T FOLLICULAR REGULATORY CELL (TFR) IN SYSTEMIC LUPUS ERYTHEMATOSUS AND ITS RELATION TO DISEASE ACTIVITY

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Systemic lupus erythematosus (SLE) is a chronic autoimmune disorder distinguished by the presence of autoantibodies causing multiorgan damages. Follicular regulatory T (Tfr) cells are mainly located in germinal centers (GCs) and play vital role in inducing Tfh suppression, sustaining B cell tolerance and inhibiting autoantibodies development. Dysregulated Tfr cells result in abnormal GCs responses and contribute to the pathogenesis of autoimmune diseases. However, the exact role of Tfr cells in SLE is still ambiguous. The present review focuses on the immunological bases and the disturbed balance of Tfr and Tfh cells in autoimmune diseases, particularly SLE. In addition to the new therapeutic strategies for SLE.

Key words: SLE, autoimmune diseases, innate immunity, Tfr, Tfh.

INTRODUCTION

Systemic lupus erythematosus (SLE) is a complex multifactorial autoimmune disorder known to primarily affect females. It’s marked by the synthesis of autoantibodies with multiple specificities, the formation of immune complexes, and end organ failure. Even though the specific pathophysiology of SLE is unknown, autoantibodies, autoreactive lymphocytes, and environmental elements are reported to be responsible. Treatment of SLE is difficult owing to complexity of the disease, ineffective drugs, in addition to drug side effects especially immunosuppressive drugs.

Genetic & Environmental Factors Affecting Patients with SLE

Genetic variations are hereditary modifications in the sequence of DNA that can affect gene transcription and functionality, resulting in phenotypic variation and pathogenicity. More than 20 loci encoding lupus-associated genes and its chromosomal sites have been reported in lupus genetics studies. Several genes have already been related to a variety of autoimmune diseases, including SLE, however the significance level of any of these genetic variations is insufficient. Due to the obvious various factors that can be involved and the large interindividual diversity in predisposition to SLE between various populations, establishing a relationship between specific environmental factors and SLE is not an easy process. Epstein Barr virus has been related to SLE pathogenesis within biologic factors, whereas UV light exposure is an established risk factor for SLE among physical agents which mutates DNA enhancing cell apoptosis. Smoking also increases risk of SLE and the production of anti-dsDNA antibodies when it comes to chemical agents, probably since smoke promotes cellular destruction.
Innate Immune Elements in SLE

Different cellular signatures related with general disease severity, as well as distinct organ presentation tendencies, have recently been discovered using genome-wide transcription sequencing of leucocytes in the blood of patients with SLE. A steady enrichment of neutrophil transcripts was reported to be associated well with higher disease severity among the various signatures. While growing neutrophil levels due to higher glucocorticoid doses during flares may have influenced this to some level, neutrophils are regarded as important cellular constituents of SLE pathogenesis. Neutrophils die by a process known as NETosis under inflammatory conditions, and during this process, they eject so-called neutrophil extracellular traps (NETs). These extracellular traps incorporate mitochondria and DNA, which are both prominent autoantibody targets in SLE. Furthermore, extracellular traps containing neutrophil-derived mitochondrial DNA (mtDNA) activate plasmacytoid dendritic cells (pDCs) to produce interferon (IFN)-α. pDCs were identified as natural IFN producing cells so linked to SLE pathogenesis. First pDCs were thought to be reduced in the circulation of SLE patients, then proved to be accumulated in the tissues of SLE patients.

Many components of the complement system are among the most well-known humoral mediators responsible for human SLE pathogenesis. Monogenic types of SLE are known to be caused by abnormalities in the components C1q, C2, and C4. Patients with homozygous complement component deficits suffer a severe lupus-like disease at early age. The C1q receptors on the surface of phagocytes play a critical role in phagocyte clearance of apoptotic cells. C1q attaches to blebs on the surface of apoptotic human keratinocytes and is required for the correct clearance of apoptotic cells, which are thought to be the principal source of self-antigens.

B Cells and Generation of Antibodies

Extrinsic cues and variables are frequently considered for the development of autoreactive B cells in SLE. Transitional B cells with increased amounts of endogenous interferon beta (IFN-β) show increased survival and activation through the B cell receptor (BCR) during peripheral B cell growth in the spleen, a hallmark correlated with more active SLE, especially in African American patients. After passing through the transitional stage, B cells fail to preserve the integrity of tolerogenic splenic marginal zone macrophages, which are responsible for removing apoptotic cell debris in a non-inflammatory manner by upregulating indoleamine pyrrole 2,3 dioxygenase.

B-Cell Tolerance

Autoantibodies against nuclear antigens are commonly found in SLE patients and provide a pathogenic role in the disease progression. Because B cells reactive to nuclear antigens have been demonstrated to be tolerated, breaking B-cell tolerance is required to produce these autoantibodies. Expressed genes in CD4+ T cells are enriched in rheumatoid arthritis whereas expressed genes in B cells are concentrated in SLE, indicating that B cell abnormalities play a key role in the development of SLE. The most frequent autoimmune disease in mice is SLE-like disease, which is caused by genetic abnormalities in B cells.

Defects in T and B Lymphocytes in SLE

B-cell surface antigen receptors (BCRs) are made up of different combinations of immunoglobulin (Ig) heavy and light chains in the bone marrow; the most of BCRs and autoantibodies in people with SLE are made up of Ig genes and combinations that are similar to those used in normal protective antibody expression. The SLE autoantibody response shows restricted clonality (similar to antibody responses to exogenous antigens) and somatic hypermutation, indicating that antigens have activated cells. In individuals with active SLE, this results in an increase in activated B cells, memory B cells, and plasma cells. In SLE, there are multiple flaws that allow autoreactive B-cell subsets to survive.

Role of T Cells in SLE

Patients with SLE have a chronic autoimmune response to self-antigens that are widely distributed and predominantly intranuclear. The presence of autoantibodies indicates that this reaction is the source of immunological complexes and activated T cells that eventually reach target organs, causing
inflammation and injury. T cells play an important role in this process. Through cytokine release and direct cellular interaction, they aid B cells and activate antigen-presenting cells, promoting the autoimmune response.

**T Cell Mechanisms that Promote SLE**

**B Cell Assistance**

T lymphocytes mature in the thymus, this reduces the number of self-reactive T cells in the repertoire. In lupus, this mechanism is unaffected; as a result, the frequency of self-reactive T cells in the peripheral blood is not higher in lupus patients and mice than in healthy controls.

During a process that is less strict than T-cell negative selection, self-reactive B cells are eliminated from the repertoire through deletion, anergy induction, or receptor modification at two checkpoints, the first in the bone marrow and the second in the periphery. In healthy adults, this is thought to reduce the frequency of self-reactive B cells from 50-75 percent to 5-20 percent. The second checkpoint in lupus patients is abnormally porous, therefore the frequency of self-reactive mature B cells is substantially higher than in controls.

**Pro-inflammatory Activities**

The production of excessive amounts of pro-inflammatory cytokines and the expression of high levels of adhesion molecules are among the phenotypic and functional abnormalities found in T cells from patients with SLE. They enter target organs and release mediators that cause local inflammation. As a result, tubulointerstitial mononuclear infiltrates in lupus nephritis-affected kidneys are a sign of poor prognosis.

**Defective Regulation**

Regulatory T cells (Tregs) are T cells whose primary role is to suppress the immunological response. Some Tregs are selected during T-cell ontogeny and acquire constitutive suppressive capabilities (e.g., CD4+ FoxP3+ cells), whereas others are standard T cells that have completed a functional differentiation process to develop immunosuppressive capacity, primarily through cytokine production. A growing evidence suggests that autoimmune disorders, including SLE, are associated to quantitative and/or qualitative deficits in Treg, although it's still unclear if these abnormalities are causal.

d. **Apoptosis Defects**

Apoptosis is major mechanism that arrases death of activated T cells at the cessation of immune responses. The critical role of this clonal interactions is example for the lymphoproliferative and autoimmune disorders that are raised by defects in apoptosis. Patients with SLE have failed T-cell apoptosis. Moreover, they produce necrotic T cells rather than apoptotic T cells. Also, the rate of spontaneous dying of resting CD4+T cells is elevated and apoptosis of activated T cells is deficient. Together, these deficiencies could promote to lymphopenia and, paradoxically, elevated survival of activated T-cell clones.

**Follicular Helper T Cells in Patients with SLE**

Follicular helper T (Tfh) cells are a kind of effector T cell that is involved in the production of high-affinity humoral responses. They generate the cytokine IL-21 and exhibit the chemokine receptor CXCR5, both of which are essential for germinal center (GC) development. Tfh cells grow out of control in a variety of animal models of systemic autoimmune disorders, as well as in individuals with these diseases. In individuals with SLE, the frequency of circulating Tfh is linked to disease activity.

**Follicular Regulatory T Cells in Patients with SLE**

Follicular regulatory T (Tfr) cells express both Foxp3 and Bcl6 and have a mix phenotype of Tregs and Tfh cells. As Tfh cells, Bcl6 and CXCR5 are essential for localization of Tfr cells to the B cell follicle. Tfr cell functions are still obscure. Tfr cells are responsible for suppressing Tfh and GC B cells numbers during the immune response and normalizing the affinity maturation of Abs. Still, Tfr cells can also stimulate the GC and Ab response in certain models. Deficiency of Tfr cells causes enhanced Tfh cell activity leading to less-strict selection of high-affinity B cell clones and expansion of low-affinity B cell clones. Tfr cells have a key role in inhibiting
autoantibodies development and sustaining tolerance during the B cell response.\textsuperscript{25&26}

**Tfh and Tfr Cells in Autoimmune Diseases**

Tfh cells deliver signals for survival, affinity maturation, proliferation, and differentiation of GC-B cells within the follicle.\textsuperscript{27} Those signals involve IL-4 and IL-21, besides cellular interface through CD40L and programmed cell death protein 1 (PD-1).\textsuperscript{28} Tfh and GC-B cells interaction is still unclear. Typically, B cells with high-affinity effectively strive for Tfh help and differentiate into Ab-producing plasma cells after several cycles of selection, while B cells lacking Tfh help may quickly die. Numerous studies have revealed that excessive Tfh cell activity is responsible for autoantibodies production.\textsuperscript{29&30} This is possibly through excessive Tfh cell signals to self-reactive B cells causing their escape from tolerance and generation of autoantibodies.\textsuperscript{31}

Contrarywise, Tfr cells have negative regulatory effect on the GC, through several mechanisms. Primarily, Tfr cells disrupt the proliferation and interaction of Tfh and B cells in GC through cytotoxic T-lymphocyte-associated protein 4 (CTLA-4).\textsuperscript{32} Moreover, Tfr cells secrete several anti-inflammatory cytokines as IL-10 which hinders GC responses as well as humoral immunity, TGF-β that restricts Tfh cell function and accumulation, in addition to interfering with activation of autoreactive B cell and autoantibody production, and granzyme B that provokes Tfh cell apoptosis.\textsuperscript{33} Furthermore, Tfr cells impair the Tfh cell stimulatory effect on B cell by changing metabolic pathways in both Tfh and B cells, thus suppressing antibody production and preserving B cell tolerance.\textsuperscript{34} Animals lacking Tfr develop autoimmune disorders on their own.\textsuperscript{35} Hence, Tfr differentiation is crucial for immunological tolerance and changes in Tfr cells numbers and functions may increase Tfh activity and impair the negative selection of autoreactive B cell, which eventually boosts the production of autoantibodies in autoimmune diseases. Balanced functions of the antagonistic regulators of GC responses, Tfh cells and Tfr cells, are crucial for immune regulation. An imbalanced Tfh/Tfr ratio leads to disrupted GC response, uncontrolled antibody production, and autoimmune diseases.\textsuperscript{36}

Owing to the difficulty of getting secondary lymphoid organs in humans, studies relied on peripheral Tfh (pTfh) and Tfr (pTfr) cells in human autoimmune diseases. These CD4+ T cells express CXCR5 and exhibit phenotypic and functional characteristics of Tfh and Tfr cells in the lymph node, thus can be indicators of GC activity and disease progression.\textsuperscript{40&41}

**Role of Tfh and Tfr Cells in SLE**

The role of Tfh and Tfr cells in the pathogenesis of SLE has become more evident. Previous studies showed that SLE patients have an increased number of pTfh cells, that directly correlated with anti-dsDNA autoantibodies titres and disease activity evaluated by the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI).\textsuperscript{32} Others have reported a decreased frequency of pTfr cell and increased Tfh/Tfr ratio in SLE patients. Serum anti-dsDNA autoantibodies titres directly associated with frequency of pTfh cells and Tfh/Tfr ratios but inversely associated with the frequency of pTfr cells. Furthermore, the disease activity was coupled with the frequency of pTfr and Tfh/Tfr ratio but not the pTfh.\textsuperscript{33}

So far, the impact of balance between Tfh and Tfr cells in SLE remains controversial due to discrepancy of the disease, differences in studied population and methodology.\textsuperscript{42&43} Thus, imbalance of Tfh and Tfr cells may have an important influence on the pathogenesis and progression of SLE.

**New Therapeutic Strategies for SLE**

The wide-ranging adverse effects impacting SLE patients due to their lifetime use of conventional immunosuppressive drugs, mainly methotrexate, azathioprine, and cyclophosphamide, urges the need for new safer drugs. Few biological drugs have been developed based on the recent understanding of SLE pathogenesis.\textsuperscript{44}

Therapeutic agents directly affecting Tfh and Tfr cells are very limited, due to insufficient knowledge about their development and activity.\textsuperscript{34} Low-dose IL-2 therapy regulated the imbalance of Treg, Tfh, and Th17 cells along with major control of disease activity in SLE patients.\textsuperscript{45} Moreover,
low-dose IL-2 therapy has been found to augment the suppressive function of Tfr cells on Tfh cells in SLE. IL-21 has an inhibitory effect on Tfr suppressive activity. Blocking of IL-21 receptor by a fusion protein reduced the clinical progression of induced arthritis in mice. These findings support the potential role of these cytokines as therapeutic targets for SLE.

Notably, regulatory B (Breg) cell induction through anti-CD40 monoclonal antibody (mAb) may have an ultimate effect on the Tfh/Tfr cells balance; yet, further research is needed. Rituximab, a B lymphocyte anti-CD20 mAb, is a promising biological agent for SLE. When used for active SLE patients, a steady decline of the SLE activity index along with clinical enhancement were found.

Recently, great attention has been given to genetic and epigenetic control of the differentiation and functions of Tfr cells and Tfh cells. In-depth epigenetic research on the Tfr/Tfh balance can help discovering novel biomarkers and clarify their role as potential therapeutic targets for SLE.

Conclusion

A balanced interplay between Tfh cells and Tfr cells is necessary to sustain proper GC response. Dysregulation of this balance results in increased production of autoantibodies and development of autoimmune diseases as SLE. Extended understanding of the development and functions of Tfh and Tfr cells and their roles in the pathogenesis of SLE will improve the exposure of new targets for treating SLE patients.

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الخلايا الجريبية التنظيمية الثانية (Tfr) في مرض الذببة الحمراء وعلاقتها بدرجة نشاط المرض

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يعتبر مرض الذببة الحمراء (SLE) اضطراب مناعي ذاتي مزمن يتميز بوجود الأجسام المضادة الذاتية التي تسبب أضراراً للكثير من الأعضاء. تتواجد الخلايا الجريبية المنظمة بشكل أساسي في المراكز الجريثومية (GCs) وتنشأ نشاط الخلايا الجريبية الجريبية المساعدة (Tfr)، والحفاظ على تحمل الخلايا البائية وتثبيت نمو الأجسام المضادة الذاتية. يؤدي عدم انتظام الخلايا الجريبية المنظمة إلى استجابات غير طبيعية للمراكز الجريثومية وساهم في التسبب في أمراض المناعة الذاتية. ومع ذلك، لا يزال الدور الدقيق للخلايا الذاتية الجريبية المنظمة في مرض الذببة الحمراء غير واضحًا. إن المقال المراجع الحالي يركز على الأسس المناعية في Tfr واضطراب توازن الخلايا الجريبية المنظمة Tfr والخلايا الجريبية المساعدة Tfr في أمراض المناعة الذاتية، وخاصة مرض الذببة الحمراء. بالإضافة إلى الاستراتيجيات العلاجية الجديدة لمرض الذببة الحمراء.

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 últimos cambios en la versión más reciente de la ley. Es importante entender que aunque esta reforma ha sido un paso importante en la protección de los derechos de los trabajadores, aún quedan áreas en donde se necesita más avance. El futuro de esta materia depende de la capacidad de las autoridades para implementar eficazmente estas nuevas leyes, así como la capacidad de los trabajadores para defender sus derechos. 