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Activating Systemic Autoimmunity: B's, T's and Tolls

Mark J. Shlomchik
Yale University School of Medicine, Departments of Laboratory Medicine and Immunobiology, Box 208035, New Haven, CT 06520

Mark J. Shlomchik: mark.shlomchik@yale.edu

Abstract

A recent advance in the treatment and understanding of autoimmune disease has been the efficacy of B cell targeted therapy. Such therapies are effective for several such diseases, with systemic autoimmunity being a prototypical example. The mechanism of action is not fully defined, but blocking B cell Ag presentation to T cells is likely to be important. T-B interactions probably engender a positive feedback loop that amplifies and sustains autoimmunity. But how is self-tolerance first broken to initiate this loop? I propose, based on recent data, a model in which autoreactive B cells are activated first, independent of T cells, but dependent upon BCR and TLR signals. These activated B cells then break T celltolerance, initiating full-blown autoimmunity.

Introduction

For many years it was assumed that autoantibodies, particularly immune complexes of them, are the main pathogenic agents in a subclass of systemic autoimmune diseases. T cells in these diseases were eventually recognized to play an important role, but only as helpers of the B cell response [1]. In both systemic autoimmune diseases, as well as organ-specific diseases such as MS and T1D [2,3], it is becoming increasingly clear that B cells play additional roles aside from autoantibody secretion. Conversely, it seems that T cells in systemic autoimmunity do more than just promote the activation of Ab-secreting B cells [4,5].

This review will focus on B cells in systemic autoimmunity, emphasizing recent basic and clinical results that provide insight into how autoreactive B cells are activated and then promote autoimmunity. It will first cover evidence that B cells have Ab-independent functions in autoimmunity. These include the ability of B cells to present Ags to T cells, which suggests the existence of a positive feedback loop between activated autoreactive B and T cells. The potential initiating events for such a positive feedback cycle will be considered next. New data suggesting that B cells may initiate autoimmunity, being the first cell type to break tolerance, will be reviewed and discussed. Emphasis will be on how and where this initial B cell activation occurs, focusing in particular on the roles of TLRs, acting independently of T cells. Together, these emerging studies suggest a new view of how systemic autoimmunity is initiated and propagated, integrating information on the effects of B cell depletion, the roles of TLRs and model systems for study of the activation of autoreactive B cells.

Evidence for Ab independent roles for B cells

Early clues that B cells indeed do more than secrete autoantibodies came from the phenotype of B cell-deficient autoimmune-prone mice [6–8]. These mice had no T cell interstitial infiltrates in their kidneys, and had a ten-fold reduction in memory phenotype CD4 and CD8 T cells. The effects of B cells were independent of autoantibody secretion, as T cell infiltration and activation was restored by B cells that could present Ag to T cells, but could not secrete Ab [9]. These Ab-independent effects on T cells are presumably due to presentation of autoAg to T cells; another potential mechanism for Ab-independent B cell influence on T cells is secretion of cytokines, including TNF-α, IL-6, IL-2 and IFN-γ [10]. Such cytokines can influence T cells and might also have direct pathogenic effects.

Regardless of whether by antigen presentation, cytokine secretion, or both, effects of autoreactive B cells on T cells have also been seen in B cell depletion settings in both humans and mice. Even in diseases that are thought to have an Ab-mediated component of pathogenesis, clinical responses are often faster than drops in Ab levels. This seems to be true in at least some RA and SLE patients [11,12]. In mice, chronic high dose administration of anti-hCD20 in hCD20 Tg lupus-prone MRL/lpr mice eventually led to B cell depletion. In these mice, the frequencies of activated and memory CD4 T cells were reduced compared to controls, but the effects were modest [13]. The limited effect might have been due to the slow pace of B cell depletion or it may indicate that once autoreactive T cells are established, they are not so dependent on B cells for either their maintenance or further expansion. Such effects of B cell depletion have also been observed in reports of rituximab treatment of lupus patients [14], in which B depletion led to a reduction of circulating T cells with an activated phentoype. Activation of transferred glutamic acid decarboxylase-specific TCR Tg T cells (BDC2.5 clonotype) was also reduced in the pancreatic LN of diabetes-prone NOD mice subsequent to B cell depletion [15]. Nonetheless, in these cases a direct linkage between effects on T cells and impact on disease was not established. Thus, more work is required on the APC and other functions of B cells in promoting the initial activation and the continuous reactivation of autoreactive T cells.

Positive feedback model and the question of initiation

We previously proposed a model of positive feedback between T and B cells to explain these observation [4,5]. In this model, T cells help autoreactive B cells and promote their clonal expansion, somatic hypermutation, isotype switch, affinity maturation and differentiation into antibody-secreting cells. Cycles of such mutual T-B interactions would lead to abundant numbers of autoreactive lymphocytes that could mediate disease via a number of mechanisms, ranging from autoantibody deposition to T cell mediated cytotoxicity.

This model has provided a useful framework for predicting and then later explaining why B cell depletion is effective in modulating various types of autoimmunity, including T cell-mediated autoimmune diseases. Indeed, this model has been supported by a substantial body of murine studies and human clinical results, as discussed above. However, one key issue that remains unclear is what starts the positive feedback cycle. This is a critical question, both for our mechanistic understanding as well as for formulating diagnosis and treatment strategies. It has been presumed, due to the mutual nature of the interaction, that B and T cell tolerance would need to be lost at the same time and in the same place at initiation of the autoimmunity cycle. Alternatively, or in addition, it has been assumed that T cells would initially be activated by dendritic cells [16], not B cells, as naïve T cells are thought to be first activated in this fashion in normal circumstances [17]. In fact, the relative role of B cells and DCs in the *initial* activation of autoreactive T cells in autoimmunity is unknown. Further, it is not clear

whether tolerance in the T or B cell compartment could be breached first, leading thereafter to breaking tolerance in the other compartment.

To help clarify this issue, BCR Tg mice with specificity for relevant autoAgs have been used to determine the requirements for initial activation and differentiation, as well as the ability of the B cells to present autoAg and secrete cytokines [18–20]. We have been studying the AM14 rheumatoid factor (RF) Tg mouse, which is specific for IgG2a of the "a" allotype ($IgG2a^a$) [21]. This Tg mouse and specificity is unique in allowing genetic control of an authentic autoAg, as well as the measurement and exogenous administration of this Ag. In the past few years we and others have made observations that suggest that initial B cell activation may procede via TLR, and not T cell-mediated signals. These in turn lead to a proposal for how B cell activation precipitates the autoimmunity positive feedback cycle, as will be discussed below.

TLRs promote the in vitro and in vivo activation of B cells with classic lupus-associated specificities

The restriction in systemic autoimmunity of domiant autoantibody specificities to nuclear Ags, along with self-IgG, suggested that there were specific receptors for components of these self-Ags that contributed to B cell activation. However, until recently the identity of such receptors remained a mystery. A critical insight into this problem was the discovery by Rifkin et al and Leadbetter et al. that AM14 RF Tg B cells were robustly stimulated to proliferate in vitro by immune complexes (ICs) comprised of IgG anti-chromatin and chromatin shed from dead cells [22,23]. Other types of immune complexes lacked this property. Hence, it was reasoned that an essential component for activation must be a receptor in B cells specific for chromatin. Leadbetter et al went on to show that this receptor signaled via MyD88, and, via a series of inhibitors, further implicated TLR9—a DNA-specific TLR. These data suggested a scheme in which BCR ligation with these ICs provided both a strong BCR signaling event as well as a means to internalize the ICs and deliver the chromatin to an intracellular compartment where TLR9 could recognize the DNA component [24]. Subsequent work extended this concept to TLR9 and anti-DNA B cells, which can recognize chromatin directly. In addition, RNA containing immune complexes were shown to stimulate RF B cells in a TLR7-dependent fashion, thus further generalizing the concept [25,26].

To investigate whether nucleic acid-specific TLRs controlled activation of anti-nuclear and RF B cells in vivo, our lab produced F2 crosses between B6 TLR9-deficient and lupus prone $MRL.Fas^{lpr}$ mice [27]. Most strikingly, mice lacking TLR9 also lacked serum Ab that causes the homogenous ANA pattern, an indicator of anti-DNA Ab. The absence of anti-chromatin Abs in TLR9-deficient mice was even more evident in the uniform absence of the mitotic chromatin staining pattern. Elements of these findings were confirmed subsequently by us in $MRL.Fas^{lpr}$ mice with a fully backcrossed homozygous TLR9-deficiency [28], and in a variety of other models of autoimmunity by other labs [29–31].

TLR7 was also investigated in several models. We produced $MRL.Fas^{lpr}$ mice homozygous for a Tlr7-null allele [28]. These mice lacked detectable Abs to RNA-containing Ags, including Sm, and RNP. Further insight was gained from mice with higher than normal TLR7 expression. It was recently shown that the Yaa allele, a Y-chromosome locus known to confer autoimmunity on a variety of genetic backgrounds, represented a duplication of a region of the X-chromosome that contained TLR7 [32,33]. Hence, mice carrying Yaa express an extra copy of TLR7; overexpression of TLR7 in Yaa mice led to increased anti-RNA Abs and systemic disease. This was reinforced by BAC Tg mice that overexpressed to varying degrees just the TLR7 locus and which had autoimmune disease characterized by anti-RNA type Abs [34].

While the effects of these TLRs is likely due at least in part to expression in B cells, their precise role in B cells and other cell-types still remains to be fully defined. Berland and colleagues studied the effects of TLR7 directly on B cells using a BCR knockin mouse with B cells specific for RNA [35]. The activation of these B cells, most of which had an anergic phenotype, was dependent on TLR7, confirming that RNA-specific B cells are regulated by TLR7. Though implicating B cells, this experiment does not formally establish a B cell-intrinsic role for TLR7. Such a role for TLR signaling in B cells was indicated in experiments with AM14 RF B cells that lacked MyD88; when transferred into wild type recipients, these cells not stimulated at all by chromatin-containing ICs in vivo [36].

It is possible that myeloid cells that express nucleic acid TLRs can contribute to B cell activation [37]. DCs provide important support for plasmablasts in vivo [38,39], though this has been called into question at least for certain T-independent responses [40]. TLR7 and TLR9 on conventional DCs (cDCs) and plasmacytoid DCs (pDCs) can elicit factors such as IFN-I and BAFF [41,42] that positively influence B cell responses [43–45] [46].

Further, signals from these TLRs can activate DCs, which may capture autoantigens by a variety of mechanisms including lectin receptors such as Mincle and DNGR-1 [47,48] or as part of ICs via activating FcRs [49,50]. These Ags can then be presented as peptides to T cells, which could provide cognate help to autoreactive B cells; in addition, DCs can present intact Ags to B cells [51,52] and may even be able to capture and present intact autoantigens, though there is no direct evidence of this to date.

TLR-dependent activation of B cells with lupus-associated specificities does not require T cells

We set out to assess the requirements for T cells in the activation of AM14 RF B cells in vivo. To test this idea directly, we used a system in which we provide IgG2a anti-chromatin Abs directly in vivo [20,36]. This is essentially an in vivo translation of the in vitro system originally described by Rothstein and colleagues [22,23]. This treatment results in a prompt and robust extrafollicular plasmablast response by the RF B cells, without a detectable GC response, thus mimicking the situation during spontaneous age-dependent activation in MRL/lpr mice. This in vivo activation pathway is TLR-dependent, enabled by both TLR7 and TLR9, and is absent when MyD88 is not expressed in responding B cells [36]. Most important for the current discussion, when IgG2a anti-chromatin Ab was administered to αβ T cell-deficient AM14 Tg MRL/lpr mice, the response was indistinguishable from that seen in T cell sufficient controls; nor did γδ T cell depletion in these animals have any impact. Hence T cells are not absolutely required for the extrafollicular AM14 RF response elicited by anti-chromatin Abs. Of interest in this regard, we found that B cell isotype switching and somatic hypermutation, while enhanced by T cells, nevertheless did not require them to achieve substantial levels ([36] and unpublished data). In a GC response, these two DNA modifications are thought to be entirely T cell-dependent. In recent years, though, it has become clear that a number of factors can promote T-independent isotype switch, at the least, including: IFN-I, TLR signals, and BAFF/APRIL signals [45,53–55]. Since these factors that promote class switch must elicit expression activation induced cytidine deaminase [56], they could also enable somatic hypermutation, which also relies on this enzyme. Presumably some of these factors are at play, substituting for T cells during the AM14 B cell response to chromatin ICs.

T-independent systemic autoimmunity

It is reasonable to question whether the lack of requirement for T cells in initial autoreactive B cells is particular to the AM14 system. One can speculate that other B cells that can gather TLR signals—most notably anti-DNA/chromatin B cells—should behave similarly, which

seems to be the case at least in vitro [25,26] and least to some extent in vivo [29]. Two other groups have recently reported T cell independent activation of autoreactive B cells and even disease [57,58]. Mackay and colleagues have produced a model of autoimmunity based on Tg-mediated overexpression of BAFF [59]. These mice display an SLE-like phenotype, including autoantibody production that does not require T cells but does require expression of TLRs in B cells [58]. Tsao et al. [57] studied B6 mice carrying the 56R H chain site directed Tg, which confers high affinity for dsDNA to most B cells [60,61], and which also has high level spontaneous anti-DNA [57]. They found that such mice lacking either CD4 or all T cells still produced these autoantibodies, and that they were also isotype switched. These three recent studies point to T-independent pathways of autoreactive B cell activation in diverse systems.

B cells can break tolerance first

From these data, we propose that, for B cells that recognize self-sturctures that contain endogenous ligands for TLRs, B cell tolerance (or ignorance) can be broken without the help of T cells. This scheme is outlined in Fig. 1. We postulate that, for such autoantigens, T cell tolerance and B cell tolerance need not be broken at the same place and time in order for autoreactive B cell clonal expansion and differentiation to ensue. Rather, in our model, the self-reactive B cell specific for autoantigens that are endogenous TLR ligands are an Achilles' heel of the immune system. Though the evolutionary advantage of TLR7-9 expression in B cells is as yet unclear, it could help to promote a rapid and robust extrafollicular plasmablast response to bacterial infection [62] that could occur prior to the steps needed for T cell priming and expansion. It was recently shown that TLR9 and BCR signals combined on a particulate Ag direct such responses in vivo [63]. The trade-off for the ability to respond without T cell help in such situations could be the possibility of autoimmunity. Indeed, this particular susceptibility to T-independent activation of DNA and RNA-specific B cells by virtue of expression of TLR9 and TLR7 could explain why systemic autoimmunity invariably targets DNA and RNA-related autoantigens to the exclusion of a vast array of other self-epitopes [64].

What about T cell contributions to autoreactive B cell activation and autoimmunity?

The notion that autoreactive B cells break tolerance in a T-independent fashion, and that this is an early step in the genesis of autoimmune disease, seems at odds with evidence demonstrating the importance of T cells in systemic autoimmunity [65–69]. (It should, however, be noted that lupus-prone mice lacking important T cell function, including ab T cells and/or CD40L, still demonstrated substantial, albeit reduced disease, perhaps consistent with a facultative role for T cells [66,68,69]). The key to resolving this paradox comes from recognizing the difference between the initial events that breach tolerance and those that are required subsequently to generate a full-blown autoimmune disease. While initiation of autoreactive B cell responses may be T-independent, for chronic disease to occur, it seems clear that T cell tolerance must also be broken. There are two non-exclusive mechanisms by which this can happen. Autoreactive T cells could be activated by myeloid APCs, such as DCs and macrophages, in a more classically accepted pathway for the activation of naïve T cells in normal immune responses [16]. Alternatively, naïve autoreactive T cells could be activated directly by cognate B cells that were previously activated in a T-independent, BCR and TLR-dependent fashion. Regardless of which mechanism causes T cell activation, after such T cells are activated, they may then modify or expand pre-existing autoreactive B cell reactions that were first incited without the help of T cells. Once this point is reached, a positive feedback cycle, as we have previously discussed [4,5], is achieved.

Aside from data showing that T-deficient or T-depleted lupus prone mice have reduced disease and autoantibody production, several studies suggest that activated T cells can promote further B cell activation in the second part of the feedback loop. When T cell help is provided to anergic anti-DNA B cells, they undergo brisk activation, with extrafollicular proliferation and AFC formation [18]. Similarly, anti-Sm T cells that had broken tolerance in an autoimmune prone background, upon transfer into normal mice that harbored anergic anti-Sm B cells, were able to promote their differentiation into AFCs [19]. In our work, we did find a partial role for T cells as well in both induced and spontaneous RF reactions, indicating that while T cells are not required for activation, when present they do modify the response. Recent work from Odegard et al. has identified a specialized T cell subset in MRL/lpr mice, with some resemblance to follicular helper T cells, but which reside at the extrafollicular site, which may be implicated this process naturally [70]. Interestingly, these T cells express high levels of ICOS and IL-21, and these two molecules may play key roles in promoting T-B interactions and the B cell response.

Can B cells activate naïve or anergic T cells?

Our model raises an important and controversial question: Can B cells present autoantigens to naïve T cells and activate these T cells from a tolerant or ignorant state? Classical studies reached the conclusion that naïve B cells cannot activate naïve T cells and may even turn them off [71,72]. However, the situations contrived in these experiments are probably not physiologically relevant to autoimmunity in vivo, as in these papers B cells were caused to present Ag without cross-linking their BCR. In autoimmunity with respect to Ag-specific B cells, it is axiomatic that B cells presenting to T cells will, at the least, have had their BCRs ligated by the relevant Ag.

In fact, a number of experiments have indicated that B cells can promote activation T cells in vivo when the B cells have received a BCR signal. CD4 T cell responses to protein Ags are impaired when B cells are eliminated, either via genetic mutation or induced depletion [15, 73,74]. However, these studies have generally been interpreted to mean that B cells amplify responses initiated by other APCs. It is more controversial whether B cells can primarily activate naïve T cells and even self-reactive T cells, which presumably could be anergic. Mamula, et al. demonstrated that priming B cells with human cytochrome c in vivo, then immunizing with mouse cytochrome c, which can be recognized by the human cytochrome-specific B cells, will allow T cell tolerance to mouse cytochrome c to be broken [75]. Similar data were obtained with snRNP-specific responses, in both normal and BCR Tg mice [19, 76]. In most of these experiments, a primary role for DCs or other APCs—perhaps enhanced by Ab that directed Ag to the FcR-bearing APCs—could not be excluded. Yan et al. however showed in mice that lacked secreted Ab that SmD protein targeted to hapten-specific B cells was effective in breaking T cell tolerance whereas the same Ag that was not targeted had no effect [77]. In elegant transfer studies, Rodriguez-Pinto and Moreno isolated APC capacity to B cells alone and showed that they could prime naïve CD4 T cells [78]. Hence, there is ample evidence that Ag-specific B cells can prime naïve T cells in vivo.

Conclusions

The cellular interactions that initiate and promote autoimmunity are still poorly understood, though both animal models and new clinical results demonstrate a key role for B cells. We have proposed that B cells could be activated before autoreactive T cells, by virtue of combined BCR and TLR signals delivered by selected autoAgs. B cells could then become a vector for activating autoreactive B cells, spreading, propagating and stabilizing autoimmunity. This second step may separate innocuous, transient autoimmunity from bona fide autoimmune disease. To what extent this scenario applies to various patients, animal models, autoimmune

diseases, and autoantigens is unclear. There may be multiple routes to the same final positive feedback situation that defines autoimmunity. In addition to investigation of further models and more mechanistic patient data, it will be useful to determine directly the roles of other APCs, chiefly DCs, in systemic autoimmunity. Ultimately, it will be necessary to devise systems to track and visualize the activation of autoreactive T cells in systemic autoimmunity in order to understand linked T and B cell activation. The goal is a more comprehensive model that will not only be able to explain disease and responses to emerging cell and molecule-specific therapies, but also to predict new such therapies.

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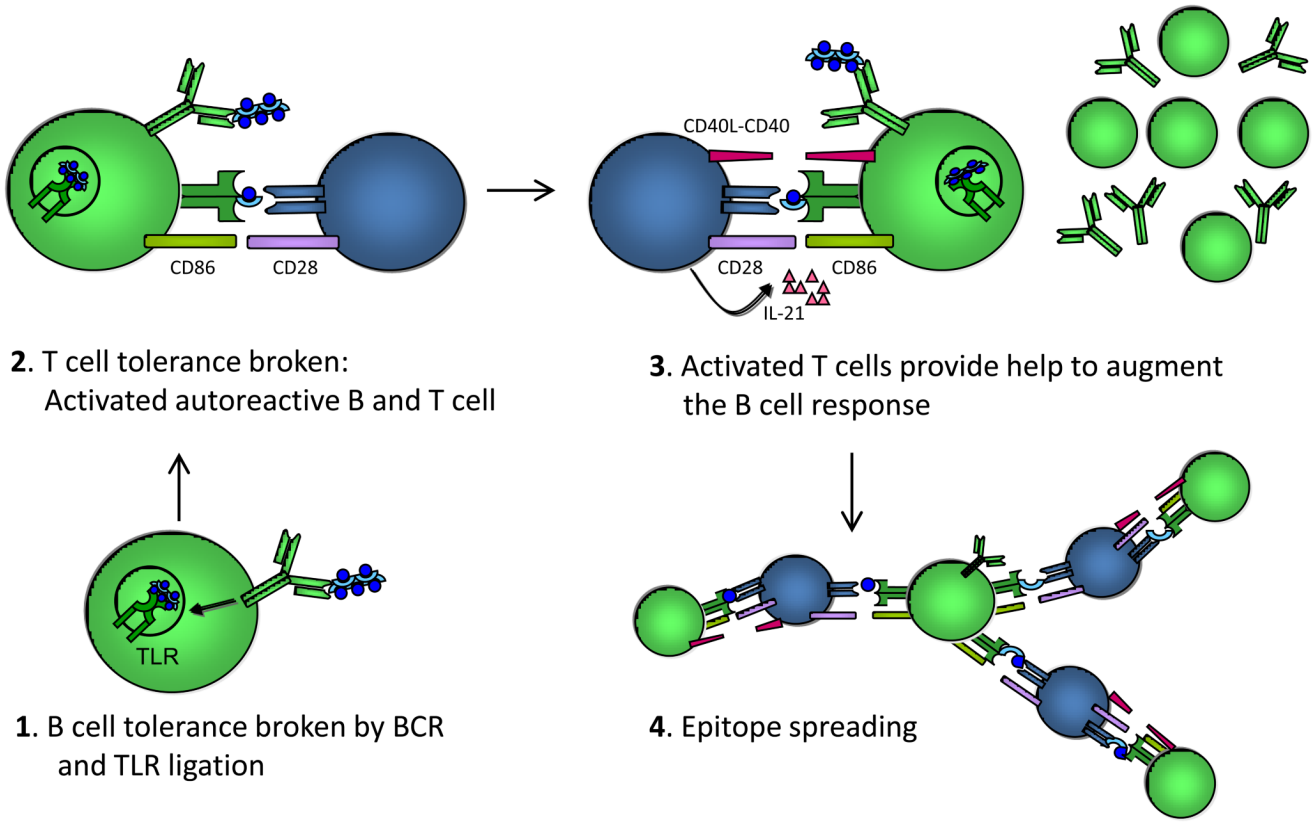

Figure 1. Proposed model for B cell-mediated, TLR-dependent initiation of the autoimmunity feedback cycle

In step 1, B cell tolerance (or ignorance) is broken in autoAg-specific B cells that recognize self-Ags that carry an endogenous TLR ligand, such as DNA. In step 2, these B cells present autoAg to any self-reactive T cell that can recognize an epitope that is contained on the self-Ag recognized by the B cell. In this case, it is the “blue circle”. The T cell becomes activated, expressing CD40L and IL-21 (shown in step 3) along with other costimulatory molecules and cytokines (not shown). In step 3, these activated T cells provide help to the cognate B cells, leading to enhanced Ab production, isotype switching, clonal expansion and somatic hypermutation. In step 4, the activated B cells can present various different epitopes to T cells with different specificity allowing these in turn to promote the activation of additional autoreactive B cells. Some of these may not need a TLR ligand for activation as they have help now from previously activated T cells. Examples of this include cells specific for the “semi-circular light blue disk” on the right of the panel.