B Cells in Health and Disease

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B cells play a key role in regulating the immune system by producing antibodies, acting as antigen-presenting cells, providing support to other mononuclear cells, and contributing directly to inflammatory pathways. Accumulating evidence points to disruption of these tightly regulated processes in the pathogenesis of autoimmune disorders. Although the exact mechanisms involved remain to be elucidated, a fundamental feature of many autoimmune disorders is a loss of B-cell tolerance and the inappropriate production of autoantibodies. Dysfunctional immune responses resulting from genetic mutations that cause intrinsic B-cell abnormalities and induction of autoimmunity in the T-cell compartment by B cells that have broken tolerance may also contribute to these disorders. These findings provide the rationale for B-cell depletion as a potential therapeutic strategy in autoimmune disorders and other disease states characterized by inappropriate immune responses. Preliminary results with the CD20-targeted monoclonal antibody rituximab indicate that rituximab can improve symptoms in a number of autoimmune and neurologic disorders (including rheumatoid arthritis, systemic lupus erythematosus, and paraneoplastic neurologic syndromes). Additional studies are warranted to further characterize the role of B cells in autoimmune diseases and the therapeutic utility of B-cell depletion.

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APC = antigen-presenting cell; IL = interleukin; RA = rheumatoid arthritis; SLE = systemic lupus erythematosus

A fully functional immune system is essential for self-preservation and good health. Healthy immune responses have several key defining attributes, including specificity in recognition of foreign bodies or antigen and mobilization of an appropriate response. A healthy immune system has a highly discriminative ability to recognize "self" from "nonself," is able to respond vigorously on initial encounter to a pathogenic antigen, and uses memory to increase the speed of subsequent responses to antigens that have been encountered previously. The system must also be self-limiting for normal immune responses.

Immune responses are orchestrated by a complex, continually evolving cooperative network of mobile cells and their products.[1,2] The high degree of complexity and interdependency among the many components of the immune system is such that it is possible for dysfunction to occur as a result of either an identifiable insult or an unknown trigger. Consequently, not all immune responses are protective; some can result in inflammatory processes, tissue destruction, and the development of autoimmune disease.[3]

Our understanding of the mechanisms involved in normal immune responses has increased substantially during the past 2 decades. One of the most important insights to emerge has been an increased appreciation of the roles that B cells play in regulating the immune system in the preservation of health. In addition to producing antibodies, critical immunoregulatory roles for B cells have been described, including direct effects on the behavior of other cells in the immune system[4] or indirect effects through antigen presentation and the production of cytokines.[5-7] Furthermore, data from animal models demonstrate that autoimmune B cells can drive responses when none should occur, resulting in disease. Elucidation of the pathways of B-cell activation raises the possibility of targeting B cells in the treatment of autoimmune disorders such as rheumatoid arthritis (RA), systemic lupus erythematous (SLE), and autoimmune neurologic disorders.[8-10]

This review examines the characteristics of B cells, including their proliferation and differentiation, and their functions in maintaining overall health. Possible mechanisms by which disordered B-cell functions can contribute to autoimmune responses and disease are discussed. Finally, the case for investigating B cells as a therapeutic target in autoimmune disease and evidence of the therapeutic benefits of B-cell depletion in autoimmune disorders are summarized.

THE IMMUNE SYSTEM AND B CELLS: FORM AND FUNCTION

Lymphocytes and antigen-presenting cells (APCs) constitute the adaptive immune system that responds to specific immune challenges, such as foreign microorganisms, and also have the potential for autoimmunity.[11,12] B lymphocytes are derived from the bone marrow, and they mature through sequential, programmed steps (Figure 1). Hematopoietic stem cells in the bone marrow mature into pro-B cells, pre-B cells, and then immature B cells. These cells enter the blood as transitional B cells and migrate to sec-
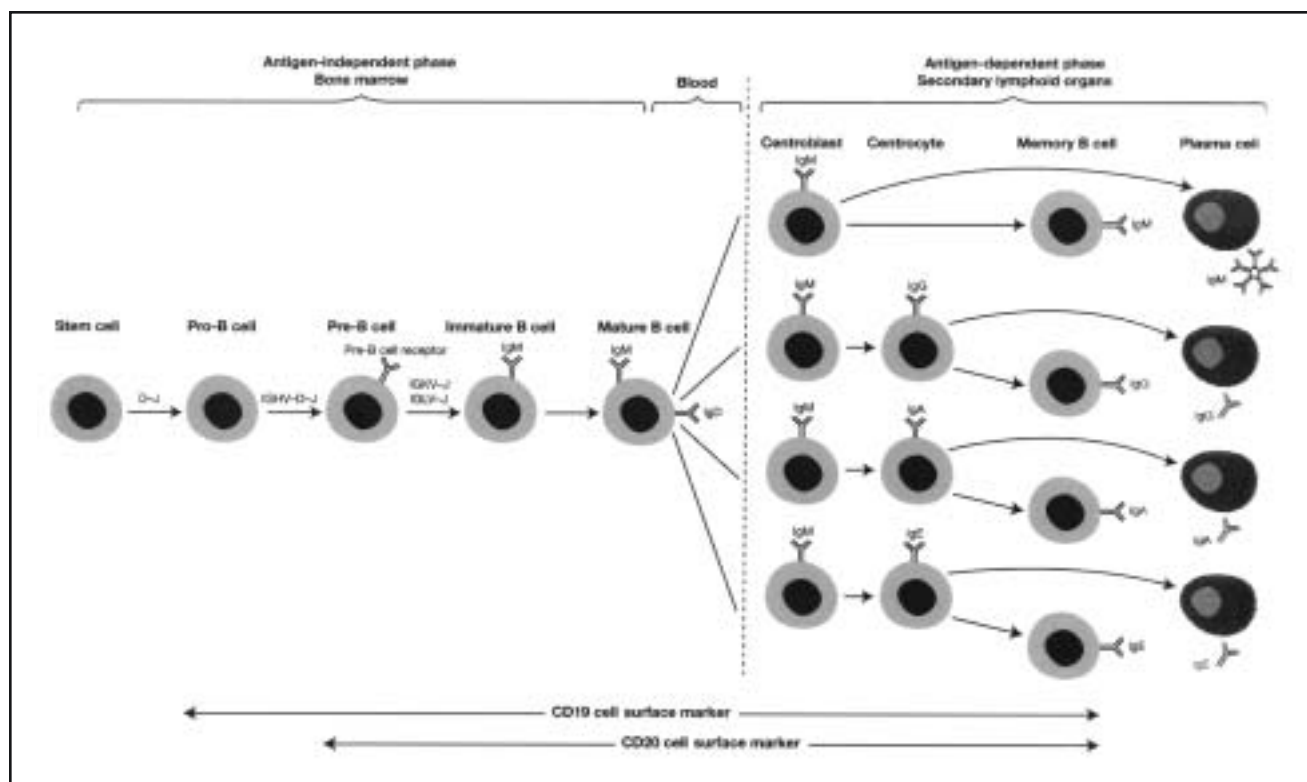
FIGURE 1. B-cell differentiation. D = diversity region; IGHV = immunoglobulin heavy chain variable region; IGKV = immunoglobulin κ light chain variable region; IGLV = immunoglobulin λ light chain variable region; J = joining region. Adapted from IMGT, the international ImMunoGeneTics information system,[13] with permission.

ondary lymphoid organs. The cells that survive past this stage become naive B cells in the periphery. Some peripheral B cells appear poised to mount a rapid but low-affinity antibody response to typical bacterial antigens, such as cell wall components. Other B cells, particularly after exposure to protein antigens, require T-cell help, generally in a structure called the germinal center, where B cells that express higher-affinity antibodies are selected and expand. Both the rapid antibody response and the T-cell–dependent pathways are regulated by APCs of different lineages. The B-cell products of the germinal center may differentiate into memory B cells or via plasmablasts into antibody-producing plasma cells. Plasma cells produce and secrete soluble antibody that is reactive with the activating antigen, whereas memory B cells carry membrane-bound antibody and are poised to mount a rapid and heightened response to subsequent antigen exposure.[11] Terminally differentiated plasma cells may survive and produce antibody in the bone marrow for years. However, the relationship among plasmablasts, memory B cells, and long-lived plasma cells remains obscure.

Clonal selection of B cells occurs during differentiation, with each clone expressing a specific antibody molecule in a membrane form (although there are exceptions). Clonal selection underpins immunobiologic memory and allows expansion of the relevant clone when reexposure to a specific antigen occurs. To cope with the challenge of a potentially limitless number of antigens, a correspondingly large number of clones are required to maintain health. This is achievable because the genes in lymphocytes that code for antigen receptor proteins can combine in a vast number of arrangements.[11] Additional antibody diversification occurs via somatic mutation of antibody during clonal expansion in the periphery, which appears to occur at a high frequency.[14,15] However, these processes also generate self-reactive molecules, creating a potential problem. The immune system has evolved such that the binding of antigen, whether self or foreign, to the membrane form of immunoglobulin (which serves as the antigen recognition receptor for the B cell) is insufficient to induce the B cell to produce antibody. Rather, the B cell must receive additional activation signals, such as binding of cytokines, ligation of costimulatory receptors on the B cell by counter receptors on activated T cells, or binding of receptors on B cells (eg, Toll-like receptors), that recognize molecular motifs specific to certain types of pathogens. Only then will the B cell

differentiate into an antibody-producing cell. Some experimental support exists for the concept that the ability to discriminate between "self" and "nonself" involves learning to respond aggressively when there are signals that suggest the presence of invasive pathogens and having effective regulatory mechanisms for suppressing inflammatory responses when such signals are absent.[16] In this context, autoimmunity could result from intrinsic B-cell abnormalities that bypass the need for extrinsic activation signals and/or from intrinsically normal B cells responding to inappropriate activation signals generated by the innate system.

B-CELL IMMUNOLOGIC FUNCTIONS: INDEPENDENT OF ANTIBODY PRODUCTION

In addition to producing antibodies, B cells can act as efficient APCs to stimulate T cells[4,17] and to allow optimal development of memory in the CD4^{+} T-cell population.[18] Compared with nonspecific uptake associated with professional APCs, selective uptake of antigen by antigen-specific B cells is markedly superior, with up to 1000-fold or greater efficiency.[17,19] Whereas other APCs take up antigen as a sampling of the extracellular environment through pinocytosis or through internalization of receptors for immune complexes, B cells capture and internalize only the antigen recognized by the membrane form of immunoglobulin that serves as the specific antigen receptor for each B cell. The internalized antigen is broken down into peptides in lysozomes, some of which bind to major histocompatibility complex class II molecules. The peptide/major histocompatibility complex class II molecules are then transported to the surface of the B cell for presentation to CD4^{+} helper T cells. As a result, the repertoire of B cells can determine which antigen is presented to T cells, particularly when the antigen concentration is low.

Furthermore, B cells produce cytokines, notably interleukin (IL) 4, IL-6, IL-10, and tumor necrosis factor α, which have regulatory effects on antigen-presenting dendritic cells or support the survival of other mononuclear cells.[5] In animal models, polarizing cytokines (IL-4, IL-10, and interferon-γ) produced by B cells are known to regulate the differentiation of T cells,[20] suggesting that the production of cytokines by B cells may also regulate immune responses to infectious pathogens. B cells can both generate and respond to chemotactic factors responsible for leukocyte migration and therefore have a major contributory role in mediating inflammatory cell infiltration processes. Additionally, B cells can synthesize membrane-associated molecules that help and support adjacent T cells.[19,21]

Collectively, these properties show that B cells are important players in combating infection by producing anti-

bodies, serving as APCs, providing help and support to other mononuclear cells, and contributing directly to inflammatory processes (Figure 2). When all these activities are appropriately coordinated and tightly regulated, the immune response is kept well honed and poised to respond. However, dysfunction may occur in B-cell–mediated regulatory functions, resulting in or contributing to various autoimmune diseases.

B CELLS AND DISEASE STATES

Given the complexity of the immune system, the development of an individual B cell is unlikely to follow a predictable and well-executed series of decision points whereby an antigen-reactive cell is expanded to a clone that produces a single antibody.[22] A more realistic perspective is that their development depends on a series of error-prone, random rearrangement events and mutations whereby specificity for the original antigen is maintained (or not) by selective pressures.[22] The development of self-reactive B cells is unavoidable given the random nature of clonal diversification; indeed, at least half of the antibodies expressed by immature human B cells are self-reactive.[23] Furthermore, some germline (ie, unmutated) immunoglobulin genes encode antibodies that are broadly reactive to a range of molecules, including self-antigens.[24] These antibodies may provide an initial form of "native" immunity to certain pathogens, such as bacteria. The presence of these self-reactive B cells in the periphery may also broaden the repertoire of antibodies available for expansion, mutation, and further selection in the periphery in response to pathogens. The process of somatic mutation in germinal centers, which increases diversity in the periphery, may also introduce mutations that result in newly created autoreactive antibodies. Thus, production of B cells that express antibodies with some capacity to bind to self-antigens may be the price of survival. Tolerance, the silencing of inappropriate production of self-reactive antibodies that arise during B-cell development, requires a crucial balance between ensuring the capacity for appropriate, diverse immune responses to pathogens and injuring the health of an individual through autoreactivity.

Selection against self-reactive antibodies occurs at multiple checkpoints during B-cell development, including in the bone marrow at the immature B-cell stage and in the periphery at the transition between new emigrant and mature B cells.[23] At least 3 mechanisms, including clonal deletion, receptor editing, and anergy, are thought to lead to tolerance during B-cell development. Clonal deletion, the negative selection and elimination of B cells that express autoantibodies that bind self-antigens strongly, has been

ORIGINAL ARTICLE

Effect of Magnetic vs Sham-Magnetic Insoles on Nonspecific Foot Pain in the Workplace: A Randomized, Double-Blind, Placebo-Controlled Trial

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OBJECTIVE: To determine whether magnetic insoles are effective for relieving nonspecific subjective foot pain in the workplace, resulting in improved job satisfaction.

SUBJECTS AND METHODS: A prospective, randomized, double-blind, placebo-controlled study of health care employees who experienced nonspecific foot pain for at least 30 days, which occurred more days than not, was conducted between February 2001 and January 2002 at the Mayo Clinic in Rochester, Minn. Participants were asked to wear either magnetic or sham-magnetic cushioned insoles for at least 4 hours daily, 4 days per week for 8 weeks. The primary outcome variable was reported foot pain (by categorical response of change from baseline and by visual analog scale) at 4 and 8 weeks. Secondary outcome variables included graded intensity of pain experienced during various daily activities and the effect of insoles on job performance and enjoyment.

RESULTS: Among 89 enrolled participants, 6 either withdrew before wearing insoles or were noncompliant with follow-up questionnaires; 83 participants remained for full statistical analysis. Participants in both treatment groups reported improvements in foot pain during the study period. No significant differences in categorical response to pain or pain intensity were seen with use of magnetic vs sham-magnetic insoles.

CONCLUSIONS: The magnetic insoles used in this study by a heterogeneous population with chronic nonspecific foot pain were not clinically effective. Findings confirmed that nonspecific foot pain significantly interferes with some employees' ability to enjoy their jobs and that treatment of that pain improves job satisfaction.

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In the past decade, the use of magnets for pain relief has increased substantially. Despite little scientific evidence (and lack of Food and Drug Administration approval for pain relief), many people have used magnets to relieve their pain, spending approximately $5 billion worldwide on magnetic pain-relieving devices.[1-3] Magnetic devices use either static or pulsed magnets. Clinically, pulsed magnets have been shown effective for treating delayed fracture healing,[4,5] for reducing pain in various musculoskeletal conditions,[6-10] and for decreasing edema associated with acute trauma,[11] although other studies have shown no benefit in these situations.[12,13]

An estimated $500 million is spent for therapeutic magnets in the United States annually.[14] The vast majority are static magnets with strengths ranging from 150 to 3000 G. Externally applied static magnets generally are considered safe and have few adverse effects,[15,16] but little is known about their mechanism of action. Most basic scientific research has focused on movement of tiny electrical volt-

ages generated via the Hall effect when blood moves in the presence of a static magnetic field. This may increase pain fiber thresholds,[17-19] although the mechanism is likely via secondary messengers affecting cell function[20] and not via gross changes in nerve-conduction velocities.[15,21,22] It is unclear how deep the magnetic fields of commonly used magnets penetrate into soft tissue, but Weintraub[19] reported a 4-cm (1.75-in) penetration depth of 475 G (multipolar triangular steep field gradient neodymium) with magnetic insoles. Many reports and advertising handouts allude to a magnetic pain-relieving effect via increasing blood flow, hyperemia, or warming of soft tissues,[23] but no in vivo evidence shows that magnets alter blood flow.[24] Also, the presence of any substantial thermal effect of static magnets is doubtful.[25]

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Scientific evidence regarding the clinical effectiveness of static magnets is accumulating with conflicting conclusions. Of 13 clinical trials identified in the current literature involving static magnets, 5 reported positive results. Magnetized insoles may be effective for patients with severely painful diabetic neuropathies, although patients with mild to moderate pain do not seem to benefit.[19,26] Magnetic pads were found to relieve multifactorial postpolio pain and tenderness immediately after use.[27] Magnetic mattress pads reportedly decreased pain and fatigue symptoms in patients with fibromyalgia.[28] Magnetic bracelets were found to decrease pain in hip and knee osteoarthritis.[29] Magnetic disks

FIGURE 2. Autoreactive B cells in health and disease. Normal regulatory mechanisms induce tolerance to autoreactive B cells in a healthy, functioning immune system. Loss of tolerance can lead to the inappropriate production of autoantibodies and/or enhancement of other autoreactive processes, ultimately leading to tissue damage. BLyS = B-lymphocyte stimulator; IFNα = interferon-α; IL-10 = interleukin 10; LTβ = lymphotoxin-β; TNF = tumor necrosis factor.

shown to mediate tolerance of B cells during the pre–B-cell to B-cell transition on exposure to self-antigen.[25,26] Receptor editing is the process whereby self-reactive B cells may escape elimination by replacing their antigen receptors. This involves genetic rearrangements induced by encounter with self-antigens, changing antigen receptor specificities from "self" to "nonself."[27,28] In some cases in which B cells have attempted to replace an autoreactive antigen receptor, the B cell may express both the replacement antigen receptor and the autoreactive antigen receptor, as has been observed in animal models.[29] Binding of antigen to the new replacement receptor can induce the B cell to produce both the antibody specific to the antigen and the

self-reactive antibody, circumventing the regulatory process. The physiologic importance of this observation is not yet clear, but it provides another potential mechanism for autoantibody production. Another mechanism, anergy, which involves functional inactivation of self-reactive B cells, may be a discrete entity or may represent a form of delayed deletion.[4,30] Anergic mechanisms in autoreactive cells can result in specific phenotypic and functional changes, such as down-regulation of membrane receptor, failure to respond to normal immune stimuli, and shortened cell survival.

A fundamental feature of autoimmune diseases is the loss of B-cell tolerance in the periphery and the inappropri-

ate production of autoantibodies. Given the large number of autoantibodies that are produced under physiologic conditions, it seems probable that even minor changes in the regulation of autoantibodies could result in increased likelihood of autoimmunity.[23] Although most strongly self-reactive antibodies are counterselected at the immature B-cell stage,[23] central tolerance is not complete under physiologic conditions. For example, self-reactive B cells can be found in the periphery in healthy humans and animals,[31] although they are generally inactive, and autoreactive antibodies remain undetectable in the serum. These clonally silent B cells could escape cell death and be induced to proliferate and secrete self-reactive antibodies in otherwise healthy individuals in the setting of a random event, such as a virus that induces especially strong activation signals (eg, cytokines), or they may be activated in disease states via inappropriate stimuli, such as binding of pathogen recognition receptors (eg, Toll-like receptors) by chromatin-containing immune complexes.[15,32,33] Moreover, if the new antigen-binding site on an autoantibody interacts with a self-antigen to generate positive cell survival signals, by stimulating or mimicking T-cell cytokine activity, the B cell may survive and proliferate. In this way, autoantibodies may drive their own production.[34] Autoimmunity may also result from overaggressiveness of the immune system to invasive pathogens, possibly due to mutations in genes that increase responsiveness.[35] Other models suggest that B cells that become autoimmune have genetic abnormalities that result in loss of tolerance. For example, genetic abnormalities that create intrinsic B-cell abnormalities can cause SLE-like diseases in animals.[36] Furthermore, some genetic predispositions identified in humans seem likely to have a direct effect on B cells and could contribute to an increased risk of autoimmune disease.[37,38]

The process of receptor revision may also introduce changes in B-cell antigen specificity. Receptor revision involves secondary rearrangement of immunoglobulin genes of B cells in the periphery, thereby changing antigen specificity.[39,40] It tends to occur after, or concurrent with, somatic mutation and generates high-affinity antibodies.[39-41] Receptor revision is likely to generate new, possibly self-reactive receptors in mature B cells, thereby complicating immune tolerance.[22]

The presence of autoreactive B cells in the periphery in humans has been demonstrated by multiple investigators.[42-44] These autoreactive B cells normally appear primarily in the immature and naive subsets of B cells and appear restricted from entering the memory or switch populations. However, both the negative selection of autoreactive B cells in the transition from the immature to the naive mature subset and the restriction of autoreactive B cells in switching may be defective in SLE and RA.[44,45] These changes allow the

production of high-affinity, switched autoantibodies in human autoimmune disease.

Accumulating evidence indicates that B cells have more essential functions in regulating immune responses than previously realized, particularly with respect to their interaction with and activation of T cells. Thus, exaggeration or dysregulation of any of these activities could potentially contribute to the development and maintenance of disease.[46] Importantly, presentation of antigen by self-reactive B cells that have broken tolerance can activate T cells that had been anergic to the self-antigen, as shown in an animal model of SLE.[31,47] Thus, autoantigen presentation by B cells may induce activation of T cells that otherwise would remain unresponsive to a particular self-antigen. This effect of B cells may play a role in epitope spreading, in which the T-cell compartment progressively becomes reactive to more sites on a given self antigen over time. Thus, loss of tolerance in the B-cell compartment can induce autoimmunity in the T-cell compartment. This appears to be the case in RA, in which the inflammatory cascade of events is traditionally considered primarily mediated by activated T cells.[48] T-cell activation has been shown to be critically dependent on B cells in RA in humans, whereas other APCs cannot maintain T-cell activation.[49,50] There is evidence for enhanced B-cell activation in RA, including isolation of B cells that have undergone receptor revision from ectopic germinal centers in inflamed tissues in patients with RA.[41,51] The activated B cells may be driving loss of tolerance in T cells in RA. However, recent animal models clearly make the point that certain antibodies are able to induce arthritis directly.[52,53] The relative contribution of production of autoantibodies and of other functions of B cells, including activation of T cells and cytokine production, to the pathogenesis of RA will be an important focus of research in the immediate future.

B CELLS AS A THERAPEUTIC TARGET IN AUTOIMMUNE DISEASE

As discussed, B cells are essential for maintaining health, particularly in their responsibility for routine surveillance for foreign pathogens throughout the body. However, their known roles in the pathogenesis of autoimmune disorders, predominantly those characterized by chronic inflammation, identify B cells as an important therapeutic target. The use of monoclonal antibody treatment to target and selectively deplete B cells is well established in the treatment of B-cell malignancies,[54,55] and a similar approach is now being investigated in the treatment of autoimmune disorders. This type of treatment may be able to reduce autoantibody production and also inhibit the ability of B cells to act

as APCs, thereby affecting the contribution of T cells to disease.

The monoclonal antibody rituximab, which is directed against the CD20 B-cell surface antigen, produces rapid, sustained, and selective depletion of circulating B cells.[56] Rituximab is believed to deplete B cells by induction of antibody-dependent cell-mediated cytotoxicity and complement-dependent cytotoxicity.[57,58] The effects of rituximab on B cells are transient, such that after treatment, B cells return to baseline levels within 6 to 12 months.[59] During this time, most circulating B cells have an immature phenotype, with a slow recovery of memory (CD27+) B cells. The characterization of the recovering B cells and their variability among treated individuals are subjects of ongoing study. Treatment with rituximab in B-cell malignancies is generally well tolerated and does not appear to be associated with increased susceptibility to infections.[54] At least with single courses of treatments, the primary adverse effects have been infusion reactions. These can include both tumor lysis syndromes in malignancies and responses to the infusion itself.

Selective B-cell depletion therapy with rituximab, as monotherapy or in combination with other agents, has been evaluated in several open-label, preliminary studies in several autoimmune diseases. Most data on the effects of rituximab on autoimmune diseases are available from studies in patients with RA, including results from one randomized, double-blind, controlled trial.[60-63] Rituximab improved disease symptoms of RA and was well tolerated in these studies. Case reports or prospective open-label studies have also reported the efficacy of rituximab in SLE and lupus nephritis,[64-67] idiopathic thrombocytopenic purpura,[68] antineutrophil cytoplasmic antibody–associated vasculitis,[69] transplant rejection,[70] and neurologic disorders such as dermatomyositis,[71] paraneoplastic neurologic syndromes,[72,73] IgM antibody–related polyneuropathies,[74,75] and demyelinating diseases[76] (the efficacy of rituximab in autoimmune diseases other than RA has been reviewed by Looney[77]). Results from these studies are encouraging, with significant sustained improvements in clinical assessments reported. Rituximab was also well tolerated in patients with these autoimmune disorders. An association between a positive clinical response and reduction in autoantibody levels after B-cell depletion with rituximab has been observed in RA[78] and IgM antibody–related polyneuropathies.[74] More controlled clinical trials using rituximab are needed to confirm the benefits observed in the preliminary trials. However, findings to date suggest that B-cell activation is not simply a bystander effect of the inflammatory process; rather, results indicate that B cells play an important role in the pathogenesis of these diseases. Interestingly, little change in total serum IgG occurs for at least a year after a single course of treatment, even though at least some autoantibodies are reduced. Since CD20 is not expressed on fully differentiated plasma cells, this suggests that at least certain autoantibodies are derived from newly generated antibody-secreting cells, whereas total serum IgG is largely produced by long-lived plasma cells. In addition, animal models suggest that different subsets of B cells are depleted at different rates after treatment with anti-CD20. For example, marginal zone B cells persist longer than follicular B cells.[79] This may become more important when repeated treatment is considered. The persistence of some percentage of marginal zone B cells, in combination with maintenance of serum IgG, may provide protection from infections, particularly pneumococcus, which might be lost with repeated B-cell depletion. As other therapeutic approaches targeting B cells are developed, such as blockade of B-lymphocyte stimulator or antibodies to other B-cell surface molecules, differences in effects on both early and late stages of B-cell differentiation may provide subtle but potentially important differences in their clinical effects.

CONCLUSIONS

B cells are essential for efficient immune responses and the maintenance of health. However, accumulating evidence points to the involvement of B cells in the pathophysiology of several autoimmune disorders, suggesting their potential as a relevant target for therapeutic intervention. Preliminary clinical evidence suggests that selective B-cell depletion is associated with clinical improvements in patients with autoimmune disease. Thus, B-cell depletion may prove to be a valuable alternative treatment modality for patients with autoimmune disease in which B cells are known (or suspected) to contribute to the disease. An important goal is to understand how B-cell depletion therapy alters the diverse mechanisms by which B cells contribute to autoimmunity. Additional studies are warranted to further characterize the role of B cells in autoimmune diseases and the therapeutic utility of B-cell depletion or other modalities that target B cells, such as those that alter B-cell activation.

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