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B lymphocytes in Rheumatoid Arthritis and Effects of anti-TNF- α agents on B lymphocytes: Review of the literature

Ozlem Pala¹, Alain Diaz², Bonnie B. Blomberg^{2,3}, Daniela Frasca²

¹Division of Rheumatology, University of Miami Miller School of Medicine, Miami, FL USA

²Department of Microbiology and Immunology, University of Miami Miller School of Medicine, Miami, FL USA

³Sylvester Comprehensive Cancer Center, University of Miami Miller School of Medicine, Miami, FL USA

Abstract

Purpose—To review published research related to B lymphocytes in Rheumatoid arthritis, their role in the pathogenesis of the disease and the effects of blocking TNF- α with TNF- α inhibitors on B lymphocytes, risk of infection and responses to vaccines.

Methods—PubMed search was conducted to review recent advances related to B lymphocytes and effects of anti-TNF- α on B lymphocytes in Rheumatoid Arthritis.

Findings—B lymphocytes play an important role in the pathogenesis of Rheumatoid Arthritis. In this review, we summarize major mechanisms by which B lymphocytes play a pathologic role in the development and propagation of the disease, as B lymphocytes are recruited to the synovial fluid where they contribute to local inflammation through the secretion of pro-inflammatory mediators (cytokines, chemokines, micro-RNAs) and present antigens to T cells. We discuss the effects of TNF- α , either direct or indirect, on B lymphocytes expressing receptors for this cytokine. We also show that total B cell numbers have been reported to be reduced in the blood of Rheumatoid arthritis patients *versus* healthy controls, but significantly increased up to normal levels in patients undergoing anti-TNF- α therapy. As for B cell subsets, controversial results have been reported with studies showing decreased frequencies of total memory B cells (and memory subsets) and other showing no differences in patients *versus* healthy controls. Studies investigating the effects of anti-TNF- α therapy also gave controversial results, with therapy found to increase or not the frequency of memory B lymphocytes, in patients with Rheumatoid arthritis *versus* healthy controls. Those highly variable results could be due to differences in patient characteristics and limited number of subjects, suggesting that there is a clear need for larger and more comprehensive studies. Finally, we summarize the effects of blocking TNF- α with anti-TNF- α

Corresponding authors: Ozlem Pala, MD, OPala@med.miami.edu, Daniela Frasca, PhD, dfrasca@med.miami.edu.

Conflict of interest statement

The research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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agents on possible infections that Rheumatoid arthritis patients may contract as well as on responses to vaccination.

Introduction

Rheumatoid arthritis is a common autoimmune disease that is associated with progressive disability, systemic complications, early death, and socioeconomic costs.¹ Rheumatoid arthritis is characterized by synovial inflammation and hyperplasia (“swelling”), autoantibody production [rheumatoid factor (RF) and anti-citrullinated protein antibody (ACPA)], cartilage and bone destruction (“deformity”), and systemic features, including cardiovascular, pulmonary, psychological, and skeletal disorders.² Cardiovascular disease and infections represent one of the leading causes of disability and mortality in Rheumatoid arthritis patients,³ which may result from compromised humoral immune response^{4, 5} and those treated with anti-TNF- α , alone or with Methotrexate (MTX), seem to be at further risk.^{6, 7} A slightly elevated risk of lymphoma and lymphoproliferative malignant disease,⁸ lung cancer,⁹ or skin cancer¹⁰ has also been associated with Rheumatoid arthritis. Non melanoma skin cancer also appears to be increased in Rheumatoid arthritis patients in the setting of use anti-TNF- α agents.¹⁰

The disease results from a complex interaction between genes and the environment, leading to a breakdown of immune tolerance and increased synovial inflammation in a characteristic symmetric pattern. Distinct mechanisms regulate inflammation and matrix destruction, including damage to bone and cartilage.¹¹ The inflammatory infiltrate in Rheumatoid arthritis includes T lymphocytes, B lymphocytes, monocytes and dendritic cells^{12–14} and in approximately 20% of patients lymphoid neogenesis develops with the formation of ectopic germinal centers (GCs).^{15–18} The pathogenic role of chronic inflammation in Rheumatoid arthritis is due to persistent immune responses during the pre-clinical and clinical phases of the disease. Chronic inflammation in Rheumatoid arthritis has recently been shown to be linked to immunometabolic requirements of innate and adaptive immune cells, as the chronic stimulation of the immune system requires a reliable supply of nutrients, oxygen, and biosynthetic precursors. Recent work has clearly indicated that the functional commitment of Rheumatoid arthritis T cells in driving persistent synovial inflammation is mechanistically connected to inefficient DNA repair/chromosome instability (shorter telomeres), and metabolic reprogramming.^{19–22} Abnormal metabolic pathways and increased oxidative stress in monocytes/macrophages also seem to be involved in altered T cell activity and development of Rheumatoid arthritis through the generation of autoantigens, as suggested by studies in both mice²² and humans.

Purpose of this review is to summarize published research on B lymphocytes in Rheumatoid arthritis, their role in the pathogenesis of the disease and the effects of blocking TNF- α with TNF- α inhibitors on B lymphocytes, risk of infection and responses to vaccines.

Search of the literature

We conducted a literature search in PubMed (MEDLINE) data base. We initially selected several appropriate keywords (examples are “Rheumatoid Arthritis”, “Inflammation”, “B

lymphocytes”, “anti-TNF- α agents”). Time frame was 1970–2018. We retrieved only the citations in English in which the selected keyword was the major focus. We had no limitations due to the type of study (experimental, clinical). References cited in our review are primary papers, as well as review articles, published in peer-reviewed journals. All references cited by the articles have also been searched and analyzed.

Role of B lymphocytes in the pathogenesis of Rheumatoid arthritis

B lymphocytes produce autoreactive pathogenic antibodies, such as RF and ACPA, which are well established indicators of disease and disease severity, as they enhance tissue injury in a pre-clinical model of autoimmune arthritis.²³ Autoreactivity to malondialdehyde (MDA) has recently been reported in Rheumatoid arthritis patients and is also linked to disease activity and synovial pathogenesis.²⁴ MDA is a naturally occurring aldehyde, produced under oxidative stress and associated with excessive generation of reactive oxygen species (ROS) which catalyze membrane lipid peroxidation. These autoreactive pathogenic antibodies in some patients can be detected many years before disease onset, suggesting that in predisposed individuals autoantibodies develop before the establishment of the inflammatory state that leads to clinically detected Rheumatoid arthritis.

Moreover, B lymphocytes are antigen-presenting cells for autoreactive T cells, as shown in both murine^{25, 26} and human studies.²⁶ A defect in early B cell tolerance has also been reported in humans, with the majority of naive B lymphocytes expressing polyreactive autoantibodies, including RF and ACPA. These B lymphocytes are resistant to Fas-induced apoptosis and therefore not suppressed by T regulatory cells (T_{REG}).²⁷

The synovial tissue is an active site of B cell accumulation, proliferation, plasma cell differentiation and autoantibody production.^{28, 29} Both synovial and intra-articular B lymphocytes are recruited to the site of inflammation from the peripheral blood, and this recruitment is driven by CC and CXC chemokines for which B lymphocytes express the receptors.³⁰ Synovial B lymphocytes are mainly localized in T-B cell area and are supported by cytokines such as proliferation-inducing ligand (APRIL) and B-lymphocyte stimulator (BLyS). Plasmablasts and plasma cells are more widely distributed in the synovium and also in juxta-articular bone marrow of Rheumatoid arthritis patients.² Because plasma cells are not targeted by Rituximab (anti-CD20 monoclonal antibodies), and autoantibody levels are variably altered after treatment, these clinical observations suggest that the role of B lymphocytes and their progeny in the pathogenesis of Rheumatoid arthritis may go beyond autoantibody production to include autoantigen presentation and cytokine production, mainly IL-6, TNF- α , lymphotoxin- β , but also IL-1, IL-4, IL-7, IL-8, IL-10, GM-CSF^{2, 31} The secretion of these pro-inflammatory cytokines by B lymphocytes is under the control of transcription factors, including NF- κ B, which is ubiquitously expressed and plays key roles in most inflammatory responses. Activated NF- κ B is not only detected in B lymphocytes but also in other cells (T cells, macrophages, macrophage-like synoviocytes) present in the synovial tissue at early and late stage of joint inflammation.³² NF- κ B activation drives both the initiation and the perpetuation of chronic inflammation in Rheumatoid arthritis, inducing tissue remodeling.³³

B lymphocytes also provide important co-stimulatory signals, required for T cell clonal expansion and effector functions.³⁴ Studies in mice have demonstrated that B lymphocytes are indispensable antigen-presenting cells for the activation of autoreactive T cells, as they can directly bind and internalize antigens through the B-cell receptor.³⁵ B lymphocytes also contribute to antigen-presentation and can form immune complexes which are then internalized by dendritic cells and macrophages through a FC γ R-mediated process.^{36, 37} This has clearly been shown in a murine model of autoimmune arthritis, proteoglycan (PG)-induced arthritis, in which both T cells (CD4+) and antigen-presenting cells cooperatively participate in the development of the disease, but only if B cells are present. B cell-deficient or IgM-deficient mice do not develop arthritis after immunization with PG.²⁶

Several findings have suggested a role for micro-RNAs (miRs) in the proliferation of resident cells in Rheumatoid arthritis joints. miRs are a group of 20–25 nucleotide noncoding RNAs which regulate gene expression post-transcriptionally, exhibit tissue-specific or developmental stage-specific expression patterns and are associated with cell proliferation, differentiation, cancer and autoimmune arthritis.^{38–40} B lymphocytes express several miRs (miR-16, miR-150, miR-150) which contribute to local inflammation and are up-regulated in synovial tissue of Rheumatoid arthritis patients.^{41,42} These miRs are also expressed by synovial fibroblasts and have been shown to be induced by TNF- α and IL-1 β .⁴³ These results suggest that these miRs significantly contribute to the pathogenesis of the disease and that the inflammatory microenvironment may alter their expression profiles in resident cells of Rheumatoid arthritis joints.

The phenotype of B lymphocytes in Rheumatoid arthritis patients has been studied in blood and in synovial tissue. The general consensus is that there is an increased presence of memory (CD27+) B lymphocytes, including swIg and LM B lymphocytes, with an activated (CD95+CD21^{low}) phenotype in both blood and synovium of Rheumatoid arthritis patients.⁴⁴ The up-regulation of CD95 (Fas ligand)⁴⁵ and the down-regulation of CD21 (complement receptor type 2, complement C3d receptor, or Epstein-Barr virus receptor)⁴⁶ have independently been associated with B lymphocyte activation and with inflammation. These cells are indeed the major producers of pro-inflammatory cytokines in Rheumatoid arthritis. In another study, a unique subset of CD23+CD21^{hi}, B cells in inflamed nodes (Bin)-like B cells, initially identified in lymph nodes draining the inflamed joints of TNF-transgenic (TNF-tg) mice⁴⁷, has also been shown to contribute to disease exacerbation and increased arthritic flare, by compromising lymph node structural integrity and lymphatic draining function.⁴⁸ The subset of B regulatory (B_{reg}) cells, both mature/memory (CD24^{hi}CD27+)⁴⁹ and immature (CD24^{hi}CD38^{hi} Transitional T2),^{50, 51} has been reported to be decreased in Rheumatoid arthritis patients, as compared to healthy controls. These cells are also functionally impaired and secrete significantly less amounts of IL-10.⁵²

The efficacy of B cell depletion therapy (BCDT) with Rituximab in Rheumatoid arthritis has led to a renewed interest in B lymphocytes and their products and the role they play in the pathogenesis of the disease.⁵³ Published data have indicated that after BCDT CD95+CD21^{low} memory B lymphocytes are increased in the peripheral blood, as compared to pre-depletion, indicating some resistance of these activated populations to Rituximab.⁴⁴ The memory populations after BCDT also expressed higher Ki-67, as compared to pre-

treatment, suggesting homeostatic proliferation of the memory B cell pool. These results altogether indicate that the response to BCDT relies on initial elimination of memory B lymphocytes, followed by a substantial repopulation, concomitant with increased frequencies of transitional B_{REG} cells, suggesting that the clinical and immunological outcome of BCDT depends on the relative ratio of protective (B_{REG}) *versus* pathogenic (memory) B cell subsets after B cell depletion and repopulation.⁴⁴ Because CD4⁺ T cell activation decrease after BCDT,⁵⁴ changes not only in B, but also in T cell subsets may underlie the response of Rheumatoid arthritis patients to therapy.

Expression of TNF- α receptors (TNFR) on B lymphocytes and effect of blocking their receptors in Rheumatoid arthritis

The effects of TNF- α are mediated by two receptors which are structurally related, but functionally distinct, TNFR1 (p55) and TNFR2 (p75).⁵⁵ Both receptors can be released from the cell surface as soluble forms and have the capacity to neutralize TNF- α .⁵⁶ TNFR1 is ubiquitously expressed, whereas TNFR2 is more restricted to lymphocytes and is more efficiently activated by transmembrane rather than by soluble TNF- α .⁵⁷ TNFR1 is the primary signaling receptor on most cell types and accounts for the majority of the pro-inflammatory effects of TNF- α . In contrast, TNFR2 primarily mediates lymphocyte activation and proliferation.⁵⁸

Both TNF- α and soluble TNFR expression are up-regulated in synovial fluids and in the synovial membrane of Rheumatoid arthritis patients, especially in areas adjacent to erosions and increased concentrations of the shed receptors appear to correlate with disease activity.⁵⁹ TNFR1 has been identified as the driving force in Rheumatoid arthritis development, and TNFR1-deficient mice show reduced development of collagen-induced arthritis,⁶⁰ suggesting that targeting TNFR1 reduces the TNF- α -induced arthritogenic pathway leaving TNFR2-mediated signals, known to be anti-inflammatory. Studies in mice have indeed shown that TNFR2-deficient mice develop aggravated arthritis and joint destruction compared with wild-type mice,⁶¹ due to increased recruitment of inflammatory cells to the synovial membrane, and likely less induction of T_{REGS}.

Direct and indirect effects of TNF- α on B lymphocytes

Levels of serum TNF- α have been shown to negatively correlate with B and T cell function. As to T cells, it has been shown that the frequencies of CD4⁺CD28⁻ T cells, which represent a proinflammatory CD4 T cell subset with characteristics of immunosenescence, are increased in the blood of individuals with inflammatory conditions (aging, Rheumatoid arthritis) and these frequencies are associated with high serum levels of TNF- α .⁶² It was shown that TNF- α was able to inhibit the activity of the CD28 promoter in CD4⁺ T cells. As to B lymphocytes, results from our laboratory have indicated that the age-related increase in plasma levels of TNF- α induces TNF- α production by unstimulated B lymphocytes, without any antigenic stimulation and that this “pre-activated” phenotype of the B lymphocytes renders them incapable of being optimally stimulated by exogenous antigens, mitogens or vaccines.⁶³ B cell function can be restored by adding an anti-TNF- α antibody to cultured B lymphocytes,⁶³ similar to what has also been shown for T cells.⁶⁴

We can speculate that indirect effects of TNF- α on B lymphocytes and B cell function may be related to the down-regulation of CD4+ T cells, for example the production of cytokines needed for optimal antibody production. It has indeed recently been shown that T Follicular Helper cells, TFH, which represents the best T helper cells for B cell responses, are expanded in the joints of Rheumatoid arthritis patients. These cells have the phenotype CD4+PD-1+CXCR5-, they are not exhausted, they express factors enabling B cell help (IL-21, CXCL13, ICOS, and MAF), induce plasma cell differentiation, and express a unique set of chemokine receptors that direct migration to inflamed sites (CCR2, CX3CR1, CCR5). Therefore, these cells seem to be specifically poised to promote B cell responses and antibody production within pathologically inflamed non-lymphoid tissues.⁶⁵

Effects of anti-TNF- α therapy on B lymphocytes in Rheumatoid arthritis patients

TNF- α plays a central role in Rheumatoid arthritis through activation of cytokine and chemokine expression, increased expression of endothelial-cell adhesion molecules, protection of synovial fibroblasts, promotion of angiogenesis, suppression of regulatory T cells, and induction of pain.^{66, 67} The central role of this cytokine has been confirmed by successful therapeutic blockade of membrane and soluble TNF- α in Rheumatoid arthritis patients. Published studies investigating the effects of TNF- α treatment on B lymphocytes and whether these could contribute to their effectiveness in Rheumatoid arthritis are summarized below.

Several TNF- α inhibitors (etanercept, infliximab, adalimumab, golimumab and certolizumab) are used alone, or together with MTX, considered the leading anti-rheumatic drug. However, TNF- α inhibitors have been shown to be inadequate in 30–40% of patients treated. In these patients, therapies with other biological agents have been used. Therapies with TNF- α inhibitors are designed to reduce inflammation, relieve pain and prevent or slow joint damage. In contrast, B cell targeted therapies (anti-CD20) have the main goal to destroy autoreactive B lymphocytes, stop autoimmune reactions and allow lymphoid tissues to be repopulated by normal, nonautoimmune B lymphocytes⁶⁹. However, these therapies may have the deleterious effect of removing B lymphocytes with innate immunosuppressive function, such as IL-10-secreting B_{REG}s, which have a crucial role in the down-regulation of inflammatory responses.^{70, 71}

Total B cell numbers have been reported to be reduced in the blood of Rheumatoid arthritis patients *versus* healthy controls, but significantly increased up to normal levels in Rheumatoid arthritis patients undergoing anti-TNF- α therapy with etanercept.⁷² This increase may be associated with modification in the B_{REG} subset. A negative correlation between disease activity and B cell counts has indeed been observed at baseline but not after treatment.⁷² Previous studies have indicated that this negative correlation reflects the lower number of B_{REG}s in the B cell pool, suggesting that B_{REG}s play a crucial role in controlling disease activity.⁴⁹

As for B cell subsets, a decrease in the frequency of circulating IgM memory B lymphocytes (IgD+CD27+), and an increase in circulating swIg memory B lymphocytes (IgD-CD27+),

has been reported in patients with Rheumatoid arthritis *versus* healthy controls, independent of disease duration. Both memory B cell subsets were found in the synovial fluids of Rheumatoid arthritis patients. Anti-TNF- α therapy with infliximab was found to increase the frequency of IgM memory B lymphocytes, suggesting that trafficking of memory B lymphocytes to the synovium is regulated by TNF- α and can be corrected using infliximab. No significant changes were seen in patients treated with only MTX.⁷³

Another study, conversely, reported decreased frequencies of IgM and swIg memory B lymphocytes after anti-TNF- α therapy with etanercept, when compared with patients treated with MTX or healthy controls.⁷⁴ The authors found reduced follicular dendritic cell networks and GC structures together with reduced CD38+ GC B cells, as compared to healthy controls and Rheumatoid arthritis patients on MTX, and concluded that anti-TNF- α therapy disrupts GC structure at least in part through an effect on follicular dendritic cells.

A study examining the influence of RF positivity on the peripheral B cell pool of Rheumatoid arthritis patients, and its modulation under anti-TNF- α therapy, has shown that RF-negative patients have significantly higher frequencies of memory B lymphocytes (total CD27+), and especially IgM memory (IgD+CD27+), as compared to healthy controls and RF-positive patients, and these frequencies increased even more in RF-negative patients undergoing anti-TNF- α therapy with etanercept. No effects of therapy were observed in RF-negative patients on MTX. This was the first study suggesting an association between RF positivity and the composition of the memory B cell pool and its modulation by TNF- α inhibition.⁷⁵ A complete evaluation of the LM B cell subset, which is the highly inflammatory B cell subset, has not yet been performed.

The frequency and the absolute number of mature/memory (CD24^{hi}CD27+)⁴⁹ and immature (CD24^{hi}CD38^{hi} Transitional T2)^{50, 51} B_{REGS} have been shown to be decreased in Rheumatoid arthritis *versus* healthy controls. IL-10-producing B_{REGS} were also found to be decreased in patients, and increased after therapy with MTX together with the anti-TNF- α agents, adalimumab or etanercept.⁵² In this study, no significant differences in the frequencies of total memory (CD27+) and IgM memory (IgD+CD27+) B lymphocytes were observed. Only the subset of CD27+CD69+ B lymphocytes, the memory B cell subset with the early B cell activation marker CD69, was found increased in Rheumatoid arthritis patients *versus* controls and significantly decreased after treatment.

Memory B lymphocytes from Rheumatoid arthritis patients show higher expression of the costimulatory molecule CD86 and lower expression of the inhibitory Fc γ receptor IIb (Fc γ RIIb), as compared to healthy controls. This is associated with high level of inflammation and circulating ACPA. Fc γ RIIb is required for feedback inhibition and its reduced expression might contribute to autoimmune responses. Anti-TNF- α therapy with adalimumab normalized the expression of these molecules, but serum levels of ACPA did not change at six months after the therapy.⁷⁶ However, other studies have reported that changes in the level of these autoantibodies can be seen after longer time on anti-TNF- α therapy.⁷⁷

Anti-TNF- α is a standard therapy also for severe inflammatory bowel disease (IBD). A few studies have shown a significant increase in the number of circulating B lymphocytes (and in the subset of IgM memory B lymphocytes), similar to what is observed in healthy controls and in active patients with IBD after anti-TNF- α therapy. Restoration of B cell numbers was correlated with the biological response to therapy.^{78, 79}

Risk of anti-TNF- α therapy: is it a safe therapy?

Multiple adverse effects of anti-TNF- α therapy have been identified through both clinical trials and post-marketing surveillance. Those include but are not limited to infusion or injection site reactions, induction of autoimmunity, demyelinating disease, malignancy, heart failure and infections. Some of those adverse effects associated with anti-TNF- α therapy are potentially serious. However, these risks must be interpreted in the context of the potential benefits associated with these medications and of the adverse effects associated with conventional therapies.

Risk of infections in Rheumatoid arthritis patients and effect of anti-TNF- α therapy

A link between infectious agents and the development of Rheumatoid arthritis has been proposed and it has been shown that Rheumatoid arthritis patients have increased antibody titers against Epstein-Barr virus^{80, 81} or cytomegalovirus⁸², as compared to healthy controls. Although in a few studies viral DNA and proteins have been detected in the synovium and joints of Rheumatoid arthritis patients,⁸³ these studies have not been confirmed and conclusions cannot be drawn from the current literature.

Recurrent infections in Rheumatoid arthritis are common,⁸⁴ including bacterial, viral and fungal infections. Abnormal distributions of peripheral B cell subsets and reduced B cell function may be responsible for the inability of Rheumatoid arthritis patients to fight infections. This reduced immunological function also include reduced antigen presentation and T cell activation. Not only systemic infections occur in Rheumatoid arthritis patients, but local infections, due to the invasion of joint spaces by a variety of microorganisms, as those for example associated with the use of prosthetic devices, represent a serious issue for Rheumatoid arthritis patients. Accumulated evidence from animal studies has shown that microbial infections can exacerbate the symptoms of the disease.^{85, 86}

Research performed in the 1950s indicates that Rheumatoid arthritis patients are more prone to have infections than healthy controls.^{87, 88} These initial studies were reinforced by subsequent cohort studies. Several controlled observational studies found that age-adjusted mortality in Rheumatoid arthritis patients was increased as compared to healthy controls, and infectious diseases were one of the three leading causes of premature death in American and European Rheumatoid arthritis patients.⁸⁹

The reasons for reduced responses to infectious agents in Rheumatoid arthritis patients are multiple: 1) Rheumatoid arthritis is a chronic disorder with immunological dysfunctions; 2) Rheumatoid arthritis is accompanied by immunocompromising comorbidities; 3)

Rheumatoid arthritis treatment is comprised of potent immunosuppressive drugs; 4) premature aging of the immune system has been described in Rheumatoid arthritis patients and has been shown to significantly contribute to a reduced protection against infectious agents. Based on our previous experience with the elderly, we can hypothesize that the high levels of chronic inflammation observed in Rheumatoid arthritis patients impair B cell function and that anti-TNF- α may improve B cell function, as also shown by our *in vitro* results⁶³. At the same time, however, anti-TNF- α agents have been associated with reduced memory B cells, inhibition of GCs and follicular dendritic cells⁷⁴ which is likely related to a deleterious effect of anti-TNF- α on T cells. Elimination of GCs from the synovial microenvironment could potentially block local antigen recognition and specific responses, leading to joint infections which have been reported in patients with Rheumatoid arthritis.

The effects of the different therapies on the risk of Rheumatoid arthritis patients to develop infections are controversial. A systematic literature search of EMBASE, MEDLINE, Cochrane Library, and electronic abstract databases of the annual scientific meetings of both the European League Against Rheumatism and the American College of Rheumatology was published in 2006. Results have shown an increased risk of serious infections and a dose-dependent increased risk of malignancies in Rheumatoid arthritis patients treated with the anti-TNF- α agents infliximab and adalimumab.⁹⁰ Other studies have also reported that anti-TNF- α therapy, in combination with MTX, induced a higher risk of infection, whereas MTX alone was safe.⁹¹ Conversely, in three placebo-controlled trials evaluating Rheumatoid arthritis patients treated with different anti-TNF- α agents (infliximab, etanercept, adalimumab), rates of infections did not increase, as compared to the placebo group.^{92–94} These contradictory results may be explained, at least in part, by the different type of infection taken into account (non-serious, serious, opportunistic infections), as well as different patient groups or different statistical parameters. As recently reviewed by Atzeni et al., in most of the studies anti-TNF- α drugs seem to be associated with an increased risk of serious infections, including tuberculosis, and of peri-operative infections after orthopedic surgeries.⁹⁵

Effect of anti-TNF- α therapy on vaccine responses in Rheumatoid arthritis patients

Published data indicate that Rheumatoid arthritis patients treated with anti-TNF- α , alone or with MTX, have decreased *in vivo* responses to the influenza vaccine,^{6, 7, 74, 96} or normal responses to both influenza and pneumococcus vaccines in Rheumatoid arthritis patients treated with anti-TNF- α .^{97–100} Other studies showed lower responses to serotypes 23F and 6B in the 23-valent polysaccharide vaccine in Rheumatoid arthritis patients treated with MTX/anti-TNF- α versus MTX or anti-TNF- α alone.^{101, 102}

Variables that may affect the response to vaccines (and explain the different published results) include age, comorbidities, concomitant immunosuppressive therapies, type of anti-TNF- α used, history of vaccinations and infections. Collectively, these results indicate that it is not clear whether anti-TNF- α induces immunosuppression and also importantly, which cell type(s) are affected (see below). The risk-benefit ratio of TNF- α inhibitors represents a

key concern, with controversial results in the literature that need to be unequivocally resolved, as the risk of infections must be balanced against the risk associated with poor disease control.

Conclusions

B lymphocytes play a significant role in the pathogenesis of Rheumatoid arthritis and BCDT has a major impact on the course of the disease. There have been great advances in treatment of Rheumatoid arthritis in recent years with development of targeted therapies. Anti-TNF- α therapies are widely and successfully used despite of potentially serious adverse effects. Total B cell numbers have been reported to be reduced in the blood of Rheumatoid arthritis patients *versus* healthy controls, but significantly increased up to normal levels in patients undergoing anti-TNF- α therapy. As for B cell subsets, controversial results have been reported with studies showing decreased frequencies of total memory B cells (and memory subsets) and others showing no differences in patients *versus* healthy controls. Studies investigating the effects of anti-TNF- α therapy also gave controversial results, with therapy found to increase or not the frequency of memory B lymphocytes, in patients with Rheumatoid arthritis *versus* healthy controls. Overall there are limited data in the literature on the effects of anti-TNF- α therapies on B lymphocytes and results are conflicting. Differences in study designs and patients characteristics also small sample sizes are likely to cause variations in the results. There is a need for larger studies to better understand the effect of newly discovered therapies on different cells of the immune system.

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