Therapeutic CD154 antibody for lupus: promise for the future?

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Systemic lupus erythematosus (SLE) is a prototypical systemic autoimmune disease characterized by the production of pathogenic autoantibodies. A new study (see the related article beginning on page 1506) demonstrates that passive antibody specific for the TNF family member, CD154, ameliorates disease by reducing levels of self-reactive antibody in the serum. This study demonstrates a substantial potential for anti-CD154 antibody in the treatment of humoral autoimmunity.


The autoimmune disease systemic lupus erythematosus (SLE) is characterized by the production of high-affinity IgG antibodies against double-stranded DNA (dsDNA). Typically, these pathogenic autoantibodies are encoded by mutated Ig genes, and the frequency and pattern of mutations strongly suggest that the source of autoreactive antibodies is B cells that have participated in the germinal center (GC) reaction. Indeed, GCs appear spontaneously in many strains of lupus-prone mice (1), and extraordinary numbers of GC B cells and their progeny are present in the blood of SLE patients (2).

GCs are organized collections of antigen-activated T and B lymphocytes in secondary lymphoid tissues (3) or sites of chronic inflammation (4, 5). Although transient GC-like reactions can be elicited by thymus-independent pathways (6–8), most GC responses are thymus-dependent. GC B cells require ongoing survival and proliferation signals that depend on CD154–CD40 signaling (9). CD154, a member of the TNF family, is inducibly expressed on the surface of CD4 T lymphocytes, whereas its ligand, CD40, is constitutively present on B lymphocytes (reviewed in ref. 10).

T cell–dependent GCs begin with the separate activation of T and B cells by antigen (11). In secondary lymphoid tissues, binding of antigen modifies B cell chemotaxis, resulting in migration toward the T cell zone (12), where cognate T cell–B cell interaction expands both lymphocyte populations. Soon afterward, activated T and B cells emigrate from T cell areas into the reticula of follicular dendritic cells (FDCs) that define the B cell follicle (Figure 1). The immigrant B lymphocytes proliferate in the FDC reticulum to generate nascent GCs and, in the process, acquire distinctive phenotypes, including expression of the CD69 activation antigen and several differentiation markers, including CD38 and CD27. Remarkably, human CD38+ GC B cells can also express variable levels of CD154, especially under conditions of chronic stimulation (13, 14), and appear to have the capacity for continued self-activation.

GCs become polarized into histologically dark and light zones (DZ, LZ). The DZ is proximal to the T cell area and contains rapidly dividing B cells called centroblasts that express little or no surface immunoglobulin. The more distal LZ contains the bulk of the activated FDC network, antigen-specific CD4 T cells, and nondividing B cells known as centrocytes. Centrocytes express surface immunoglobulin and are thought to be the progeny of DZ centroblasts. In turn, selected centrocytes likely reenter the DZ and regain the centroblast form.

The enzyme AID (activation-induced cytidine deaminase) drives both somatic hypermutation (SHM) and immunoglobulin class switch recombination (CSR) in GC B cells (15). SHM introduces point mutations and occasional small deletions into the

V(D)J regions of transcriptionally active immunoglobulin genes. Mutations are probably introduced in centroblasts and accumulate during repeated rounds of centroblast/cenetrocyte migration between the DZs and LZs (Figure 1). Intense selection for higher-affinity mutants establishes GCs as foci of rapid somatic evolution and antibody affinity maturation. CSR ensures that mutated, high-affinity antibodies acquire various effector activities and can be delivered throughout the body.

With time, centrocytes leave GCs and enter into at least two phenotypically distinct compartments of memory cells (Figure 1). One compartment, comprising antigen-specific, small, resting B lymphocytes, defines the classical memory B lymphocyte. The other contains long-lived plasmacytes that secrete high-affinity antibody and are capable of maintaining significant levels of serum antibody for years, if not decades. Characteristically, fully differentiated human plasmacytes express high levels of CD38.

**Passive CD154 antibody therapy for lupus**

In this issue of the JCI, Amrie Grammer and colleagues demonstrate that administration of a humanized monoclonal antibody specific for CD154 (16) produced significant clinical benefit in a small group of active SLE patients (17). Anti-CD154 promptly decreased levels of dsDNA serum autoantibody and also diminished the numbers of GC B cells and plasmacytes circulating in the blood. While dsDNA antibody levels rebounded after treatment, other objective measures of disease including proteinuria and scores on a standardized index of SLE activity (SLEDAI) remained significantly suppressed over a 20-month post-treatment period. These promising results are tempered by the fact that this clinical study was prematurely ended due to adverse side effects (16). Nonetheless, the substantial clinical improvements observed by Grammer et al. (17) observe that passive anti-CD154 reduces the numbers of IgD+CD38+ GC precursors (I and II) and CD38high plasmacytes (III and V) but has little effect on circulating GC (IgD-CD38+) cells (IV). The primary effect(s) of CD154 antibody in SLE patients may be to interrupt the early initiation (I) and/or migration steps (II) of the GC reaction.

**Potential cell targets for anti-CD154**

There are at least three stages of humoral immune responses in which interruption of CD154-CD40 signals can abrogate the GC reaction and antibody responses (Figure 1). Anti-CD154 can (i) interrupt the initial, cognate interaction between antigen-
specific T and B lymphocytes within and/or adjacent to the T cell areas of lymph nodes or spleen. Blockade of this interaction prevents both the formation of GCs and the initial production of plasmacytes. Later in the response, anti-CD154 disrupts GCs either by (ii) preventing homotypic CD154-CD40 interaction among proliferating DZ centroblasts; or (iii) interfering with collaboration between LZ T cells and centrocytes. Of note, Grammer et al. (17) find that anti-CD154 elicits relatively little change in the numbers of IgD-CD38- blood cells in SLE patients but rather causes substantial reductions in the numbers of IgD-CD38+ cells and CD38high plasmacytes. Reductions in these pre-switch GC cells and plasmacytes correlate with reductions in autoantibody titers and DNA-specific blood plasmacytes (16). The biased effects on pre-switch cells and plasmacytes suggests that the primary benefit of anti-CD154 treatment in SLE may be the disruption of plasmacyte generation/differentiation, perhaps at a point before B cells become fully differentiated into centroblasts or centrocytes.

Even though passive CD154 antibody did not reduce the number of IgD-CD38+ GC cells in the blood of SLE patients, the ability to abrogate active GC responses remains an attractive feature of this therapy (17). In SLE, IgG autoantibodies against dsDNA appear to descend from B cell clones that do not react to DNA. These pathogenic autoantibodies arise by SHM, not in response to DNA but to some other antigen or by some nonantigenic stimulus that supports SHM. In bone marrow, autoreactive cells generated during B lymphocyte development are deleted by apoptosis (18), enfeebled (19), or given a new reactivity by receptor editing (20). SHM in GC B cells must occasionally produce autoreactive antigen-receptor absents from the tolerizing environment of the bone marrow. Although GCs may possess the capacity to remove self-reactive lymphocytes by apoptosis (21), this process does not appear particularly efficient (22). Thus, anti-CD154 therapy may carry the additional benefit of halting the generation of autoreactive B cells or their affinity maturation into pathogenic forms.

Regardless of where, or even how, passive antibody to CD154 acts to reduce SLE disease, Grammer and colleagues have taken an important step toward defining the promise of CD154-CD40 interaction as a target of therapy (17). The future is not clear — the antibody used in these studies elicited unacceptable side effects — but the direction of study has been clearly defined. If different autoimmune diseases represent distinct errors of immune regulation, analogous approaches to other therapeutic targets are surely in store.