THERAPEUTICS TO BLOCK AUTOANTIBODY INITIATION AND PROPAGATION IN SYSTEMIC LUPUS ERYTHEMATOSUS AND RHEUMATOID ARTHRITIS

Chapter 13

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ABSTRACT

Most current therapies for the autoimmune diseases systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA), as well as many of the drugs in the therapeutic pipeline, reduce the autoimmune inflammatory process but lead to a general immunosuppression. The goal of the next generation of therapies should be to reduce autoimmunity while at the same time maintaining immunocompetence. We propose three approaches for accomplishing this goal: (i) modulate antigen presentation to the adaptive immune system, (ii) alter B cell selection in the germinal center and (iii) use decoy antigens to prevent the formation of proinflammatory immune complexes.

These approaches are based on recent advances in the field: We now appreciate the role of dendritic cell function in autoimmune disease and the importance of citrullinated proteins as neoantigens in RA. There is also new recognition that most pathogenic autoantibodies are produced by B cells that have matured within the germinal center and that immune complexes in both diseases contain ligands for toll like receptors. We propose that treatments that target these newly revealed aspects of RA and SLE will decrease systemic inflammation without immunocompromising patients.

INTRODUCTION

Systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA) are autoimmune diseases that affect approximately 0.1 and 1 % of the population, respectively. Because individuals in early to mid adulthood are affected by these diseases, their associated morbidity constitutes a considerable economic burden and diminishes quality of life over decades. Moreover, both diseases are associated with early death. Although the prognosis for these diseases has improved substantially over the past decades, overall outcome remains inadequate. Even while on a therapeutic regimen, most patients continue to be symptomatic, and current treatments produce undesirable immunosuppression. For these reasons, there is a number of novel therapeutics under development. Hopefully, some of these will demonstrate dramatic benefit and low toxicity. Here, we propose concepts for therapy that we believe are closely aligned with disease pathogenesis.

In considering the next generation of therapeutic regimens, it is important to consider the full spectrum of therapeutic goals. Certainly, we need to protect against irreversible tissue damage, primarily in the kidney and brain in SLE, and in joints in RA. We also need to prevent the accelerated atherosclerosis that occurs in both SLE and RA and which is believed to be consequent to an ongoing systemic inflammation, which remains active

despite current treatments. We also need to maintain immunocompetence while suppressing autoreactivity and systemic inflammation. Ultimately, an ideal therapy would effect sufficient immunomodulation that patients experienced a prolonged disease-free, drug-free existence, despite their genetic predisposition to disease.

One important therapeutic question is: When do we intervene? In principle, it would be best to intervene at a preclinical stage of disease when there is evidence of abnormal immune activation, but no clinical disease and no detectable target organ injury. This would obviate the need to understand the nature of tissue injury and the contribution of tissue-resident cells to organ damage. Preventive strategies for RA are currently being tested in a clinical trial but they may necessitate treating individuals who may never develop disease with potentially toxic agents. For example, first degree relatives of autoimmune patients can exhibit elevated titers of disease-associated autoantibodies or aberrant cytokine profiles, but most will never develop disease. Individuals with anti-citrullinated protein antibodies (ACPA) who present with painful joints (arthralgia) or undifferentiated arthritis, however, have a high risk of RA. For these people, immunomodulation might prevent full-blown disease.

Treatment after the onset of clinical symptoms needs to ameliorate disease by inhibiting tissue injury, reducing active inflammation and maintaining prolonged immune inactivity in lymphoid tissue. Strategies designed to reduce organ damage would differ considerably from treatments that induce immune quiescence. Moreover, their use would be intermittent, at times of tissue inflammation but not during the prolonged periods of systemic inflammation that are thought to contribute to accelerated cardiovascular disease. In contrast, therapeutics that decreases ongoing activation of autoreactive cells when target organ inflammatory is under control could reduce systemic inflammation without suppressing immunity against pathogens, and prevent acute exacerbations and further organ damage.

To successfully modulate immune responses in SLE and RA, we must understand what cellular interactions or molecular pathways are aberrantly regulated in these diseases. Gene expression analyses based on genome wide association studies (GWAS) suggest that dendritic cell (DC) and B cell intrinsic pathways may be dysregulated in SLE, and T cell intrinsic pathways dysregulated in RA (1). Nonetheless, each disease is promoted by an aberrant repertoire of adaptive immune cells, triggered, we propose, by altered antigen presentation and abnormal B cell selection in the germinal center (GC). Disease activity is sustained, in part, by the pro-inflammatory properties of the disease-specific immune complexes. We suggest that these pathways of disease initiation and propagation will be the targets of next generation therapies.

Should we explore antigen-specific therapeutic interventions or more global immune modulation? The answer may depend on the disease. In SLE, there are diverse autoantibodies and the T cell autospecificities have not been clearly defined. Thus, we may not yet have adequate knowledge of the pathogenic T and B cells in this disease, and targeting cells specific for one or two antigens may not be sufficient. Thus, we favor a global immuno-modulatory therapeutic approach for SLE. In RA, it may be time to explore antigen-specific therapy targeted to citrullinated proteins. Although ACPA are present in only 70% of patients, they clearly contribute to systemic inflammation and to tissue damage.

INITIATION OF AUTOIMMUNITY

DCs are key controllers of immune responses that present both self and foreign antigens to T cells in either a tolerogenic or immunogenic fashion, thereby delicately balancing immune activation and tolerance (2). As SLE and RA are both associated with autoantibodies produced through T cell – B cell collaboration, the DC is thought to play a major role in the initiation of such responses. The regulatory function of DCs is affected by environmental cues such as the microbiome, prior pathogen exposure and cytokines. Thus, mature DCs are stimulated by pattern recognition receptors to upregulate co-stimulatory molecule expression and drive effector T cell responses against pathogens, while immature DCs or DCs exposed to apoptotic cells normally induce T cell anergy or promote regulatory T cells (Treg) differentiation through secretion of cytokines such as TGF and IL-10 (3, 4). Therefore, the activation and differentiation pathways of autoreactive T cells depend on the exact nature of DC activation and presentation of self peptides. Treatments that inhibit DC activation or change the peptides presented by HLA class II molecules could represent new avenues to prevent the initiation of autoimmunity.

MODULATING DC ACTIVATION.

A variety of receptors, including pattern recognition receptors, complement receptors, and Fc receptors can modulate activation of DCs by self antigen (5, 6). DCs express several inhibitory receptors, including LAIR1, a recently identified receptor for the complement component C1q (7). C1q deficiency is a monogenic risk factor for SLE development (8), and low levels of C1q correlate with increased disease activity (9). C1q prevents autoreactivity in at least two ways. It acts as an opsonin of apoptotic cells to facilitate clearance of cell debris, and it regulates DC maturation and cytokine production (10, 11). C1q blocks DC differentiation from monocytes through LAIR1 (7). Monocyte-derived DCs are

immunogenic; after stimulation, they express high levels of co-stimulatory molecules and secrete proinflammatory cytokines, which determine T effector cell phenotype (12). Monocytes from SLE patients show accelerated differentiation (13). C1q also inhibits activation of conventional DCs (cDCs) and plasmacytoid DCs (pDCs) by preventing the engagement of endosomal toll like receptors (TLRs)by immune complexes that contain TLR ligands (14).

Thus, C1q and C1q-like agonists could be potent therapeutics to prevent activation of monocytes, cDCs and pDCs, thereby reducing the proinflammatory, proimmunogenic milieu in autoimmune patients. Additional strategies to modulate activation of DCs may emerge through studies of the microbiome and pathogen-induced immune deviation (15-17), but the data are not yet sufficient to enable the design of therapeutics.

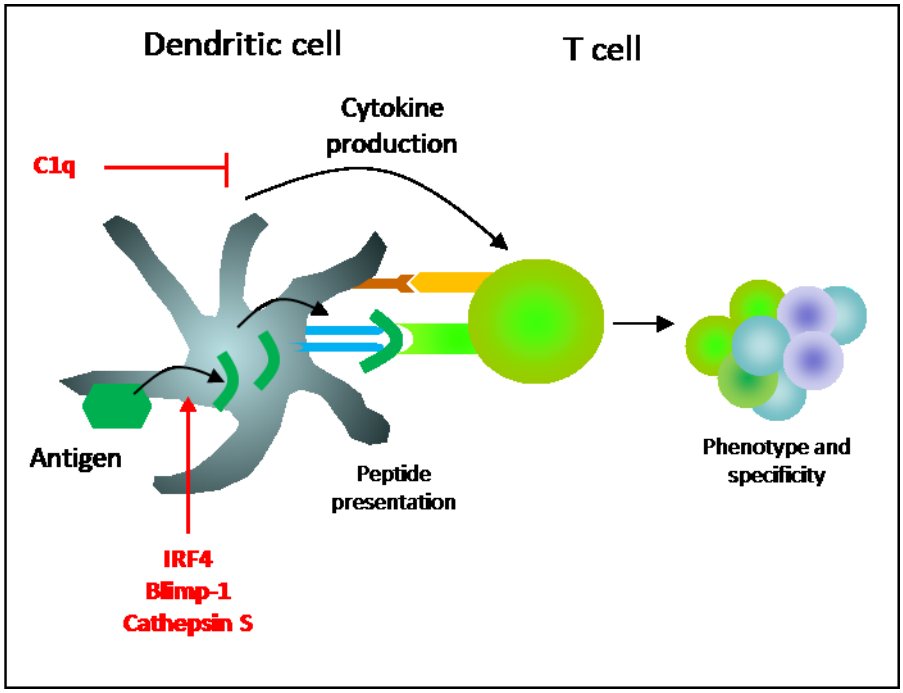

Figure 1. Targets for next-generation therapeutics: DC–T cell interactions. C1q or other ligands for inhibitory receptors on DCs can modulate DC activation by blocking the up-regulation of costimulatory molecules and cytokine production (top red arrow), modulating T cell phenotype and activation. Altering the levels or activity of IRF4, Blimp-1, and cathepsin S (bottom red arrow) could change the peptides presented by DCs through HLA, modulating T cell specificity.

DC ANTIGEN PRESENTATION (MODULATING T CELL REPERTOIRE).

SLE and especially RA have a strong association with certain HLA-DR risk alleles, suggesting that presentation of particular antigens to CD4+ T cells are crucial components of the initiation of autoreactive responses (18). Transcription factors IRF4 and Blimp-1 and the lysosomal protease cathepsin S normally participate in the process of antigen presentation (19). Cathepsin S degrades the MHC class II invariant chain to allow peptide loading to the MHC II molecules in antigen presenting cells (20, 21). Cathepsin S also degrades endo-lysosomal antigens, shaping the spectrum of peptides presented on DCs (22, 23). As cathepsin S has both positive and negative effects on T cell selection in the thymus and the periphery, its abundance needs to be tightly controlled (24). For example, too much cathepsin S in the thymus can destroy immunodominant epitopes of self antigens, leading to escape of autoreactive T cells from negative selection (25). Low Blimp-1 levels in DCs, as occurs in individuals with the SLE Blimp-1 risk allele, can lead to elevated cathepsin S (26). A therapeutic agent that diminishes autoantigen presentation and autoreactive T cell differentiation by modulating levels of Blimp-1, IRF4 or cathepsin S could be an effective strategy against autoimmunity that would not result in global immunosuppression. This approach could be effective even without knowing the self peptides that drive autoreactive T cells in SLE.

Antigen presentation of specific peptides not only initiates autoreactive T cell responses; it also contributes to the production of autoantibodies. This role in autoantibody generation is evidenced by the fact that patients with ACPA+ and ACPA- RA tend to have different HLA class II risk alleles, and recently citrulline-specific effector T cells were shown to be present in those individuals carrying the HLA risk allele associated with ACPA+ RA (27, 28). (Citrulline-specific T cells are also present in healthy individuals, but there these CD4+ T cells exhibit a Treg phenotype. In RA patients, citrulline-specific T cells have a proinflammatory phenotype, suggesting that additional genetic or environmental risk factors allow these T cells to differentiate to effector cells in the periphery where inflammation induces the citrullination of proteins.

Numerous intracellular and extracellular proteins undergo post translational citrullination, with extracellular proteins undergoing citullination primarily in an inflammatory milieu. The exact citrullinated epitopes that activate T cells in RA has only recently begun to be revealed. More knowledge on the processing and presentation of citrullinated antigens in the thymus and periphery is needed before we can design ways to modulate the T cell repertoire in RA patients by inhibiting presentation of citrullinated antigens to CD4+ T cells. Even now, however, we might consider decreasing the formation of citrullinated proteins by interfering with the expression or function of the peptidylarginine deiminases (PAD) that catalyze citrulline formation, especially PAD4, which is found in inflammatory synovium.

PROPAGATION OF AUTOIMMUNITY

Although autoreactive B cells can be directly activated by self-antigens, B cell survival and the generation of memory B cells and long-lived plasma cells in the GC are promoted by proinflammatory cytokines from immunogenic DCs and require help from T follicular help (Tfh) cells (29). Autoantibody-containing immune complexes feed forward to inflammatory responses by activating complement receptors and Fc receptors on DCs and other innate immune cells (30). Furthermore, immune complexes deposited on follicular DCs could serve as a self-antigen reservoir that sustains autoreactive GC reactions (31). Interventions that can break down this detrimental feedback mechanism will be a part of the future therapeutic armamentarium for SLE and RA.

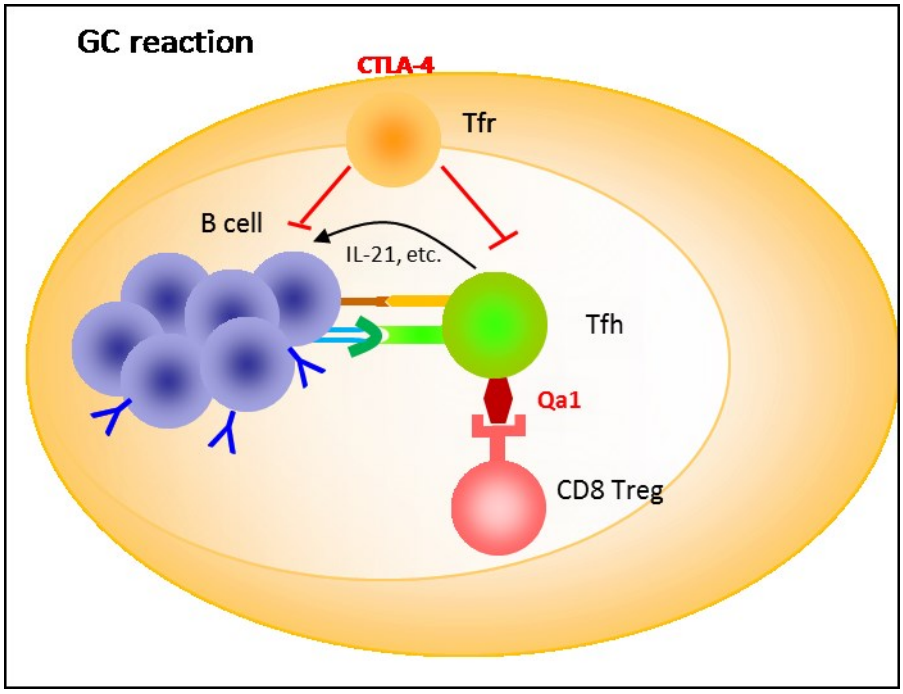

Figure 2. Targets for next-generation therapeutics: GC reactions. The interaction of follicular T helper cells and B cells in the GC leads to propagation of autoimmunity. Enhancing the function of Treg cells in the germinal center may diminish this process. Qa1-restricted $CD8^+$ Treg cells (red cell) and CTLA-4 expressing T follicular regulatory cells can inhibit these GC reactions.

In the GC, antigen-activated B cells undergo random somatic hypermutations in immunoglobulin genes that can cause an increase or a decrease of affinity of B cell receptors. The rate of hypermutation and cell division is proportionally increased with the amount of antigen captured and presented by GC B cells to Tfh cells (32). Thus, B cells

expressing higher affinity B cell receptors are selected to expand and differentiate into memory B cells or long-lived plasma cells. B cells can also gain de novo self-reactivity through the process of hypermutation. These autoreactive B cells normally undergo negative selection, a result of the lack of cognate Tfh cells. But in both SLE and RA, somatic mutations contribute significantly to the generation of autoreactive memory B cells, suggesting that they interacted with Tfh cells in a GC response (33, 34).

Autoreactive B and T cells are normally suppressed by regulatory cells. The regulatory cell population is highly heterogeneous, including Foxp3+ CD4 Treg, Qa-1 restricted CD8 Treg cells and IL-10- and IL-35-producing regulatory B cells (35-37). Even the CD4 Tregs are comprised of functionally diverse subsets that selectively regulate Th1, Th2 or Th17 responses (38, 39). Therefore, the enhancement of a specific subset of regulatory cells in SLE or RA patients may suppress certain GC reactions without globally affecting immunity. For example, Qa-1 is specifically expressed by Tfh cells, and Qa-1 restricted CD8 Tregs inhibit spontaneous GC reactions by targeting autoreactive Tfh cells (40). Reduced numbers and dysfunctional CD4 Tregs have been reported in SLE and RA patients (41, 42).

Whether other regulatory cells are perturbed in SLE or RA patients has not been well studied. More effort is required to develop agents that boost a specific population of regulatory cells and maintain their function in chronic inflammatory condition in SLE and RA patients. One specific Treg subset found in GCs --- T follicular regulatory cells (Tfr) --- inhibit Tfh differentiation and GC B cell responses; their inhibition of Tfh requires expression of CTLA-4 (43, 44). CTLA-4-Ig fusion proteins (abatacept), which block CD28 binding to CD80 and CD86 and thus preventing second signal for T cell activation, have been successfully used to treat RA (45). Abatacept has not been able to prevent flares in SLE patients; howeverthis may be because SLE patients already have increased serum levels of soluble CTLA-4. Moreover, a major B cell tolerance checkpoint is in the GC where a specifically targeted CTLA-4 agonist may boost Tfr function and suppress autoreactivity in GC B cell responses in SLE.

NEUTRALIZATION OF AUTOANTIBODIES

Because autoantibodies are thought to be critical for the pathogenesis of SLE and RA through their generation of immune complexes, another way to neutralize the proinflammatory effects of those complexes without affecting the immunocompetence of patients would be the use of decoy antigens.

In SLE, anti-DNA antibodies contribute to kidney disease and neurotoxicity by through their cross-reactivity to glomeruli and neurons. Moreover, the DNA-containing immune

complexes can activate TLRs and thereby in turn activate myeloid cells and permit DNA-reactive B cells to escape tolerance mechanisms (46). We have developed a small molecule peptidomimetic that prevents the anti-DNA antibody from binding to target organs and from activating TLR 9 (47, 48). Such mimetics are ideal agents to treat autoimmunity as they specifically suppress autoantibody-mediated tissue injury and autoantibody-mediated systemic inflammation, without inhibiting immunity against pathogens.

In RA, citrullination is a central event in the pathogenesis of ACPA+ RA, contributing to both T- and B-cell activation, and induction of chronic inflammation through formation of ACPA immune complexes, which can activate Fc receptors and TLR4 (49, 50).

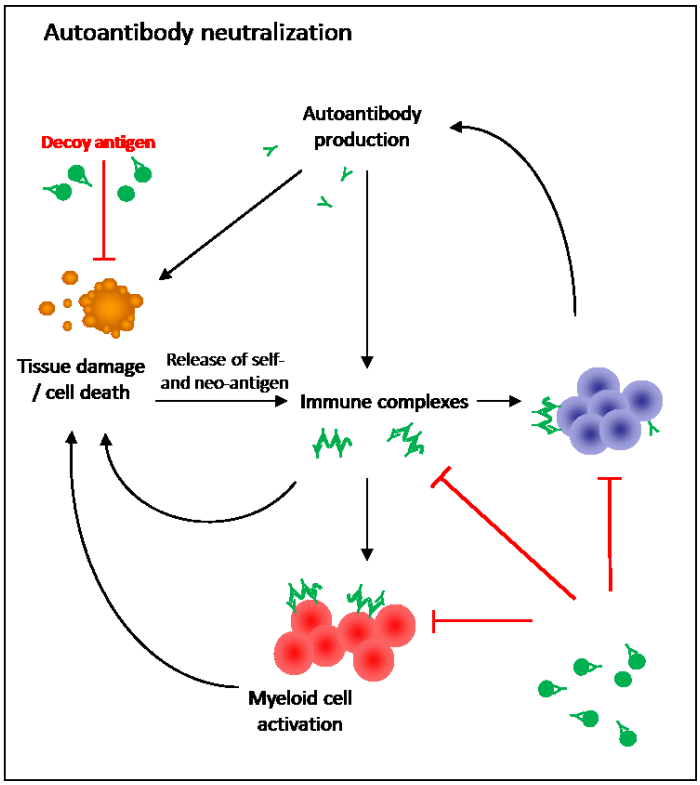

Figure 3. Targets for next-generation therapeutics: Autoantibody neutralization. Autoantibodies induce a chronic cycle of events leading to cell and tissue damage, either through direct effects on tissue or through formation of immune complexes. Decoy antigens can inhibit each of these processes by reducing antibody binding in tissues, inhibiting formation of immune complexes, and interfering with B cell activation.

The use of decoy antigens for citrullinated peptides in RA could reduce activation of myeloid cells, thereby directly reducing inflammatory responses in the joint. Activation of myeloid cells by ACPA can lead to cell death and release of PAD enzymes or citrullinated proteins in the joint (51, 52). This drives an incessant cycle of antigen recognition, immune activation, and further release of PAD enzymes and creation of citrullinated antigens in the joint. Decoy antigens mightn block the formation of inflammatory ACPA immune complexes, thereby preventing myeloid cell activation. In addition, ACPA-producing

plasmablasts are continuously generated in the joint, suggesting that B cells are activated to undergo terminal differentiation to plasma cells by joint-localized citrullinated antigens. Decoy antigens could compete with citrullinated antigens for binding to the B cell receptor; by inhibiting crosslinking of the B cell receptor, these decoys could block B cell activation as well as prevent generation of autoantibody producing plasma cells.

Citrullinated proteins also occur in atherosclerotic plaques (53). Therefore, prevention of ACPA binding to citrullinated targets may reduce the atherosclerotic complications of chronic inflammation in RA patients, in addition to decreasing acute inflammatory responses in the joint.

PRECISION MEDICINE

In both SLE and RA, patients vary in the exact constellation of signs and symptoms that they present, likely reflecting differences in underlying genetic risk and pathogenic pathways. Therefore, the approaches presented here should be applied in a patient-specific manner. For example, modulation of DC activation and antigen presentation may be most useful in SLE patients with a Blimp-1 risk allele, and modulation of the processing of citrullinated antigens in RA may be most effective in those harboring the HLA shared epitope alleles.

As more is learned of autoimmune disease pathogenesis, our ability to modulate the immune response will become more refined. The approaches suggested here represent a starting point. Modulation of antigen presentation and the GC response will interfere with key events in the induction of autoimmunity. Employing decoy antigens can lessen the proinflammatory milieu. Agents that can be used in these strategies are available and deserve serious consideration.

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