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REVIEW ARTICLE

A perspective on B-cell-targeting therapy for SLE

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Abstract In recent years, large controlled trials have tested several new agents for systemic lupus erythematosus (SLE). Unfortunately, none of these trials has met its primary outcome. This does not mean progress has not been made. In fact, a great deal has been learned about doing clinical trials in lupus and about the biological and clinical effects of the drugs being tested. Many of these drugs were designed to target B cells directly, e.g., rituximab, belimumab, epratuzumab, and transmembrane activator and calcium modulator and cyclophilin ligand interactor–immunoglobulin (TACI–Ig). The enthusiasm for targeting B cells derives from substantial evidence showing the critical role of B cells in murine models of SLE, as well promising results from multiple open trials with rituximab, a chimeric anti-CD20 monoclonal antibody that specifically depletes B cells (Martin and Chan in Immunity 20(5):517–527, 2004; Sobel et al. in J Exp Med 173:1441–1449, 1991; Silverman and Weisman in Arthritis Rheum 48:1484–1492, 2003; Silverman in Arthritis Rheum 52(4):1342, 2005; Shlomchik et al. in Nat Rev Immunol 1:147–153, 2001; Looney et al. in Arthritis Rheum 50:2580–2589, 2004; Lu et al. in Arthritis Rheum 61(4):482–487, 2009; Saito et al. in Lupus 12(10):798–800, 2003; van Vollenhoven et al. in Scand J Rheumatol 33(6):423–427, 2004; Sfikakis et al. Arthritis Rheum 52(2):501–513, 2005). Why have the controlled trials of B-cell-targeting therapies failed to demonstrate efficacy? Were there flaws in design or execution of these trials? Or, were promising animal studies and open trials misleading, as so often happens? This perspective discusses the current state of B-cell-targeting therapies for human lupus and the future development of these therapies.

Keywords Atacicept · Belimumab · B lymphocytes · Bortezomib · Rituximab · Systemic lupus erythematosus (SLE)

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Background

Autoantibodies are of major importance in systemic lupus erythematosus (SLE). Autoantibodies are usually present for many years before the diagnosis of SLE [11]. Over time, autoantibodies in an individual developing lupus evolve to have higher affinity, undergo isotype switching, and spread to recognize new epitopes. In SLE, autoantibodies are not passive bystanders but critical effectors responsible for many manifestations, including cytopenias, thromboembolic disease, neuronal damage, skin rashes, arthritis, and renal disease.

Plasma cells, the source of circulating autoantibodies, present a difficult challenge for B-cell-targeting therapies (Fig. 1). Although some plasma cells are susceptible to conventional therapies, most are not. Furthermore, biologic agents with one exception (atacicept) have had relatively little effects on plasma cells. Anti-double-stranded-DNA (anti-dsDNA)-producing plasma cells are unusual in that these plasma cells are often (but not always) susceptible to conventional therapies. Thus, the titer of anti-dsDNA antibodies can plummet precipitously with high-dose steroids while at the same time total immunoglobulin and the levels of most autoantibodies, e.g., anti-Ro or anti-Sm, are unaffected. This rapid response of anti-dsDNA antibodies

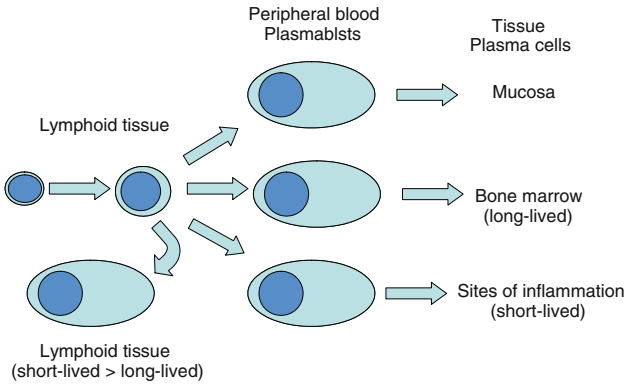

Fig. 1 Plasma-cell biology. Plasmablasts are generated in secondary lymphoid tissues where they differentiate into plasma cells or migrate into peripheral blood and home to various tissues based on their adhesion molecules and chemokine receptors. Bone marrow plasma cells tend to be long lived, whereas plasma cells in secondary lymphoid tissue tend to be short lived. Plasma cells that home to sites of inflammation are often dependant on continued inflammation to provide an appropriate microenvironment

may be explained by short-lived plasma cells whose generation is blocked by therapy. Alternatively anti-dsDNA-specific plasma cells could preferentially home to sites of inflammation, and treatments decreasing inflammation could disrupt this microenvironment, inducing plasma-cell apoptosis [12].

In the 1980s, interferon-α levels were found to be increased in patients with active SLE [13]. Production of interferon-α is important for constitutional and organ-specific symptoms, and in addition, interferon-α can induce hyperactivity of the immune system, as seen with active SLE [14]. It is now clear that autoantibodies binding RNA- or DNA-containing autoantigens induce the production of interferon-α in SLE [15–19]. These immune complexes are internalized by Fc-gamma-receptor IIa (FcγRIIa) on plasmacytoid dendritic cells then bind to intracellular toll-like receptor (TLR)-7, 8, or 9, triggering massive interferon-α production. Similarly, by binding to cell-surface autoantibodies then to TLR-7 or 9 within B cells, RNA- or DNA-containing autoantigens selectively trigger activation and proliferation of autoantibody-producing B cells [20–23].

B cells also influence autoimmune disease through multiple antibody-independent mechanisms [24, 25]. For example, through antigen presentation and production of cytokines and costimulatory factors, B cells regulate T-cell activation and polarization. Moreover, B cells induce lymphoid neogenesis through surface-bound lymphotoxin, which recruits and activates follicular dendritic cells, thus generating lymphoid follicles. B cells can also affect myeloid dendritic cells and regulatory T cells.

The relative importance of autoantibody production vs. autoantibody-independent effects of B cells is usually difficult to assess but was clearly demonstrated in a clever animal model from Mark Shlomchik's lab, where B cells were engineered to express only membrane immunoglobulin-M (mIgM). Mice with only mIgM have mature B cells, but these B cells are incapable of secreting immunoglobulin. The development of lupus in MRL/*lpr* mice with mIgM-only B cells was compared with the development of lupus in MRL/*lpr* mice with no B cells and MRL/*lpr* mice with normal B cells [26–28]. There was a significant difference in survival between mice in each of the three groups. In the group with normal B cells, 50% survival was reached at 32 weeks, whereas in the group with mIgM-only B cells, 50% of animals survived until 56 weeks; p value <0.0007. In contrast, 90% of MRL/*lpr* mice with no B cells were still alive at 56 weeks: p value <0.0001 for this group compared with either of the other two groups. Thus, even without secreting immunoglobulin B cells significantly influenced the course of lupus in this particular animal model. These results raise the possibility that therapies targeting B cells but not affecting plasma cells, immunoglobulins, or autoantibodies, e.g., anti-CD20 antibodies, such as rituximab, might nevertheless be effective for lupus.

Of significant relevance to the concept of B-cell targeting, not all of the effects of B cells promote autoimmunity. Indeed, B cells suppress disease in some models of autoimmunity, such as in experimental allergic encephalitis [29–35]. In many of these models, interleukin-10 (IL-10) produced by B cells suppresses dendritic-cell production of IL-12, thus blocking T-helper-1 (Th1) cell responses. OX40L expression by B cells may also suppress disease by inducing immune deviation toward Th2 cells. Furthermore, natural autoantibodies, germ-line IgM autoantibodies, may also play an important role in suppressing autoimmunity by clearing immune complexes and promoting tolerance. A recent clinical trial using rituximab in alloantigen-poly-sensitized patients awaiting transplantation emphasized the potential dangers from depleting B cells. This trial was halted because of concerns about worsening rejection after B-cell depletion [36].

Rituximab

Rituximab is a chimeric monoclonal antibody with variable regions derived from a mouse anti-human CD20 antibody and constant regions from human IgG1κ. CD20 is expressed on immature, naïve, and memory B cells but is not expressed on mature plasma cells or B-cell precursors. Thus, when used in patients with lymphomas, rituximab depleted normal and malignant B cells but had little effect on serum IgG. A decade ago when several of the open trials using rituximab for SLE were began, the rationale for using rituximab was based the studies from Mark Shlomchik's

lab showing the importance of the antibody-independent effects of B cells in murine lupus, plus the observation that anti-dsDNA could respond rapidly to steroids, suggesting a dependence on short-lived plasma cells [27]. Since that time, there has been considerable success using rituximab in human autoimmune diseases, including several large phase II/III trials in rheumatoid arthritis (RA) and a phase II trial in relapsing−remitting multiple sclerosis [37, 38]. The success in RA and in multiple open clinical trials for SLE led to considerable enthusiasm for randomized trials in nonrenal and renal SLE [7].

EXPLORER was a randomized, double-blind, placebo-controlled trial of rituximab for nonrenal SLE. Preliminary results were presented at the American College of Rheumatology (ACR) 2008 meeting [39]. A total of 257 patients with active SLE were randomized to rituximab vs. placebo (2:1 rituximab:placebo). Patients had to fulfill at least one British Isles Lupus Assessment Group (BILAG) A (severe activity in an organ system) or two BILAG B (moderate activity in two organ systems) and also had to be on a stable dose of an immunosuppressive drug (azathioprine, mycophenolate, or methotrexate). Patients continued their baseline immunosuppressive drug and received a 10-week course of increased glucocorticoids (0.5–1.0 mg/kg). Patients received either placebo or 1,000 mg of rituximab on days 1 and 15. Rituximab treatments were repeated at 6 months. The BILAG was used to define flares (severe flare = one new BILAG A or two new BILAG B, and moderate flare = a new BILAG B), This flare definition was used to create criteria for major or partial clinical response (MCR or PCR). It was expected that patients would respond to the course of steroids, so differences might only become apparent as steroids were tapered. As expected, both groups responded similarly with a significant improvement in clinical activity. However, even after the steroid taper, there was no difference between groups, i.e., at week 52, MCR = 15.9 vs. 12.4% for placebo vs. rituximab; PCR = 12.5 vs. 17.2%; and neither MCR nor PCR = 71.6 vs. 70.4%. There were also no differences between the groups in any of the secondary endpoints. An ethnicity analysis did find a benefit of rituximab in African Americans and Hispanics compared with other subjects: 33.8% of African Americans and Hispanics had an MCR or a PCR vs. 15.7% of non-African Americans and non-Hispanics. Moreover, patients entering the study on methotrexate also appeared to benefit from rituximab. Interestingly, and perhaps importantly, the rituximab-treated group had much better serological response, i.e., anti-dsDNA, C3, and C4 were better in the rituximab group, with p values = 0.005, 0.0029, and 0.0045, respectively, at 1 year. Thus, despite promising results in many open trials, rituximab failed when fairly tested as an add-on therapy to standard of care in moderate to severe nonrenal lupus.

Although EXPLORER is clearly the best trial so far on the use of rituximab for SLE, it does have problems. It was designed to enter patients who were very active and therefore treated aggressively with moderate- to high-dose glucocorticoids. This makes detecting a benefit difficult over the short term, and one could argue that for lupus, a year is short term when looking for flares. Thus, a longer time of follow-up would have been very helpful. The serological response in the rituximab-treated group is one hint that some benefit might have accrued in the long run, as serologically active but clinically quiescent lupus patients are at high risk of flares [40]. A second problem is the outcome measures used. Although the BILAG has many virtues, it is still unproven as an outcome measure for clinical trials. In fact, there are no generally accepted outcome measures for nonrenal lupus. At this point, the SLE responder index (SRI) developed in the belimumab trials is probably the best-supported outcome measure for nonrenal lupus. Another potential problem is that all patients were systematically retreated with rituximab or placebo at 6 months, irrespective or response. If one accepts the idea that one important benefit is to repopulate with regulatory/protective cells, then patients doing so and then retreated would be deprived of that benefit, and a long-term response would be delayed, thus compounding the problem with short-term follow-up [41]. A final problem is the heterogeneous nature of SLE itself. Different manifestations of SLE have different mechanisms. Therefore, one therapy, especially one targeted therapy, is unlikely to be effective for all patients. Limiting entry to a few different manifestations that are felt to have the same pathophysiology might be necessary.

The LUNAR trial was a randomized, double-blind, placebo-controlled trial of rituximab for proliferative lupus nephritis. Rituximab or placebo was added on to standard-of-care therapy using myccophenolate and high-dose steroids. The results for this trial are only available from a press release, but again, the results were disappointing: neither the primary outcome nor any of the secondary outcomes were achieved. However, the trial had some of the same problems seen in EXPLORER. The primary outcome was at 1 year, which is very unlikely to be long enough, especially in a nephritis trial and when all the patients are treated with high-dose steroids and mycophenolate. Moreover, as in EXPLORER, all patients were re-treated at 6 months, irrespective of response. On the other hand, the outcome measure for the LUNAR trial, renal function, is a very good outcome that almost everyone would accept. Another problem with the LUNAR trial and also with EXPLORER is the use of immunosuppressive agents other than cyclophosphamide. The most impressive results with rituximab for SLE have been in refractory patients treated with one or two low doses of cyclophosphamide at the same time rituximab

was given [7]. As both rituximab and cyclophosphamide target B cells, there should be synergy, and this may be crucial in determining response. The BELONG trial, a phase III trial of the humanized anti-CD20 ocrelizumab for lupus nephritis, is still recruiting patients, and both mycophenolate and cyclophosphamide are being used in that trial. Thus, the BELONG trial may determine whether cyclophosphamide rather than mycophenolate should be used with anti-CD20.

Belimumab

Belimumab is a fully human monoclonal antibody against B-lymphocyte stimulator/B-cell-activating factor (BLyS/BAFF). An initial phase I trial demonstrated tolerability, safety, and an effect on B-cell numbers [42]. Subsequently, belimumab was evaluated in a phase II, dose-escalating, double-blind, placebo-controlled trial enrolling 449 subject with a Safety of Estrogens in Lupus Erythematosus National Assessment−Systemic Lupus Erythematosus Disease Activity Index (SELENA−SLEDAI) ≥ 4. The results from this study were presented at the ACR meetings in 2006, 2007, and 2008 [43–45]. The primary endpoint was improvement in the SELENA−SLEDAI at week 52. The trial did not meet this endpoint. Unfortunately, 28% of patients were not serologically active, i.e., not positive for antinuclear antibodies (ANA) or anti-dsDNA at study entry. When only the serologically active subgroup was included in the analysis, there was a statistically significant but modest benefit in terms of the primary outcome, i.e., a 28% greater reduction in the SELENA/SLEDAI in the belimumab-treated compared with the control group; $p = 0.044$. In addition, secondary endpoints were also better in the belimumab-treated group, e.g. the physician global assessment and physical component of the 36-question short-form health survey (SF-36) both improved in the belimumab group; $p = 0.001$ and 0.02, respectively. The data from this trial were used to develop a SLE responder index (SRI = improvement of SELENA−SLEDAI > 4 plus no worsening of physician global assessment plus no BILAG flares). In the belimumab-treated group, 46% of patients met the SRI at 1 year vs. 29% in the placebo group; $p = 0.0056$. During long-term, open-label follow-up, the SRI increased to 55% by week 76 [44]. In the first year of the study, 62% of belimumab-treated, seropositive subjects had SELENA−SLEDAI flares, but this declined dramatically so that during the third year of the study, only 7% of subject had a SELENA−SLEDAI flare. These results suggest that long-term therapy with belimumab may have substantial benefits [43]. Thus, the results of the phase II trial and follow-up open trial of belimumab are certainly encouraging. Two phase III trials of belimumab are ongoing and have completed recruitment.

Other agents

Anti-CD22

Epratuzumab is a monoclonal antibody against CD22, a B-cell-specific surface antigen. An initial open trial of 360 mg/m^2 every 2 weeks for four doses treated 14 subjects [46, 47]. The drug was well tolerated, and there was improvement in the BILAG. There was a modest decrease in B cells, i.e., a 35−44% decrease, and no change in immunoglobulin levels. SL0003 and SL0004 [48] were two randomized clinical trials that were prematurely discontinued due to problems with manufacturing. However, 90 patients were entered. Combined data from these two trials suggests that anti-CD22 was safe and might have been shown to be effective had the trials not been terminated.

TACI−Ig

Atacicept, a chimeric molecule with the extracellular domain of transmembrane activator and calcium modulator and cyclophilin ligand interactor (TACI), which binds both BLyS and a proliferation-inducing ligand (APRIL), fused to the Fc domains of human IgG1, is the one biologic therapy with dramatic effects on plasma cells [49]. After seven doses of 420 mg every 2 weeks in patients with RA, total IgG decreased by 21%, IgA by 37%, and IgM by 54%. IgG, IgA, and IgM rheumatoid factors all decreased between 41% and 44% and anti-cyclic citrullinated peptide (anti-CCP) antibodies decreased by 25% [50]. These rapid changes in immunoglobulin and autoantibody levels can only be explained by effects on plasma cells. Atacicept was well tolerated in an SLE phase I study [51]. However, a phase II study of atacicept in combination with mycophenolate for lupus nephritis was terminated because of infections. A phase II/III trial of atacicept for nonrenal lupus is ongoing and still recruiting. Because of its effects on plasma cells, TACI−Ig would appear to have great potential in autoantibody-mediated diseases. The challenge will be to eliminate autoantibody-producing plasma cells without increasing the risk of infection that we presume may be due to an effect on protective antibody.

New targets

Anti-CD19

CD19 is expressed on virtually all B cells and is lost at a later stage than CD20 as B cells differentiate into plasma cells. However, mature plasma cells are CD19 negative. In animal models of autoimmunity, anti-CD19 depletes a

wider spectrum of B cells than anti-CD20, e.g., peritoneal B cells, including B1 B cells, are depleted with anti-CD19 but spared with anti-CD20 [52]. Moreover, treatment with anti-CD19 in these models was associated with a decrease in total immunoglobulins, indicating an effect on plasma cells. One caveat with this interpretation is that the strain of mice used expresses human CD19, which induces autoimmunity and hypergammaglobulinemia. Whether similar effects on immunoglobulins would be seen in mouse strains using anti-murine CD19 or with anti-CD19 in humans is unclear.

Proteasome inhibitors

Bortezomib, a low-molecular-weight drug that inhibits proteasomes, has been approved for to treat multiple myeloma, a plasma-cell malignancy. In models of lupus, bortezomib improves survival, decreases renal disease, decreases titers of autoantibodies, and induces apoptosis of both short-lived and long-lived plasma cells [53]. Bortezomib has not yet been tested in human lupus. As bortezomib is associated with a painful peripheral neuropathy, such trials may be difficult to justify [54]. SLE-prone mice lacking TLR-9, which binds DNA complexes, fail to make anti-dsDNA antibodies, whereas SLE-prone mice lacking TLR-7, which binds RNA complexes, fail to make anti-Sm antibodies [22]. These results indicate TLRs are critical for determining the selection of autoantibodies. Thus, agents inhibiting TLR-7, 8, and 9 more effectively than currently available antimalarials are likely to be a major advance in treatment. Such inhibitors are currently in development.

B cell and homeostasis

B-cell-depleting therapies such as rituximab can cause dramatic shifts in B-cell populations. In most patients treated with rituximab, peripheral-blood B cells disappear completely for several months then reconstitute after 6–12 months. During reconstitution, most patients also have a dramatic shift in B-cell subsets, i.e., memory B cells (CD19+ CD27+) remain at very low levels, and conversely, levels of naïve and immature B cells (both CD19+ CD27−) increase [55–59]. Nonresponse and early relapse in RA patients treated with rituximab has been associated with increased proportion of memory B cells [57–59]. Moreover, in our own small series of patients with lupus treated with rituximab, long-term responders, patients who remained in remission for several years off therapy, had very low levels of memory B cells and very high levels of naïve and transitional B cells [56, 60]. At least under some conditions, stimulated naïve human B cells have been found to produce high levels of IL-10, whereas stimulated memory B cells produce high levels of lymphotoxin and tumor necrosis factor alpha (TNFα) [61–63]. Thus, we speculate that our long-term responders had both a decrease in the availability of autoantigen-specific memory B cells and an increase in anti-inflammatory naïve and transitional B cells. Improving depletion of memory B cells, decreasing production or proliferation of memory B cells, and increasing production of transitional and naïve B cells by bone marrow may all be important strategies for improving the outcome of B-cell-depletion therapy [41].

Anti-CD20 antibodies are usually effective in depleting B cells from peripheral blood, but their success at depleting B cells from other sites such as lymph nodes or tertiary lymphoid tissues may be highly variable [1, 64]. Failure to deplete in these tissue sites may lead to nonresponse or early relapse. Defects in effector mechanisms such as a low numbers of FcγR-bearing cells, the presence of FcγRs with low affinity for IgG1 or blockade of FcγR may be responsible for failure to deplete. The development of new anti-CD20 antibodies with increased binding to FcγR might increase depletion [65]. Increased levels of survival factors may also be important for survival of memory B cells in tissue. Moreover, systemic levels of BLyS/BAFF often increase markedly after treatment with rituximab, which might also contribute to the survival of memory B cells [66]. In animal models, combining anti-CD20 with inhibition of BLyS/BAFF is synergistic and has been shown to improve depletion in tissues [64]. Therefore, combining anti-CD20 or other B-cell-depleting autoantibodies with anti-BLyS or perhaps TACI−Ig may improve depletion of memory B cells and increase the likelihood of clinical response.

The rate of B-cell lymphopoiesis by bone marrow can have profound effects on B-cell populations. Resting memory B cells transferred to animals unable to produce B cells have increased proliferation, increased activation markers, and increased B-cell death [67, 68]. These animals remain B-cell lymphopenic. Mice chimeric for bone marrow competent to generate B cells (a mixture of B cells immunodeficient and wild-type bone marrow) are also lymphopenic and have B cells with increased proliferation and activation markers [69]. Thus, in animal models, failure to generate adequate numbers of B cells is associated with B cell lymphopenia and a shift to an activated phenotype (production of pro- vs. anti-inflammatory cytokines in these systems has not to our knowledge been studied). In human SLE B cells, lymphopenia is frequently seen. Some of this lymphopenia may be due to cytotoxic antibodies. Indeed, cytotoxic autoantibodies to B220 expressed on naïve B cells have been described [70]. In addition, interferon-α, which is a signature cytokine for lupus, profoundly inhibits B-cell lymphopoiesis [71, 72]. Anti-interferon-α monoclonal antibodies are now in clinical trials for lupus.

Studies of B-cell subsets and B-cell lymphopoiesis as part of these trials would be extremely interesting.

Lymphopenia, particularly lymphopenia due to poor B-cell lymphopoiesis, can be associated with increased proliferation and activation of B cells, giving them a memory phenotype [67, 68]. In addition, germinal-center reactions also increase production and proliferation of memory B cells. Thus, increasing B-cell lymphopoiesis or decreasing germinal-center reactions may decrease the number of memory B cells. Indeed, etanercept was recently shown to decrease peripheral blood memory B cells by about 50% and to decreased germinal-center reactions in tonsils [73]. Other anti-TNF therapies do not have this effect on memory B cells presumably because, unlike etanercept, they do not also target lymphotoxin. BLyS is extremely important for transitional and naïve B-cell survival. Thus, in a trial of anti-BLyS, naïve and transitional B cells in peripheral blood are disproportionately affected compared with memory B cells [74, 75]. However, after 6 months, the number of effector memory B cells (CD19+IgD-CD27−) also declines, and after 1 year, memory B cells and plasmablasts modestly decline. These late effects on B cells may be important in the delayed therapeutic effects of anti-BLyS on lupus activity.

Plasma-cell homeostasis

As plasma cells are the source of circulating antibodies, they are critical for the autoantibody-dependent effects of B cells in SLE. Plasma-cell survival is dependent on residence in an appropriate microenvironment [76–78] (Fig. 2). Autoantibody-producing plasma cells established in an environment allowing long-term survival can be very difficult to eradiate [79, 80]. Depletion of B cells with rituximab or other agents will have no effect on autoantibodies produced by long-lived plasma cells. Moreover, when generation of both autoimmune and normal plasmablasts is blocked, there can be no new normal plasma cells to "knock" long-lived autoimmune plasma cells out of their niche [79].

There may be drugs that selectively decreased the production of autoantibody-producing but not normal plasmablasts. After a long period of time, this selective inhibition may result in decreased autoantibody titers as the long-lived plasma-cell compartment turns over. Certainly, drugs that inhibit TLR-7 and TLR-9 should selectively affect the generation of autoimmune plasmablasts. Obviously, normal homeostatic mechanisms also have this selective effect, which is why normal individuals make autoantibodies transiently, if at all. If production of autoantibodies in lupus is due to immune system hyperactivity, then a number of immunosuppressive agents may inhibit

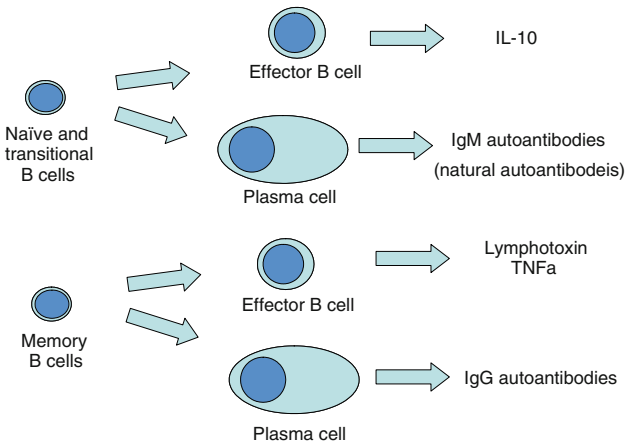

Fig. 2 Naïve and transitional vs. memory B cells. Activated naïve B cells secrete interleukin-10 (IL-10) or differentiate into plasma cells that produce natural autoantibodies. Both IL-10 and natural autoantibodies can suppress autoimmunity. In contrast, activated memory B cells secrete lymphotoxin and tumor necrosis factor alpha (TNFα) or differentiate into plasma cells producing pathogenic autoantibodies

generation of autoimmune plasmablasts, thus inhibiting immune system hyperactivity. The "right" level of immunosuppression would be when autoimmune but not normal plasmablasts are inhibited. These studies may also have to characterize plasmablasts to see whether they are capable of competing for the long-lived plasma-cell compartment [81].

An alternative approach for eradicating autoantibodies produced by long-lived plasma cells would be therapies that can directly kill long-lived plasma cells. Killing long-lived plasma cells would provide an opportunity and a need to repopulate their compartment with normal plasma cells. There is solid evidence from elegant experiments in murine lupus that proteasome inhibitors can kill long-lived plasma cells [53]. Similar studies using TACI−Ig would be of great interest. The vigorous application of therapies that kill long-lived plasma cells does have its downside, i.e., protective antibodies may also be eliminated. Thus, ideally, depletion of long-lived plasma cells will be combined with therapies that selectively block the generation of autoimmune plasmablasts, thereby preventing repopulation of the long-lived compartment with autoimmune plasma cells.

Overview

So far, the results from controlled clinical trials of B-cell-targeting agents in SLE have been disappointing. One explanation for these negative results may have been trial design and outcome measures. Some potential lessons about trial design and outcomes from belimumab and rituximab trials include:

1. The importance of entry criteria. The belimumab trial would have been positive had it required patients to be serologically active at entry.
2. The potential for concomitant medications to obscure benefits. Moderate- to high-dose steroids and immunosuppressive medications were used as concomitant therapy in both EXPLORER and LUNAR trials. In contrast, the belimumab trial did not use a course of steroids at study entry.
3. Long-term follow-up is essential. The primary outcomes for the rituximab studies and the belimumab study were all at 1 year. Although there was no clinical benefit at 1 year in EXPLORER, serological response clearly favored rituximab. This serological response may have translated into clinical benefit with longer follow-up. In lupus nephritis, long-term follow-up is especially important, and thus it is not surprising that in the LUNAR trial no difference was found at 1 year [82–87]. The effects of belimumab on B-cell subsets took more than a year to fully develop, and the clinical benefits appeared to increase with time during the open follow-on trial.
4. Problems with outcome measures and the potential benefit of composite outcomes. There is a suspicion that using the BILAG definition of flares in EXPLORER may have overestimated the rate of clinically important flares; >70% of subjects flared, and yet the BILAG score improved rapidly in the first 4 weeks of treatment and continued to improve over the course of the year (mean BILAG was approximately 14 at baseline, 9 at 1 month, 6 at 1 year). Additional analysis of outcomes in this study may be helpful. Data from the belimumab trial has been used to develop an SLE responder index (SRI), which appears to be more robust than its individual components. However, the SRI still needs to be validated in a prospective trial.
5. Targeted therapies may work better for targeted manifestations. Given that immunopathogenesis is probably as heterogeneous as the manifestations of lupus, it is unrealistic to expect that a targeted therapy will work for all aspects of disease. Thus, targeting a limited number of manifestations (inflammatory arthritis, skin disease, immune thrombocytopenia, refractory central nervous system disease) makes sense mechanistically and may make outcomes easier to measure. Of course, such a strategy did not work with proliferative lupus nephritis in the LUNAR trial, but this trial had other problems (see 2 and 4 above).

A second potential explanation for negative results of B-cell targeting clinical trials in SLE may be that the therapies being tested are inadequate and that new therapies are needed that can:

1. Reduce the number of long-lived plasma cells. Autoantibody-producing plasma cells, especially long-lived plasma cells, present a major therapeutic challenge for B-cell-targeting therapies. Fortunately, drugs targeting plasma cells are in development. Bortezomib, a proteosome inhibitor, triggers plasma cells by inducing an unfolded protein response. In murine lupus, bortezomib has shown impressive efficacy. Additional proteosome inhibitors are now in clinical trials for hematologic malignancies. TACI−Ig targets plasma cells by inhibiting both APRIL and BLyS. In human trials for RA and SLE, the effects of TACI−Ig on plasma cells has been demonstrated by a rapid drop in serum immunoglobulins and autoantibodies.
2. Selectively block activation of autoimmune B cells and generation of autoimmune plasmablasts. Therapies that selectively block activation of autoimmune B cells and the generation of autoimmune plasmablasts would be very attractive, as normal B cells would be relatively unaffected. One approach would be to target TLRs. Since TLR-7 and TLR-9 promote activation and proliferation of B cells binding autoantigens that contain RNA or DNA, inhibitors of these TLRs should have a selective effect on autoantibody-specific B cells in SLE. Antimalarials such as hydroxychloroquine and quinacrine are weak inhibitors of these TLRs. Using antimalarials has been associated with increased renal responses and reduced renal disease, and optimal management of these medications has been associated with decreased exacerbations [88–91]. Potent inhibitors for TLR-7, 8, and 9 are in development [92]. Combining a brief course of agents that kill long-lived plasma cells with chronic administration of TLR-7 and TLR-9 inhibitors may help assure the long-lived plasma-cell compartment is not repopulated with autoimmune plasma cells. Measuring the number of circulating autoantibody-producing plasmablasts (antibody-secreting cells) vs. the total number of plasmablasts may provide early evidence on whether TLR inhibition has been appropriately selective. A similar strategy could be use of biologic agents and conventional immunosuppressives [93].
3. Promote the number and activity of regulatory B cells. Regulatory B cells capable of preventing disease or promoting recovery have been demonstrated in animal models of autoimmunity [31, 32]. B-cell production of IL-10 appears to be particularly important in these models and is also produced by human B cells [63]. Identification of the different regulatory B-cell subsets in human autoimmune disease, determining the effects of B-cell-targeting therapies on these cells, and discovering methods to promote regulatory B-cell activity are all areas of active investigation.

4. Regulate memory B cells. Characterizing memory B-cell subsets and the factors that determine homeostasis and activation of these subsets will also be important for future studies [24]. Interferon-α is likely to be one such factor. By activating resting B cells and preventing B-cell lymphopoiesis in the bone marrow interferon-α may induce generation and proliferation of memory B cells and at the same time prevent expansion of transitional and naïve B cells that might otherwise complete for space of growth factors with memory B cells, and by producing IL-10, suppress immune activation and inflammation.

In summary, the controlled trials of B-cell-targeting agents for SLE have so far been unsuccessful, but these trials have contributed to our understanding of how to do trials in lupus and of basic human B-cell biology. The lessons learned now need to be applied to ongoing and future trials. New therapies, especially agents capable of affecting long-lived plasma cells, are also needed. Improved understanding of B-cell and plasma-cell homeostasis—and in future trials, careful studies of B-cell subsets, plasma cells, autoantibodies, and protective antibodies—will all be needed to optimize the use of B-cell- and plasma-cell-targeting therapies.

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