165. Mallikarjuna P, Raviprakash TS, Aripaka K, et al. Interactions between TGF-β type I receptor and hypoxia-inducible factor-α mediates a synergistic crosstalk leading to poor prognosis for patients with clear cell renal cell carcinoma. Cell Cycle 2019; 18(17): 2141-2156. doi: 10.1080/15384101.2019.1642069.
166. Deng W, Feng X, Li X, et al. Hypoxia-inducible factor 1 in autoimmune diseases. Cell Immunol. 2016; 303: 7-15. doi: 10.1016/j.cellimm.2016.04.001.
167. Kyriakopoulos AM, McCullough PA, Nigh G, et al. Potential mechanisms for human genome integration of genetic code from SARS-CoV-2 mRNA vaccination: implications for disease. J Neurol Disord 2022; 10: 519.
168. Seneff S, Kyriakopoulos AM, Nigh G, et al. A potential role of the spike protein in neurodegenerative diseases: A narrative review. Cureus 2023; 15(2): e34872. doi: 10.7759/cureus.34872.
169. Tang YY, Wang DC, Wang YQ, et al. Emerging role of hypoxia-inducible factor-1α in inflammatory autoimmune diseases: A comprehensive review. Front Immunol 2023; 13: 1073971. doi: 10.3389/fimmu.2022.1073971
170. Xu M, Pang Q, Xu S, et al. Hypoxia-inducible factor-1α activates transforming growth factor-β1/Smad signaling and increases collagen deposition in dermal fibroblasts. Oncotarget 2017; 9(3): 3188-3197. doi: 10.18632/oncotarget.23225.
171. Samson M, Audia S, Janikashvili N, et al. Brief report: inhibition of interleukin-6 function corrects Th17/Treg cell imbalance in patients with rheumatoid arthritis. Arthritis Rheum 2012; 64(8): 2499-503. doi: 10.1002/art.34477.
172. Pesce B, Soto L, Sabugo F, et al. Effect of interleukin-6 receptor blockade on the balance between regulatory T cells and T helper type 17 cells in rheumatoid arthritis patients. Clin Exp Immunol 2013; 171(3): 237-42. doi: 10.1111/cei.12017.
173. Manel N, Unutmaz D, Littman DR. The differentiation of human T(H)-17 cells requires transforming growth factor-beta and induction of the nuclear receptor RORgammat. Nat Immunol 2008; 9(6): 641-9. doi: 10.1038/ni.1610.
174. Khan S, Shafiei MS, Longoria C, et al. SARS-CoV-2 spike protein induces inflammation via TLR2-dependent activation of the NF-ϰB pathway. eLife 2021; 10: e68563. doi: 10.7554/eLife.68563.
175. Park DW, Lyu JH, Kim JS, et al. Role of JAK2-STAT3 in TLR2-mediated tissue factor expression. J Cell Biochem 2013; 114(6): 1315-21. doi: 10.1002/jcb.24472.
176. Fontes-Dantas FL, Fernandes GG, Gutman EG, et al. SARS-CoV-2 Spike protein induces TLR4-mediated long-term cognitive dysfunction recapitulating post-COVID-19 syndrome in mice. Cell Rep 2023; 42(3): 112189. doi: 10.1016/j.celrep.2023.112189.
177. Palakkott AR, Alneyadi A, Muhammad K, et al. The SARS-CoV-2 spike protein activates the epider-

mal growth factor receptor-mediated signaling. Vaccines (Basel) 2023; 11(4): 768. doi: 10.3390/vaccines11040768.
178. Yu H, Pardoll D, Jove R. STATs in cancer inflammation and immunity: a leading role for STAT3. Nat Rev Cancer 2009; 9(11): 798-809. doi: 10.1038/nrc2734.
179. Wang Y, van Boxel-Dezaire AH, Cheon H, et al. STAT3 activation in response to IL-6 is prolonged by the binding of IL-6 receptor to EGF receptor. Proc Natl Acad Sci U S A 2013; 110(42): 16975-80. doi: 10.1073/pnas.1315862110.
180. McGeachy MJ, Bak-Jensen KS, Chen Y, et al. TGF-beta and IL-6 drive the production of IL-17 and IL-10 by T cells and restrain T(H)-17 cell-mediated pathology. Nat Immunol 2007; 8(12): 1390-7. doi: 10.1038/ni1539.
181. Yasuda K, Takeuchi Y, Hirota K. The pathogenicity of Th17 cells in autoimmune diseases. Semin Immunopathol. 2019; 41(3): 283-297. doi: 10.1007/s00281-019-00733-8.
182. Bailey SR, Nelson MH, Himes RA, et al. Th17 cells in cancer: the ultimate identity crisis. Front Immunol 2014; 5: 276. doi: 10.3389/fimmu.2014.00276.
183. Lee AR, Woo JS, Lee SY, et al. SARS-CoV-2 spike protein promotes inflammatory cytokine activation and aggravates rheumatoid arthritis. Cell Commun Signal 2023; 21: 44. doi: 10.1186/s12964-023-01044-0.
184. Buergin N, Lopez-Ayala P, Hirsiger JR, et al. Sex-specific differences in myocardial injury incidence after COVID-19 mRNA-1273 booster vaccination. Eur J Heart Fail 2023; 25(10): 1871-1881. doi: 10.1002/ejhf.2978.
185. Schwab C, Domke LM, Hartmann L, et al. Autopsy-based histopathological characterization of myocarditis after anti-SARS-CoV-2-vaccination. Clin Res Cardiol 2023; 112(3): 431-440. doi: 10.1007/s00392-022-02129-5.
186. Wang J, Liu T, Chen X, et al. Bazedoxifene regulates Th17 immune response to ameliorate experimental autoimmune myocarditis via inhibition of STAT3 activation. Front Pharmacol 2021; 11: 613160. doi: 10.3389/fphar.2020.613160.
187. Barmada A, Klein J, Ramaswamy A, et al. Cytokinopathy with aberrant cytotoxic lymphocytes and profibrotic myeloid response in SARS-CoV-2 mRNA vaccine-associated myocarditis. Sci Immunol 2023; 8(83): eadh3455. doi: 10.1126/sciimmunol.adh3455.
188. Xu L, Zhang T, Liu Z, et al. Critical role of Th17 cells in development of autoimmune hemolytic anemia. Exp Hematol 2012; 40: 994–1004. doi: 10.1016/j.exphem.2012.08.008.
189. Barcellini W, Clerici G, Montesano R, et al. In vitro quantification of anti-red blood cell antibody production in idiopathic autoimmune haemolytic anaemia: effect of mitogen and cytokine stimulation. Br J Haematol 2000; 111: 452–460. doi: 10.1046/j.1365-2141.2000.02380.x.
190. Ahmad E, Elgohary T, Ibrahim H. Naturally occurring regulatory T cells and interleukins 10 and 12 in the pathogenesis of idiopathic warm autoimmune hemolytic anemia. J Investig Allergol Clin Immunol 2011; 21:297–304, 2011.
191. Yonker LM, Swank Z, Bartsch YC, et al. Circulating spike protein detected in post-COVID-19 mRNA vaccine myocarditis. Circulation 2023; 147(11): 867-876. doi: 10.1161/CIRCULATIONAHA.122.061025.
192. Mohiddin SA, Guttmann O, Marelli-Berg F. Vaccine-triggered acute autoimmune myocarditis: Defining, detecting, and managing an apparently novel condition. J Am Heart Assoc 2022; 11(21): e026873. doi: 10.1161/JAHA.122.026873.
193. Sung SS Monocyte-derived dendritic cells as antigen-presenting cells in T-cell proliferation and cytokine production. Methods Mol Med 2008; 138: 97-106. doi: 10.1007/978-1-59745-366-0_9.
194. Sanjabi S, Oh SA, Li MO. Regulation of the immune response by TGF-β: From conception to autoimmunity and infection. Cold Spring Harb Perspect Biol 2017; 9(6): a022236. doi: 10.1101/cshperspect.a022236.
195. Sriwastava S, Sharma K, Khalid SH, et al. COVID-19 vaccination and neurological manifestations: A review of case reports and case series. Brain Sci 2022; 12(3): 407. doi: 10.3390/brainsci12030407.
196. Abu-Abaa M, Dawood G, Arshad H, et al. A possible case of autoimmune encephalitis after mRNA

COVID-19 booster vaccine: A case report. Cureus 2022; 14(11): e31118. doi: 10.7759/cureus.31118.
197. Vidarsson G, Dekkers G, Rispens T. IgG subclasses and allotypes: from structure to effector functions. Front Immunol 2014; 5: 520. doi: 10.3389/fimmu.2014.00520.
198. Irrgang P, Gerling J, Kocher K, et al. Class switch toward noninflammatory, spike-specific IgG4 antibodies after repeated SARS-CoV-2 mRNA vaccination. Sci Immunol 2023; 8(79): eade2798. doi: 10.1126/sciimmunol.ade2798.
199. Uversky VN, Redwan EM, Makis W, et al. IgG4 antibodies induced by repeated vaccination may generate immune tolerance to the SARS-CoV-2 spike protein. Vaccines (Basel) 2023; 11(5): 991. doi: 10.3390/vaccines11050991.
200. Kiszel P, Sík P, Miklós J, et al. Class switch towards spike protein-specific IgG4 antibodies after SARS-CoV-2 mRNA vaccination depends on prior infection history. Sci Rep 2023; 13: 13166. doi: 10.1038/s41598-023-40103-x.
201. Liu J, Yin W, Westerberg LS, et al. Immune dysregulation in IgG4-related disease. Front Immunol 2021; 12: 738540. doi: 10.3389/fimmu.2021.738540.
202. Zhang X, Lu H, Peng L, et al. The role of PD-1/PD-Ls in the pathogenesis of IgG4-related disease. Rheumatology (Oxford) 2022; 61(2): 815-825. doi: 10.1093/rheumatology/keab360.
203. Akiyama M, Suzuki K, Kassai Y, et al. Resolution of elevated circulating regulatory T cells by corticosteroids in patients with IgG4-Related dacryoadenitis and sialoadenitis. Int J Rheum Dis 2016; 19(4): 4302. doi: 10.1111/1756185X.12725
204. Kusuda T, Uchida K, Miyoshi H, et al. Involvement of inducible costimulator and interleukin 10-positive regulatory T cells in the development of IgG4-related autoimmune pancreatitis. Pancreas 2011; 40(7):1120-1130. doi:10.1097/MPA.0b013e31821fc796.
205. Uchida K, Okazaki K. Current status of type 1 (IgG4-related) autoimmune pancreatitis. J Gastroenterol 2022; 57(10): 695-708. doi: 10.1007/s00535-022-01891-7.
206. Yu T, Wu Y, Liu J, et al. The risk of malignancy in patients with IgG4-related disease: a systematic review and meta-analysis. Arthritis Res Ther 2022; 24: 14. doi: 10.1186/s13075-021-02652-2.
207. Kalra RK, Jayadeep S, Ball AL. Acute pancreatitis in an adolescent following COVID vaccination. Clin Pediatr (Phila) 61(3): 236-240, 2022. doi: 10.1177/00099228211067678.
208. Kantar A, Seminara M, Odoni M, et al. Acute mild pancreatitis following COVID-19 mRNA vaccine in an adolescent. Children (Basel) 2021; 9(1): 29. doi: 10.3390/children9010029.
209. Walter T, Connor S, Stedman C, et al. A case of acute necrotising pancreatitis following the second dose of Pfizer-BioNTech COVID-19 mRNA vaccine. Br J Clin Pharmacol 2022; 88(3): 1385-1386. doi: 10.1111/bcp.15039.
210. Goldman S, Bron D, Tousseyn T, et al. Rapid progression of angioimmunoblastic T cell lymphoma following BNT162b2 mRNA vaccine booster shot: a case report. Front Med (Lausanne) 2021; 8: 798095. doi: 10.3389/fmed.2021.798095.
211. Cavanna L, Grassi SO, Ruffini L, et al. Non-Hodgkin lymphoma developed shortly after mRNA COVID-19 vaccination: Report of a case and review of the literature. Medicina (Kaunas) 2023; 59(1): 157. doi: 10.3390/medicina59010157.
212. Mizutani M, Mitsui H, Amano T, et al. Two cases of axillary lymphadenopathy diagnosed as diffuse large B-cell lymphoma developed shortly after BNT162b2 COVID-19 vaccination. J Eur Acad Dermatol Venereol 2022; 36(8): e613-e615. doi: 10.1111/jdv.18136.
213. Tachita T, Takahata T, Yamashita S, et al. Newly diagnosed extranodal NK/T-cell lymphoma, nasal type, at the injected left arm after BNT162b2 mRNA COVID-19 vaccination. Int J Hematol 2023; 1–5. doi: 10.1007/s12185-023-03607-w.
214. Zamfir MA, Moraru L, Dobrea C, et al. Hematologic malignancies diagnosed in the context of the mRNA COVID-19 vaccination campaign: A report of two cases. Medicina (Kaunas) 2022; 58(7): 874. doi: 10.3390/medicina58070874.
215. Eens S, Van Hecke M, Favere K, et al. B-cell lymphoblastic lymphoma following intravenous BNT162b2 mRNA booster in a BALB/c mouse: A case report. Front Oncol 2023; 13: 1158124. doi: 10.3389/fonc.2023.1158124.