Killer B lymphocytes: the evidence and the potential

Steven K. Lundy

Division of Rheumatology, Department of Internal Medicine, University of Michigan Medical School, Ann Arbor, MI 48109-2200, USA

Steven K. Lundy: sklundy@umich.edu

Abstract

Immune regulation plays a critical role in controlling potentially dangerous inflammation and maintaining health. The Fas ligand/Fas receptor axis has been studied extensively as a mechanism of killing T cells and other cells during infections, autoimmunity, and cancer. FasL expression has been primarily attributed to activated T cells and NK cells. Evidence has emerged that B lymphocytes can express FasL and other death-inducing ligands, and can mediate cell death under many circumstances. Among B cell subsets, the expression of both Fas ligand and IL-10 is highest on the CD5⁺ B cell population, suggesting that CD5⁺ B cells may have a specialized regulatory function. The relevance of killer B cells to normal immune regulation, disease pathogenesis, and inflammation is discussed.

Keywords

B lymphocytes; Immune regulation; Fas ligand; Th cell apoptosis; Cell-based therapy

Introduction

Inflammation is a necessary mechanism used by the immune system to control infectious microorganisms. In the majority of instances, the coordination of the immune response to a specific stimulus is directed by the activity of CD4⁺ T helper (Th) cells. Unfortunately, misdirected or poorly regulated immune inflammatory reactions can lead to serious complications including atopy, anaphylaxis, autoimmunity, cachexia and sometimes death. Thus, the regulation of Th cells following an inflammatory event is a critical factor in maintaining the health of the individual. Th cells receive antigen-specific stimulation through interaction of the T cell receptor with peptide antigens bound to Class II MHC molecules on the surface of professional antigen presenting cells (APC), including activated B lymphocytes.

Control of inflammatory reactions consists of both passive and active immune suppression. Passive mechanisms of suppression include antigen clearance or sequestration, cytokine withdrawal, decreased co-stimulation and/or T cell anergy. One mechanism of active immune regulation is the release of immune suppressive cytokines including, IL-10 and TGF β , by regulatory T cells and other cells. Another major mechanism of immune regulation is activation-induced cell death (AICD or apoptosis) mediated by death-inducing ligands. These include: Fas ligand (FasL; CD178), tumor necrosis factor-related apoptosis-inducing ligand (TRAIL: CD253), and programmed death ligands 1 and 2 (PD-L1: CD274

Correspondence to: Steven K. Lundy, sklundy@umich.edu.

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and PD-L2: CD273) [5,6,81]. The critical importance of active immune suppression to the control of inflammation and autoimmunity has been highlighted by studies performed in mice lacking one or more of these suppressive mechanisms [79,88].

There has been some renewed interest in production of IL-10 and TGF β by 'regulatory' B cells, and recent studies have shown that B cell subsets differ in their ability to produce these cytokines [45,56,63,103]. Although the cytokine-based regulatory properties of B cells have been a focus of increasing attention, the expression of death ligands by 'killer' B cells and their ability to regulate Th cell-mediated reactions has gone relatively unnoticed.

Expression of FasL has primarily been reported in activated T cells, NK cells, tumor cells from multiple sources, and at sites of immune privilege such as the eye and reproductive organs. Although most studies of FasL-mediated apoptosis have focused on T cells as the effector cells, a growing body of evidence suggests that activated B cells can express FasL and mediate cell death [30,49,67]. In addition to FasL, B cells have been reported to express the other death-inducing ligands: TRAIL, PD-L1 and PD-L2 [35,54,108].

An especially interesting finding is that the CD5⁺ B cell subsets may have multiple regulatory properties including: constitutive FasL and PD-L2 expression, and a propensity to produce IL-10 upon activation by bacterial and parasitic antigens [48,71,86,103,108]. The unique tissue distribution, antibody production, and evidence of immune deviation found in mice lacking CD5⁺ B cells suggest that these cells may play an important role in controlling mucosal immune reactions and autoimmunity [27,47,62].

Death-inducing ligand expression by B cells, the relative importance of killer/regulatory B cells in disease and immune homeostasis, and the potential therapeutic uses of killer B cells have not been reviewed previously. This article is intended to present in detail the many lines of evidence supporting a killer function of B cells, highlight some of the gaps in our current understanding, and suggest some possible uses of killer B cells in cell-based immune therapy.

Death ligand expression by B cells

Leukemia and lymphoma

The expression of death-inducing ligands by tumor cells is thought to be an important mechanism of escape from immune surveillance. In 1998, three independent groups reported expression of death ligands on non-Hodgkin's B cell lymphoma and leukemia [53,66,92]. The first group studied expression of FasL and TRAIL on a panel of human and mouse T and B cell lymphoma and leukemia lines and found that either one and sometimes both death ligands were expressed on transformed B cells [53]. In addition, FasL expression was found to be higher in more aggressive forms of B cell lymphoma such as diffuse large B cell lymphoma and Burkitt's lymphoma than in other less aggressive lymphomas [66]. In 27 of 30 patients with B chronic lymphocytic leukemia (B-CLL), CD19⁺ B cells stained positive for FasL, and each of the FasL⁺ lines tested were able to mediate cell death of a susceptible T cell line [92]. This killing effect of B-CLL lines could be blocked by neutralizing antibodies against either Fas or FasL. Several subsequent studies have reported the expression of FasL by B cell leukemia and lymphoma, and have linked FasL expression to the escape of B cell neoplasms from immune attack and worse cancer prognosis [39,74,110]. It is noteworthy that some B cell neoplasms that are not high expressors of FasL tend to be less aggressive [29,66].

A series of studies have demonstrated that FasL and TRAIL are upregulated on malignant plasma cells in multiple myeloma patients [82,83,97]. Stage III myeloma cells had much

higher expression of FasL and lower expression of Fas than lower stage myeloma plasma cells [83]. Similar results were found for TRAIL, and the high expression of FasL and TRAIL correlated with increased erythroblast apoptosis and anemia in advanced stage myeloma patients. In addition, myeloma patients with erosive bone disease were found to have higher expression of FasL and TRAIL on plasma cells in the bone marrow, and osteoblasts from these patients were highly susceptible to apoptosis mediated by the FasL⁺ and TRAIL⁺ myeloma cell line, MCC-2 [82].

To date, there has been only one published report of expression of PD-L1 on the A20 B lymphoma cell line [22]. PD-L2 expression on B cell leukemia or lymphoma cells has not been demonstrated. However, constitutive expression of these death ligands on non-transformed B cells would suggest that malignant B cells may also express PD-L1 and PD-L2, and use these ligands to induce apoptosis and escape immune surveillance [35,108].

Death ligands on non-transformed B Cells

In 1996, Hahne et al. [30] published the first report of FasL expression on non-transformed, mouse B lymphocytes. This brief yet thorough study demonstrated that PMA/Ionomycin-stimulated mouse splenocytes displayed upregulated surface expression of FasL on T and B cells. Specificity of the anti-FasL antibody used for flow cytometry was demonstrated by partial blockade by a peptide derived from FasL. Activation of purified B cells with lipopolysaccharide (LPS) alone or in combination with PMA/Ionomycin resulted in increased FasL mRNA, and elevated protein levels of FasL were measured by flow cytometry and Western blotting. Further evidence of functional FasL expression by LPS-stimulated, purified B cells came from chromium release assays in which the Fas⁺ A20 lymphoma line was sensitive to target cell lysis by B cells from C3H mice but not *gld*-C3H (FasL-deficient) animals.

Several other studies have demonstrated the expression of FasL on B cells freshly isolated from human lymphoid organs [40,68,87]. In one of these studies, expression of FasL was low in the thymus and most lymph nodes, but was detected in the spleen, tonsil and mucosal-associated lymphoid tissue, primarily on Ig⁺ plasma cells [87]. Interestingly, the FasL protein was most abundant in para-nuclear compartments within the cytoplasm of the plasma cells, suggesting that FasL might be released in its soluble form by plasma cells.

Earlier studies of TRAIL and FasL expression on transformed T and B cells were also extended to activated non-transformed B cells [54]. It was found that approximately 25% of freshly isolated B220⁺ cells from healthy mice were positive for TRAIL, and that both TRAIL and FasL were expressed by activated B220⁺ cells. As further proof of the activation-induced expression of TRAIL on B cells, CpG oligodeoxynucleotides (CpG-ODN), bacterial ligands for toll-like receptor 9, were capable of inducing TRAIL expression on human B cells [36]. Tumor cell lysis mediated by CpG-ODN-stimulated B cells was completely blocked by a TRAIL-R2.Fc fusion protein but not by a FasR.Fc construct. It was also determined that IFN- α alone or in combination with anti-CD40 antibody stimulation was an effective means of inducing TRAIL on CD19⁺ human B cells.

PD-L1 and PD-L2 are differentially expressed members of the B7 costimulatory family that can confer death signals to cells expressing the PD-1 receptor [14,35,81]. PD-L1 is constitutively expressed by T and B lymphocytes, as well as being found on dendritic cells, macrophages, mast cells and many types of non-hematopoietic cells. Like FasL, PD-L1 is expressed at sites of immune privilege and has been shown to play a role in protection from autoimmune diseases [12,81]. PD-L2 expression has been reported to be restricted to activated dendritic cells and macrophages; however, a recent report has shown that a subset of splenic and peritoneal CD5⁺ B cells have constitutively high expression of PD-L2 [108].

In this study, PD-L2 expression was not stimulated on CD5⁺ or CD5⁻ B cells by a panel of stimuli including: cytokines, TLR ligands, CD40 ligand or B cell receptor. In contrast, LPS, CpG and CD40 ligand treatment decreased PD-L2 expression on CD5⁺ B cells. These data suggests that PD-L2 expression is an intrinsic property of resting CD5⁺ B cells that is lost upon TLR or costimulatory activation.

Regulators of Fas ligand expression by B cells

In addition to FasL expression induced on B cells by mitogens such as PMA/Ionomycin and LPS, several groups, including our own have discovered upregulation of FasL on B cells during parasitic and viral infections [49,75,90,111].

Studies of mice infected with the helminth parasite, *Schistosoma mansoni*, revealed that FasL expression on B cells increased in parallel with the severity of granulomatous inflammation [49]. Depletion of B cells from schistosome egg antigen- (SEA-) stimulated splenocyte cultures led to decreases in T cell apoptosis, and T cell survival and granuloma formation were exacerbated in B cell-deficient mice. Other notable findings in the schistosome model were that FasL was constitutively expressed by splenic CD5⁺ B cells, and that FasL expression and CD5⁺ B cell killer function were upregulated by stimulation with antigens extracted from schistosome eggs [48]. SEA-stimulated FasL expression on CD5⁺ B cells was further enhanced by IL-4 and IL-10. Other studies have shown that FasL is induced on B cells during infection with *Trypanosoma cruzi* [1,111].

Several viral infections, including human immunodeficiency virus, murine leukemia virus and Epstein-Barr virus (EBV) have been reported to induce B cells to express FasL [73,75,77,90]. The mechanisms by which viruses induce FasL expression have not been studied.

Ligation of surface CD54 (ICAM-1) on Burkitt lymphoma cell lines resulted in the upregulation of FasL by the B cell lymphomas, and induction of Fas-dependent cell death [38]. A subsequent study by the same group demonstrated that activation of B7-H4 on EBV-transformed B cell lines led to increased FasL expression and apoptosis [85]. In both of these studies, activation of reactive oxygen species was shown to be an integral part of the pathway leading to FasL expression on the B cell lines. These studies have not yet been carried forward to non-transformed B cells, nor has it been directly demonstrated that ICAM-1- or B7-H4-induced FasL expression on these transformed B cell lines might confer a protective effect by inducing T cell apoptosis. The demonstrated susceptibility of transformed B cells to FasL-induced fratricide does have therapeutic implications, however, it remains to be seen whether induction of FasL on transformed B cells will have the same effect in vivo.

Other factors that induce FasL expression on B cells that are not associated with infection include: stimulation with toxic mistletoe lectins and cockroach allergen [11,47]. Mistletoe lectins have been studied experimentally to induce tumor cell apoptosis, and have been shown to stimulate FasL expression by both T cells and B cells [11]. In the cockroach allergen model of asthma, FasL expression was induced on lung CD5⁺ B cells after repeated airway antigen exposure, and mice lacking CD5⁺ B cells had a marked reduction in lung T cell apoptosis [47].

Another interesting finding was the upregulation of FasL on B cells in mice deficient for the MHC Class II-transactivator, CIITA, which is involved in the regulation of MHC Class I and II, invariant chain expression, and suppression of IL-4 gene transcription [28]. The expression of FasL on CIITA^{-/-} B cells was upregulated by LPS but inhibited when IL-4 was added. This crosstalk between FasL expression, CIITA, and IL-4 has also been found in

mice infected with *T. cruzi* [1]. In a separate study, ligation of MHC Class II on human B cells resulted in the upregulation of Fas ligand protein within the B cells, and increased B cell fratricide [93]. Table 1 summarizes the factors that have been shown to upregulate or control Fas ligand expression on B cells.

Killer/regulatory B cells in health and disease

Many lines of evidence have implicated B cells as an important regulatory cell population during infections, autoimmunity and immune tolerance. Much of the early work done in this field was based on the experimental models performed in B cell depleted or deficient mice in which Th cell activity was consistently upregulated. However, it was not until relatively recently that the mechanisms underlying B cell-mediated regulation were elucidated. Many of these recent studies have correctly been focused on the expression of IL-10 and TGF β by B cells, and have been well reviewed [23,56,63]. As noted in the previous section, FasL expression on B cell malignancies is often associated with poorer prognosis. Following are additional lines of evidence that suggest that FasL expression by B cells may also play a role in immune regulation during infections and immune-mediated diseases. Table 2 summarizes the known B cell malignancies, infections and disease models in which death ligand expression on B cells has been demonstrated.

Parasitic infections

Much of the early evidence of a regulatory role for B cells came from studies of experimental infection with *S. mansoni* helminth worms. In this model, eggs produced by the adult female become trapped primarily in the post-sinusoidal capillaries of the host liver and induce a T cell-mediated granulomatous inflammation [10]. It has long been recognized that a fundamental property of the schistosome granuloma is that chronic infection causes spontaneous down modulation of the granulomatous response to freshly implanted eggs [9]. Depletion of B cells from mice infected with either *S. mansoni* or *S. japonicum* did not change disease pathology in the acute reaction phase, but drastically impaired granuloma down modulation in the chronic phase of infection [13]. This regulatory function of B cells was originally thought to be mediated by regulatory antibodies or interactions with suppressor T cells. Although these effector functions should not be ruled out, a number of reports have now brought attention to B cell-mediated regulation by IL-10 and Fas ligand [48,49,96]. It was demonstrated that lacto-N-fucopentaose (LNFP-III), a component of schistosome eggs, was able to stimulate IL-10 production from splenic B cells [96]. Subsequent studies showed that FasL expression on B cells, particularly CD5⁺ B cells, was inducible by schistosome egg antigens and was upregulated by IL-10 and IL-4 [48]. Gaubert et al. [27] demonstrated that schistosome infection of X-linked immunodeficiency (XID) mice, which lack CD5⁺ B cells, had increased granulomatous inflammation, reduced IL-10 production, increased expression of IL-4 and IFN- γ , and higher mortality rates. T cell apoptosis was not reported in that study, however, our lab has found that intravenous injection of schistosome eggs into XID mice resulted in decreased T cell apoptosis in the lung during acute inflammation as compared to wild-type mice (unpublished observation).

Studies of other parasitic infections in XID mice have also confirmed that B cells are important producers of IL-10 and regulators of T cell activation [4,62,71]. Some reports have shown that infection with *T. cruzi* stimulates antigen-specific B cells to express FasL and results in increased B cell apoptosis [111]. Unlike what was found in the schistosome infection model, IL-4 played a down-regulatory role on B cell FasL expression following *T. cruzi* infection [1]. This result was attributed to the upregulation of CIITA, a negative regulator of FasL in B cells [1,28].

It has been suggested that reductions in parasitic infections are correlated to the increases in allergic and autoimmune diseases noted over the past several decades [21,100]. Schistosome infection or injection of schistosome eggs has been shown to be protective in several experimental models of allergy and autoimmunity [16,20,42,51,80]. A study of the direct role that FasL expression by B cells plays in helminth-induced immune tolerance to secondary immune challenges has yet to be performed.

Viral and bacterial infections

A limited number of studies have shown that B cells have regulatory functions in viral infections. Splenocytes from mice infected with herpes simplex virus type 1 (HSV-1) were resistant to mitogen-induced proliferation compared with uninfected mice [76]. The suppressor activity disappeared when HSV-1 infection was performed in CD5⁺ B cell-deficient XID mice; however, the mechanism of regulation was not determined, leaving open the possibility that suppressive cytokines, death-inducing ligands, or both mechanisms were important in this model. In a different study, human peripheral blood T cells exposed to EBV, another member of the herpes virus family, upregulated Fas (CD95) on several lymphoid populations, but only CD4⁺ T cells exhibited increased susceptibility to undergo apoptosis [90]. Among the lymphocyte populations tested, only CD20⁺ B cells displayed an EBV-mediated increase in FasL expression. These data suggests that EBV-infected B cells expressing FasL may selectively kill CD4⁺ T cells.

In an in vitro study of peripheral blood lymphocyte apoptosis of HIV-infected individuals, B cells were the predominant FasL-expressing lymphocyte population [75]. Likewise, in an experimental infection using the SHIV C2/1 virus to infect cynomolgus monkeys, a model of HIV infection in humans, FasL upregulation was noted on both CD8⁺ T cells and HLA-DR⁺/CD20⁺ B cells [77]. Studies of murine acquired immunodeficiency syndrome (MAIDS) in XID mice have revealed that CD5⁺ B cells are an important mediator of disease initiation and progression [34,89].

Purified lymphocyte subsets including B cells but not dendritic cells from AKR.H-2^b mice, which spontaneously produce MuLV retrovirus, interfered with the virus-specific killing efficacy of CD8⁺ T cells [73]. This effect was mediated by FasL/Fas interactions and B cells were shown to have higher FasL expression in AKR.H-2^b mice than either CD4⁺ or CD8⁺ T cells. What is lacking from these studies showing B cell FasL expression during HIV or MuLV infection are direct killing assays using purified populations of B cells as effectors and T cells as targets.

Much less is known about the effects of bacterial infection or exposure to gut flora on the killer/regulatory function of B cells. In the original description of FasL expression by activated B cells, bacterial LPS was shown to be one of the stimuli that induced functional FasL on mouse B cells [30]. A more recent study has demonstrated that murine B cells activated with LPS expressed higher levels of both FasL and TGF- β , and were able to protect NOD/scid mice from the induction of diabetes mediated by adoptive transfer of T cells from diabetic NOD mice [91]. It is not yet clear whether the effect of LPS-stimulated B cells on diabetes induction was mediated by FasL, TGF- β or a combination of signals from both molecules. It has also not been established whether gram-negative, bacterial LPS naturally stimulates killer/regulatory B cell phenotype in vivo.

Infection of B cell-deficient (μ MT) mice with *Mycobacterium tuberculosis* led to exacerbated lung granuloma formation marked by increases in lung T cells and neutrophils as compared to wild-type mice [50]. Interestingly, the increased lung inflammatory response in μ MT mice could not be attributed to the lack of local IL-10 production, since this immune suppressive cytokine was upregulated in the lungs of MTb-exposed B cell-deficient

mice. A recent report suggested that IL-10 produced by B cells in the spleen rather than at the local site of inflammation could be responsible for T cell regulation mediated by the MTb found in complete Freund's adjuvant [103]. However, it also remains possible that death ligand expression is induced on B cells following activation by MTb [36].

In a study of adjuvant-induced arthritis (AIA), a disease model initiated by injection of MTb at the base of the tail in Lewis rats, B cells genetically engineered to express a mycobacterial 65-kd heat shock protein- (Hsp65-) IgG fusion protein had a protective effect against the development of AIA [78]. Along with an increase in Hsp65-specific antibody titers, a marked reduction of Hsp65-specific T cell activation was noted in mice that received Hsp65-IgG engineered B cells. It was not determined whether the decrease in antigen-specific T cell response was mediated by cell death. Further studies of the effects of LPS and mycobacterial infections on B cell activation, particularly the expression of FasL and other death-inducing ligands, and the ability of bacteria-activated B cells to kill antigen-specific T cells is warranted.

B cells in allergy, autoimmunity, and transplantation tolerance

In addition to the involvement of killer/regulatory B cells during infections, there is an increasing amount of data to suggest that B cells are involved in the prevention or control of several T cell-mediated diseases including, airway inflammation and asthma, organ-specific and systemic autoimmunity, and transplant rejection. Many of these studies have focused on the ability of B cells to produce IL-10 and/or stimulate the induction of T regulatory cells [18,24,45,52,57,64,99,107]. Some experimental evidence suggests that FasL expression and death induction mediated by B cells may also play an important role in maintaining or inducing tolerance.

It has been suggested that the role of Fas ligand-bearing cells in asthma is to preferentially eliminate Th1-type T cells, allowing the Th2 cell response to dominate and cause airway inflammation and asthma [3,58]. A study of cockroach allergen-induced airway inflammation in XID and wild-type CBA/J mice revealed that CD5⁺ B cells in the inflamed lung were the highest expressors of Fas ligand, and that lung CD4⁺ T cell apoptosis was dramatically impaired in XID mice [47]. XID mice also had increased airway inflammation, lung eosinophilia, and mucus hypersecretion compared with controls. In addition, levels of the Th2 cytokines, IL-5 and IL-13, were significantly elevated in the lungs of XID mice, with levels of IL-4 and IFN- γ also higher in the lungs of some, but not all XID mice. Surprisingly, levels of IL-10 were also significantly higher in the lungs of XID mice than in controls, suggesting that CD5⁺ B cells exerted control over both Th1 and Th2 cells in the lung, independent of IL-10 but correlated with Th cell apoptosis. A separate study related to the lung mucosa demonstrated that T cell tolerance induced by intranasal injection of antigen was mediated by antigen-specific B lymphocytes [94]. This group primarily looked at antigen-specific cell proliferation, and did not address whether T cell apoptosis mediated by B cells played a role in the findings. It will be interesting to determine whether airway T cell tolerance induction is related to the death-inducing activity of lung resident B lymphocytes.

Thus far, the clearest and most direct evidence of a role for FasL⁺ B lymphocytes in inducing T cell tolerance came from a study conducted by Minagawa et al. [61]. These authors studied the role of Fas ligand in a transplantation model in which H-Y containing male skin grafts were rejected by naïve, syngeneic, female mice, but accepted when the recipient mice had previously been injected with male spleen cells. With the use of wild-type and Fas-deficient, *lpr*, female mice, and wild-type, *lpr*, and FasL-deficient, *gld*, male mice, it was elegantly shown that tolerance to H-Y was mediated by FasL⁺ splenocytes. Further analysis using splenic B cells purified by magnetic bead separation revealed that

FasL⁺ B cells were sufficient for the induction of tolerance and acceptance of skin grafts. It is perhaps important to note that other than positive selection using anti-CD19 magnetic beads, the splenic B cells used in the above study were not treated before injection into the female recipients. This result suggests that naïve, splenic B cells in healthy mice may be capable of inducing T cell tolerance, a result similar to what was found in the schistosome model [48].

Data regarding the natural ability of FasL⁺ B cells to tolerize against self antigens is currently lacking. One report has described FasL and TGF β expression on LPS-stimulated B cells resulting in the suppression of diabetes in NOD mice [91]. Unfortunately, the relative importance of FasL versus TGF β was not assessed in this study nor can it be inferred that LPS originating from gut flora induces killer/regulatory B cell function. It has been demonstrated that B cells from patients with systemic lupus erythematosus constitutively expressed Fas ligand [67]. B cells isolated from lupus-prone (*lpr*) mice also spontaneously expressed FasL and had cytotoxic activity greater than or equal to T cells and NK cells [8]. In the case of lupus, FasL expression by B cells was postulated to limit the suppressive effects of T cells, and confer protection on the autoantibody-secreting FasL⁺ B cells, thus leading to poorer disease prognosis [8,67].

In summary, FasL expression by B cells appears to be of functional importance in parasitic, viral, and bacterial infections, as well as in immune-mediated diseases. B cell FasL expression appears to play a role in the induction of T cell tolerance, and this may lead to either protection or pathogenicity depending on the disease being studied.

The CD5⁺ B cell debate

The CD5⁺ B cell compartment seems to possess several important immune regulatory properties [48,70,74,103,108]. B lymphocyte subpopulations, like T cell subsets, have been divided according to the expression of cell surface markers. The four main subsets of B cells are B-1a, B-1b, B-2 and marginal zone (MZ) B cells. These subpopulations can be distinguished by cell surface markers, and have been shown to be phenotypically distinct [55]. Traditionally, CD5 was thought to be a distinctive marker of the B-1a cell group, which is highly represented in the peritoneal and pleural cavities. As such, many studies have been performed to determine the phenotypic differences between CD5⁺ and CD5⁻ B cell subsets. Some of the major differences in functional properties of B-1a cells and follicular B-2 cells are outlined in Table 3.

Some controversy has arisen regarding the true identity and origin of CD5⁺ B cells because of studies showing that CD5 expression could be transiently induced by the activation of previously CD5⁻ splenic B cells [15,26]. In fact, splenic CD5⁺ B cells have been shown to be phenotypically different from both splenic B-2 cells and CD5⁺ B-1a cells found in the peritoneum [25,95]. More recently, three distinct precursors have been identified that develop into *bona fide* B-1a cells, B-1b cells and B-2 cells, respectively [65]. It may well be that CD5 is a poor marker to distinguish conventional B-1a cells from CD5⁺ B cells found in the spleen. Unfortunately, most of the previous studies regarding the functional properties of B cells only used CD5 as a marker, and did not distinguish between *bona fide* CD5⁺/CD11b⁺ B-1a cells and CD5⁺ B cells originating from non-B-1a cell compartments, thus complicating data interpretation.

Despite this controversy, splenic CD5⁺ B cells have been shown to produce IL-10 and to express FasL at levels higher than splenic CD5⁻ B cells [48,103]. These data, combined with many studies showing increased T cell activation in CD5⁺ B cell-deficient (XID) mice, suggest that one, if not all of the CD5⁺ B cell populations can regulate T cell activation. It will be interesting to determine whether the splenic CD5⁺/CD21⁺ B (B10) cells that were

recently shown to produce high levels of IL-10, are also high expressors of FasL. If so, then this subset of cells may be especially potent at regulating T cell-mediated inflammation and may be a good target for developing antigen-specific, cell-based immunotherapy strategies.

The potential of killer B cells in the treatment of autoimmunity and other pathogenic immune reactions

Presentation of antigens by killer/regulatory B cells

Activated B cells are professional APC that have been shown to be critical for T cell activation [46,69,109]. Some studies have demonstrated that CD5⁺ B cells are more potent APC than conventional follicular B-2 cells [109]. One of the most striking differences between B-2 cells and CD5⁺ B cells is that the latter tend to produce antibodies with specificities for non-protein antigens, such as carbohydrates, lipids and glycolipids [7,60]. Many of these polyreactive, “natural” antibodies are also self antigen-specific, implicating CD5⁺ B cells as a major source of autoreactive antibodies [17,32,33]. Because B cells acquire most of the antigen that they present via MHC Class II molecules by first binding the antigen through surface immunoglobulin, autoreactive CD5⁺ B cells would be very likely to present autoantigens to Th cells. Autoantigen presentation in the context of death ligand expression would be expected to induce cell death of autoreactive T cells (Fig. 1) Therefore, the killer/regulatory phenotype of CD5⁺ B cells may normally serve to protect against the development of autoimmunity. Reciprocally, biological forces that decrease or abolish B cell-mediated regulatory molecule expression may not only result in a release of autoreactive T cells from death or regulation, but might convert autoreactive B cells into proinflammatory APC, thus becoming inducers of auto-reactivity (Fig. 1) Whether or not such a dramatic switch in B cell phenotype occurs has yet to be determined, but factors that would control such a switch would be attractive targets for therapeutic intervention.

B cells have long been implicated as a pathogenic population in autoimmune diseases. Depletion of B cells using antibodies directed against CD20, has been shown to be effective at inhibiting autoimmunity in murine models [2,102,104]. The utility of B cell depletion as a therapy for rheumatoid arthritis patients has also reached clinical trials, with favorable results thus far [19,44]. Interestingly, a study by Hamaguchi et al. [31] demonstrated that B cells in the peritoneal cavity of mice, particularly CD5⁺ B-1a cells, were resistant to depletion by anti-CD20 antibodies. Since B-1a cells may be especially high producers of IL-10 and Fas ligand, these data would suggest that partial B cell depletion could result in a relative increase in killer/regulatory B cells. Similarly, early reconstitution of B cells in humans following B cell depletion therapy also seems to favor CD5⁺ B cells [72]. It will be interesting to determine whether or not these repopulating human B cells are able to express FasL and whether they are able to prevent recurrent inflammation and autoimmunity via T cell apoptosis.

Lessons from engineered FasL⁺ APC

A series of studies has demonstrated the treatment potential of forcing expression of FasL on professional APC [37,41,106]. Genetic engineering resulting in the overexpression of FasL on either dendritic cells or B cells, and subsequent transfer into mice, led to antigen-specific T cell tolerance and amelioration of collagen-induced arthritis or contact hypersensitivity [37,41,106]. A potential hazard of injecting cells engineered to express FasL is the induction of liver toxicity, due to high Fas expression on hepatocytes. However, systemic introduction of FasL-expressing APC did not induce significant liver damage, despite inducing death of T cells infiltrating several organs, including the liver [105]. These data suggest that the specific interactions of T cells with FasL⁺ APC are necessary for cell death induction. Thus, the upregulation of FasL specifically on APC, including B cells, may be a relatively safe

way to target antigen-specific T cells without affecting non-specific T cells or non-hematopoietic cells.

Toward a non-engineered approach to FasL upregulation on B cells

There are foreseeable drawbacks to engineering FasL expression on B cells or other APC as a treatment modality. First, there are safety issues associated with the viral vectors used to introduce the FasL gene. Second, the diversity of MHC Class I and II expression for each individual will necessitate tailoring each treatment to use APC derived from that particular patient. Third, in vitro manipulation of the APC and testing for FasL expression will be complex and time-consuming. These issues can be expected to make FasL-transfection cost prohibitive and unlikely to be developed into mainstream therapy. Therefore, it is much more attractive to find a way to manipulate the endogenous B cells to acquire and/or maintain FasL expression either in vivo, or with limited induction ex vivo followed by adoptive transfer. A potential benefit to the ex vivo approach is that the killer B cells could be loaded with specific peptides prior to reintroduction into the recipient, thus allowing for control over the specificity of the T cells to be targeted. Alternatively, targeting of specific antigens to killer/regulatory B cells in vivo might be achieved by conjugation of the genes to B cell tropic retroviruses [101]. This type of approach has been demonstrated to induce T cell tolerance in animal models of multiple sclerosis and diabetes, and to be dependent on B cell expression of FasL [59]. A third alternative may be to block the signals that either decrease regulatory molecule expression by B cells or that are involved in the conversion of regulatory B cells to a more proinflammatory phenotype. These approaches will require a better understanding of the factors that control expression of death ligands, and suppressive or proinflammatory cytokine production by B cells.

Conclusions

The demonstrated ability of transformed and non-transformed B lymphocytes to express functional death-inducing ligands suggests that B cells should be included with cytotoxic T cells and NK cells among the ranks of killer cells. The interaction between T cell receptor and peptide/MHC Class II may make death ligand-expressing, killer B cells particularly well suited to mediate antigen-specific Th cell death. Beyond death ligand expression, B cells have also been shown to express IL-10 and TGF- β , suggesting that B cells may also have regulatory functions similar to T regulatory cells [23,43,84,98]. Interestingly, IL-10 and FasL expression have both been reported on the small pool of splenic CD5⁺ B cells, and may not be mutually exclusive [48,103]. In fact IL-10 and FasL may cooperate in making CD5⁺ B cells highly effective killer/regulatory cells. However, it remains to be seen whether or not the same B cells that express death ligands are also the main producers of immune suppressive cytokines. These intrinsic properties of CD5⁺ B cells may have implications for mucosal immune homeostasis and regulation of diseases such as asthma and inflammatory bowel disease. The potential of Fas ligand-expressing B cells to be useful in the treatment of Th cell-mediated diseases will need further investigation, but may hold great promise based on evidence from studies of genetically engineered FasL⁺ APC.

Conversely, FasL expression by CD5⁺ B cells may play a role in the pathogenesis of B-CLL, HIV infection and systemic lupus erythematosus. Therefore, inhibition of death ligand expression on B cells in these contexts may become a potent treatment strategy. Further studies focused on the factors that control death ligand expression and production of immune suppressive cytokines by B cells, and the role that killer/regulatory B cells play in immune homeostasis and disease are expected to have important clinical implications.

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References

1. Acosta Rodriguez EV, Zuniga E, Montes CL, Gruppi A. Interleukin-4 biases differentiation of B cells from *Trypanosoma cruzi*-infected mice and restrains their fratricide: role of Fas ligand down-regulation and MHC class II-transactivator up-regulation. *J Leukoc Biol.* 2003; 73:127–36. [PubMed: 12525570]
2. Ahuja A, Shupe J, Dunn R, Kashgarian M, Kehry MR, Shlomchik MJ. Depletion of B cells in murine lupus: efficacy and resistance. *J Immunol.* 2007; 179:3351–61. [PubMed: 17709552]
3. Akdis M, Trautmann A, Klunker S, Daigle I, Kucuksezer UC, Deglmann W, et al. T helper (Th) 2 predominance in atopic diseases is due to preferential apoptosis of circulating memory/effector Th1 cells. *FASEB J.* 2003; 17:1026–35. [PubMed: 12773485]
4. Al-Qaoud KM, Fleischer B, Hoerauf A. The Xid defect imparts susceptibility to experimental murine filariasis—association with a lack of antibody and IL-10 production by B cells in response to phosphorylcholine. *Int Immunol.* 1998; 10:17–25. [PubMed: 9488152]
5. Alderson MR, Lynch DH. Receptors and ligands that mediate activation-induced death of T cells. *Springer Semin Immunopathol.* 1998; 19:289–300. [PubMed: 9540157]
6. Anel A, Bosque A, Naval J, Pineiro A, Larrad L, Alava MA, et al. Apo2L/TRAIL and immune regulation. *Front Biosci.* 2007; 12:2074–84. [PubMed: 17127445]
7. Arnold LW, McCray SK, Tatu C, Clarke SH. Identification of a precursor to phosphatidyl choline-specific B-1 cells suggesting that B-1 cells differentiate from splenic conventional B cells in vivo: cyclosporin A blocks differentiation to B-1. *J Immunol.* 2000; 164:2924–30. [PubMed: 10706678]
8. Bonardelle D, Benihoud K, Kiger N, Bobe P. B lymphocytes mediate Fas-dependent cytotoxicity in MRL/lpr mice. *J Leukoc Biol.* 2005; 78:1052–9. [PubMed: 16204618]
9. Boros DL. Immunoregulation of granuloma formation in murine schistosomiasis mansoni. *Ann N Y Acad Sci.* 1986; 465:313–23. [PubMed: 3089103]
10. Boros DL. T helper cell populations, cytokine dynamics, and pathology of the schistosome egg granuloma. *Microbes Infect.* 1999; 1:511–6. [PubMed: 10603567]
11. Bussing A, Stein GM, Pfuller U, Schietzel M. Induction of Fas ligand (CD95L) by the toxic mistletoe lectins in human lymphocytes. *Anticancer Res.* 1999; 19:1785–90. [PubMed: 10470116]
12. Carter LL, Leach MW, Azoitei ML, Cui J, Pelker JW, Jussif J, et al. PD-1/PD-L1, but not PD-1/PD-L2, interactions regulate the severity of experimental autoimmune encephalomyelitis. *J Neuroimmunol.* 2007; 182:124–34. [PubMed: 17182110]
13. Cheever AW, Byram JE, Hieny S, von Lichtenberg F, Lunde MN, Sher A. Immunopathology of *Schistosoma japonicum* and *S. mansoni* infection in B cell depleted mice. *Parasite Immunol.* 1985; 7:399–413. [PubMed: 3875829]
14. Collins M, Ling V, Carreno BM. The B7 family of immune-regulatory ligands. *Genome Biol.* 2005; 6:223. [PubMed: 15960813]
15. Cong YZ, Rabin E, Wortis HH. Treatment of murine CD5⁺ B cells with anti-Ig, but not LPS, induces surface CD5: two B-cell activation pathways. *Int Immunol.* 1991; 3:467–76. [PubMed: 1716972]
16. Cooke A, Tonks P, Jones FM, O'Shea H, Hutchings P, Fulford AJ, et al. Infection with *Schistosoma mansoni* prevents insulin dependent diabetes mellitus in non-obese diabetic mice. *Parasite Immunol.* 1999; 21:169–76. [PubMed: 10320614]
17. Duan B, Morel L. Role of B-1a cells in autoimmunity. *Autoimmun Rev.* 2006; 5:403–8. [PubMed: 16890894]
18. Duddy M, Niino M, Adatia F, Hebert S, Freedman M, Atkins H, et al. Distinct effector cytokine profiles of memory and naive human B cell subsets and implication in multiple sclerosis. *J Immunol.* 2007; 178:6092–9. [PubMed: 17475834]

19. Edwards JC, Szczepanski L, Szechinski J, Filipowicz-Sosnowska A, Emery P, Close DR, et al. Efficacy of B-cell-targeted therapy with rituximab in patients with rheumatoid arthritis. *N Engl J Med*. 2004; 350:2572–81. [PubMed: 15201414]
20. Elliott DE, Li J, Blum A, Metwali A, Qadir K, Urban JF Jr, et al. Exposure to schistosome eggs protects mice from TNBS-induced colitis. *Am J Physiol Gastrointest Liver Physiol*. 2003; 284:G385–91. [PubMed: 12431903]
21. Elliott DE, Summers RW, Weinstock JV. Helminths as governors of immune-mediated inflammation. *Int J Parasitol*. 2007; 37:457–64. [PubMed: 17313951]
22. Elpek KG, Lacelle C, Singh NP, Yolcu ES, Shirwan H. CD4⁺ CD25⁺ T regulatory cells dominate multiple immune evasion mechanisms in early but not late phases of tumor development in a B cell lymphoma model. *J Immunol*. 2007; 178:6840–8. [PubMed: 17513732]
23. Fillatreau S, Gray D, Anderton SM. Not always the bad guys: B cells as regulators of autoimmune pathology. *Nat Rev Immunol*. 2008; 8:391–7. [PubMed: 18437156]
24. Fillatreau S, Sweeney CH, McGeachy MJ, Gray D, Anderton SM. B cells regulate autoimmunity by provision of IL-10. *Nat Immunol*. 2002; 3:944–50. [PubMed: 12244307]
25. Fischer GM, Solt LA, Hastings WD, Yang K, Gerstein RM, Nikolajczyk BS, et al. Splenic and peritoneal B-1 cells differ in terms of transcriptional and proliferative features that separate peritoneal B-1 from splenic B-2 cells. *Cell Immunol*. 2001; 213:62–71. [PubMed: 11747357]
26. Gagro A, McCloskey N, Challa A, Holder M, Grafton G, Pound JD, et al. CD5-positive and CD5-negative human B cells converge to an indistinguishable population on signalling through B-cell receptors and CD40. *Immunology*. 2000; 101:201–9. [PubMed: 11012773]
27. Gaubert S, Viana da Costa A, Maurage CA, Lima EC, Fontaine J, Lafitte S, et al. X-linked immunodeficiency affects the outcome of *Schistosoma mansoni* infection in the murine model. *Parasite Immunol*. 1999; 21:89–101. [PubMed: 10101719]
28. Gourley TS, Patel DR, Nickerson K, Hong SC, Chang CH. Aberrant expression of Fas ligand in mice deficient for the MHC class II transactivator. *J Immunol*. 2002; 168:4414–9. [PubMed: 11970984]
29. Grulich C, Richter M, Exner S, Finke J. Fas ligand is not constitutively expressed in low-grade B-cell lymphoma and B-lymphoblastoid cells. *Eur J Haematol*. 2003; 71:184–8. [PubMed: 12930319]
30. Hahne M, Renno T, Schroeter M, Irmeler M, French L, Bornard T, et al. Activated B cells express functional Fas ligand. *Eur J Immunol*. 1996; 26:721–4. [PubMed: 8605944]
31. Hamaguchi Y, Uchida J, Cain DW, Venturi GM, Poe JC, Haas KM, et al. The peritoneal cavity provides a protective niche for B1 and conventional B lymphocytes during anti-CD20 immunotherapy in mice. *J Immunol*. 2005; 174:4389–99. [PubMed: 15778404]
32. Hayakawa K, Asano M, Shinton SA, Gui M, Allman D, Stewart CL, et al. Positive selection of natural autoreactive B cells. *Science*. 1999; 285:113–6. [PubMed: 10390361]
33. Hayakawa K, Asano M, Shinton SA, Gui M, Wen LJ, Dashoff J, et al. Positive selection of anti-thy-1 autoreactive B-1 cells and natural serum autoantibody production independent from bone marrow B cell development. *J Exp Med*. 2003; 197:87–99. [PubMed: 12515816]
34. Hitoshi Y, Okada Y, Sonoda E, Tominaga A, Makino M, Suzuki K, et al. Delayed progression of a murine retrovirus-induced acquired immunodeficiency syndrome in X-linked immunodeficient mice. *J Exp Med*. 1993; 177:621–6. [PubMed: 8382252]
35. Ishida M, Iwai Y, Tanaka Y, Okazaki T, Freeman GJ, Minato N, et al. Differential expression of PD-L1 and PD-L2, ligands for an inhibitory receptor PD-1, in the cells of lymphohematopoietic tissues. *Immunol Lett*. 2002; 84:57–62. [PubMed: 12161284]
36. Kemp TJ, Moore JM, Griffith TS. Human B cells express functional TRAIL/Apo-2 ligand after CpG-containing oligodeoxynucleotide stimulation. *J Immunol*. 2004; 173:892–9. [PubMed: 15240676]
37. Kim SH, Kim S, Oligino TJ, Robbins PD. Effective treatment of established mouse collagen-induced arthritis by systemic administration of dendritic cells genetically modified to express FasL. *Mol Ther*. 2002; 6:584–90. [PubMed: 12409256]
38. Kim YS, Park GB, Song HK, Hur I, Lee HK, Kang JS, et al. Cross-linking of CD54 on Burkitt lymphoma cell line Raji and Ramos induces FasL expression by reactive oxygen species and

- apoptosis of adjacent cells in Fas/FasL interaction. *J Immunother.* 2007; 30:727–39. [PubMed: 17893565]
39. Kojima Y, Tsurumi H, Goto N, Shimizu M, Kasahara S, Yamada T, et al. Fas and Fas ligand expression on germinal center type-diffuse large B-cell lymphoma is associated with the clinical outcome. *Eur J Haematol.* 2006; 76:465–72. [PubMed: 16494623]
 40. Kondo E, Yoshino T, Nishiuchi R, Sakuma I, Nishizaki K, Kayagaki N, et al. Expression of Fas ligand mRNA in germinal centres of the human tonsil. *J Pathol.* 1997; 183:75–9. [PubMed: 9370951]
 41. Kosiewicz MM, Krishnan A, Worthington MT, Matriano JA, Ross WG. B cells engineered to express Fas ligand suppress pre-sensitized antigen-specific T cell responses in vivo. *Eur J Immunol.* 2002; 32:1679–87. [PubMed: 12115651]
 42. La Flamme AC, Ruddenklau K, Backstrom BT. Schistosomiasis decreases central nervous system inflammation and alters the progression of experimental autoimmune encephalomyelitis. *Infect Immun.* 2003; 71:4996–5004. [PubMed: 12933842]
 43. Lampropoulou V, Hoehlig K, Roch T, Neves P, Gomez EC, Sweenie CH, et al. TLR-activated B cells suppress T cell-mediated autoimmunity. *J Immunol.* 2008; 180:4763–73. [PubMed: 18354200]
 44. Leandro MJ, Cambridge G, Ehrenstein MR, Edwards JC. Reconstitution of peripheral blood B cells after depletion with rituximab in patients with rheumatoid arthritis. *Arthritis Rheum.* 2006; 54:613–20. [PubMed: 16447239]
 45. Lund FE, Garvy BA, Randall TD, Harris DP. Regulatory roles for cytokine-producing B cells in infection and autoimmune disease. *Curr Dir Autoimmun.* 2005; 8:25–54. [PubMed: 15564716]
 46. Lund FE, Hollifield M, Schuer K, Lines JL, Randall TD, Garvy BA. B cells are required for generation of protective effector and memory CD4 cells in response to *Pneumocystis* lung infection. *J Immunol.* 2006; 176:6147–54. [PubMed: 16670323]
 47. Lundy SK, Berlin AA, Martens TF, Lukacs NW. Deficiency of regulatory B cells increases allergic airway inflammation. *Inflamm Res.* 2005; 54:514–21. [PubMed: 16389573]
 48. Lundy SK, Boros DL. Fas ligand-expressing B-1a lymphocytes mediate CD4(+) T-cell apoptosis during schistosomal infection: induction by interleukin 4 (IL-4) and IL-10. *Infect Immun.* 2002; 70:812–9. [PubMed: 11796615]
 49. Lundy SK, Lerman SP, Boros DL. Soluble egg antigen-stimulated T helper lymphocyte apoptosis and evidence for cell death mediated by FasL(+) T and B cells during murine *Schistosoma mansoni* infection. *Infect Immun.* 2001; 69:271–80. [PubMed: 11119515]
 50. Maglione PJ, Xu J, Chan J. B cells moderate inflammatory progression and enhance bacterial containment upon pulmonary challenge with *Mycobacterium tuberculosis*. *J Immunol.* 2007; 178:7222–34. [PubMed: 17513771]
 51. Mangan NE, Fallon RE, Smith P, van Rooijen N, McKenzie AN, Fallon PG. Helminth infection protects mice from anaphylaxis via IL-10-producing B cells. *J Immunol.* 2004; 173:6346–56. [PubMed: 15528374]
 52. Mann MK, Maresz K, Shriver LP, Tan Y, Dittel BN. B cell regulation of CD4⁺ CD25⁺ T regulatory cells and IL-10 via B7 is essential for recovery from experimental autoimmune encephalomyelitis. *J Immunol.* 2007; 178:3447–56. [PubMed: 17339439]
 53. Mariani SM, Krammer PH. Differential regulation of TRAIL and CD95 ligand in transformed cells of the T and B lymphocyte lineage. *Eur J Immunol.* 1998; 28:973–82. [PubMed: 9541592]
 54. Mariani SM, Krammer PH. Surface expression of TRAIL/Apo-2 ligand in activated mouse T and B cells. *Eur J Immunol.* 1998; 28:1492–8. [PubMed: 9603453]
 55. Martin F, Kearney JF. B1 cells: similarities and differences with other B cell subsets. *Curr Opin Immunol.* 2001; 13:195–201. [PubMed: 11228413]
 56. Mauri C, Ehrenstein MR. The ‘short’ history of regulatory B cells. *Trends Immunol.* 2008; 29:34–40. [PubMed: 18289504]
 57. Mauri C, Gray D, Mushtaq N, Londei M. Prevention of arthritis by interleukin 10-producing B cells. *J Exp Med.* 2003; 197:489–501. [PubMed: 12591906]
 58. Meiler F, Zimmermann M, Blaser K, Akdis CA, Akdis M. T-cell subsets in the pathogenesis of human asthma. *Curr Allergy Asthma Rep.* 2006; 6:91–6. [PubMed: 16566857]

59. Melo ME, Qian J, El-Amine M, Agarwal RK, Soukhareva N, Kang Y, et al. Gene transfer of Ig-fusion proteins into B cells prevents and treats autoimmune diseases. *J Immunol.* 2002; 168:4788–95. [PubMed: 11971030]
60. Mercolino TJ, Arnold LW, Hawkins LA, Haughton G. Normal mouse peritoneum contains a large population of Ly-1⁺ (CD5) B cells that recognize phosphatidyl choline. Relationship to cells that secrete hemolytic antibody specific for autologous erythrocytes. *J Exp Med.* 1988; 168:687–98. [PubMed: 3045250]
61. Minagawa R, Okano S, Tomita Y, Kishihara K, Yamada H, Nomoto K, et al. The critical role of Fas–Fas ligand interaction in donor-specific transfusion-induced tolerance to H-Y antigen. *Transplantation.* 2004; 78:799–806. [PubMed: 15385797]
62. Minoprio P, el Cheikh MC, Murphy E, Hontebeyrie-Joskowicz M, Coffman R, Coutinho A, et al. Xid-associated resistance to experimental Chagas' disease is IFN-gamma dependent. *J Immunol.* 1993; 151:4200–8. [PubMed: 8409396]
63. Mizoguchi A, Bhan AK. A case for regulatory B cells. *J Immunol.* 2006; 176:705–10. [PubMed: 16393950]
64. Mizoguchi A, Mizoguchi E, Takedatsu H, Blumberg RS, Bhan AK. Chronic intestinal inflammatory condition generates IL-10-producing regulatory B cell subset characterized by CD1d upregulation. *Immunity.* 2002; 16:219–30. [PubMed: 11869683]
65. Montecino-Rodriguez E, Leathers H, Dorshkind K. Identification of a B-1 B cell-specified progenitor. *Nat Immunol.* 2006; 7:293–301. [PubMed: 16429139]
66. Mullauer L, Mosberger I, Chott A. Fas ligand expression in nodal non-Hodgkin's lymphoma. *Mod Pathol.* 1998; 11:369–75. [PubMed: 9578088]
67. Nagafuchi H, Wakisaka S, Takeba Y, Takeno M, Sakane T, Suzuki N. Aberrant expression of Fas ligand on anti-DNA autoantibody secreting B lymphocytes in patients with systemic lupus erythematosus: "immune privilege"-like state of the autoreactive B cells. *Clin Exp Rheumatol.* 2002; 20:625–31. [PubMed: 12412192]
68. Nilsson N, Ingvarsson S, Borrebaeck CA. Immature B cells in bone marrow express Fas/FasL. *Scand J Immunol.* 2000; 51:279–84. [PubMed: 10736097]
69. Noorchashm H, Reed AJ, Rostami SY, Mozaffari R, Zekavat G, Koeberlein B, et al. B cell-mediated antigen presentation is required for the pathogenesis of acute cardiac allograft rejection. *J Immunol.* 2006; 177:7715–22. [PubMed: 17114442]
70. O'Garra A, Howard M. IL-10 production by CD5 B cells. *Ann N Y Acad Sci.* 1992; 651:182–99. [PubMed: 1376039]
71. Palanivel V, Posey C, Horauf AM, Solbach W, Piessens WF, Harn DA. B-cell outgrowth and ligand-specific production of IL-10 correlate with Th2 dominance in certain parasitic diseases. *Exp Parasitol.* 1996; 84:168–77. [PubMed: 8932766]
72. Pers JO, Daridon C, Bendaoud B, Devauchelle V, Berthou C, Saraux A, et al. B-cell depletion and repopulation in autoimmune diseases. *Clin Rev Allergy Immunol.* 2008; 34:50–5. [PubMed: 18270858]
73. Rich RF, Cook WJ, Green WR. Spontaneous in vivo retrovirus-infected T and B cells, but not dendritic cells, mediate antigen-specific Fas ligand/Fas-dependent apoptosis of anti-retroviral CTL. *Virology.* 2006; 346:287–300. [PubMed: 16337984]
74. Sampalo A, Navas G, Medina F, Segundo C, Camara C, Brieva JA. Chronic lymphocytic leukemia B cells inhibit spontaneous Ig production by autologous bone marrow cells: role of CD95-CD95L interaction. *Blood.* 2000; 96:3168–74. [PubMed: 11049999]
75. Samuelsson A, Sonnerborg A, Heuts N, Coster J, Chiodi F. Progressive B cell apoptosis and expression of Fas ligand during human immunodeficiency virus type 1 infection. *AIDS Res Hum Retroviruses.* 1997; 13:1031–8. [PubMed: 9264290]
76. Sasaki H, Schmitt DA, Matsumoto K, Pollard RB, Suzuki F. Demonstrations of a B-cell population that regulates the immune response in spleens of mice infected with herpes simplex virus type I. *Clin Immunol Immunopathol.* 1993; 66:169–75. [PubMed: 8453787]
77. Sasaki Y, Ami Y, Nakasone T, Shinohara K, Takahashi E, Ando S, et al. Induction of CD95 ligand expression on T lymphocytes and B lymphocytes and its contribution to apoptosis of CD95-up-

- regulated CD4⁺ T lymphocytes in macaques by infection with a pathogenic simian/human immunodeficiency virus. *Clin Exp Immunol.* 2000; 122:381–9. [PubMed: 11122244]
78. Satpute SR, Soukhareva N, Scott DW, Moudgil KD. Mycobacterial Hsp65-IgG-expressing tolerogenic B cells confer protection against adjuvant-induced arthritis in Lewis rats. *Arthritis Rheum.* 2007; 56:1490–6. [PubMed: 17469108]
 79. Sedger LM, Glaccum MB, Schuh JC, Kanaly ST, Williamson E, Kayagaki N, et al. Characterization of the in vivo function of TNF-alpha-related apoptosis-inducing ligand, TRAIL/Apo2L, using TRAIL/Apo2L gene-deficient mice. *Eur J Immunol.* 2002; 32:2246–54. [PubMed: 12209637]
 80. Sewell D, Qing Z, Reinke E, Elliot D, Weinstock J, Sandor M, et al. Immunomodulation of experimental autoimmune encephalomyelitis by helminth ova immunization. *Int Immunol.* 2003; 15:59–69. [PubMed: 12502726]
 81. Sharpe AH, Wherry EJ, Ahmed R, Freeman GJ. The function of programmed cell death 1 and its ligands in regulating autoimmunity and infection. *Nat Immunol.* 2007; 8:239–45. [PubMed: 17304234]
 82. Silvestris F, Cafforio P, Tucci M, Grinello D, Dammacco F. Upregulation of osteoblast apoptosis by malignant plasma cells: a role in myeloma bone disease. *Br J Haematol.* 2003; 122:39–52. [PubMed: 12823344]
 83. Silvestris F, Tucci M, Cafforio P, Dammacco F. Fas-L up-regulation by highly malignant myeloma plasma cells: role in the pathogenesis of anemia and disease progression. *Blood.* 2001; 97:1155–64. [PubMed: 11222356]
 84. Singh A, Carson WF, Secor ER Jr, Guernsey LA, Flavell RA, Clark RB, et al. Regulatory role of B cells in a murine model of allergic airway disease. *J Immunol.* 2008; 180:7318–26. [PubMed: 18490731]
 85. Song H, Park G, Kim YS, Hur I, Kim H, Ryu JW, et al. B7–H4 reverse signaling induces the apoptosis of EBV-transformed B cells through Fas ligand up-regulation. *Cancer Lett.* 2008; 266:227–37. [PubMed: 18417276]
 86. Spencer NF, Daynes RA. IL-12 directly stimulates expression of IL-10 by CD5⁺ B cells and IL-6 by both CD5⁺ and CD5[–] B cells: possible involvement in age-associated cytokine dysregulation. *Int Immunol.* 1997; 9:745–54. [PubMed: 9184920]
 87. Strater J, Mariani SM, Walczak H, Rucker FG, Leithauser F, Krammer PH, et al. CD95 ligand (CD95L) in normal human lymphoid tissues: a subset of plasma cells are prominent producers of CD95L. *Am J Pathol.* 1999; 154:193–201. [PubMed: 9916933]
 88. Takahashi T, Tanaka M, Brannan CI, Jenkins NA, Copeland NG, Suda T, et al. Generalized lymphoproliferative disease in mice, caused by a point mutation in the Fas ligand. *Cell.* 1994; 76:969–76. [PubMed: 7511063]
 89. Tang Y, Kim WK, Holmes KL, Hugin AW, Kenny JJ, Chattopadhyay SK, et al. Contribution of B cell subsets to delayed development of MAIDS in Xid mice. *Cell Immunol.* 1995; 165:1–6. [PubMed: 7545546]
 90. Tanner JE, Alfieri C. Epstein–Barr virus induces Fas (CD95) in T cells and Fas ligand in B cells leading to T-cell apoptosis. *Blood.* 1999; 94:3439–47. [PubMed: 10552954]
 91. Tian J, Zekzer D, Hanssen L, Lu Y, Olcott A, Kaufman DL. Lipopolysaccharide-activated B cells down-regulate Th1 immunity and prevent autoimmune diabetes in nonobese diabetic mice. *J Immunol.* 2001; 167:1081–9. [PubMed: 11441119]
 92. Tinhof I, Marschitz I, Kos M, Henn T, Egle A, Villunger A, et al. Differential sensitivity of CD4⁺ and CD8⁺ T lymphocytes to the killing efficacy of Fas (Apo-1/CD95) ligand + tumor cells in B chronic lymphocytic leukemia. *Blood.* 1998; 91:4273–81. [PubMed: 9596676]
 93. Truman JP, Choqueux C, Tschopp J, Vedrenne J, Le Deist F, Charron D, et al. HLA class II-mediated death is induced via Fas/Fas ligand interactions in human splenic B lymphocytes. *Blood.* 1997; 89:1996–2007. [PubMed: 9058721]
 94. Tsitoura DC, Yeung VP, DeKruyff RH, Umetsu DT. Critical role of B cells in the development of T cell tolerance to aero-allergens. *Int Immunol.* 2002; 14:659–67. [PubMed: 12039917]

95. Tumang JR, Hastings WD, Bai C, Rothstein TL. Peritoneal and splenic B-1 cells are separable by phenotypic, functional, and transcriptomic characteristics. *Eur J Immunol.* 2004; 34:2158–67. [PubMed: 15259013]
96. Velupillai P, Harn DA. Oligosaccharide-specific induction of interleukin 10 production by B220⁺ cells from schistosome-infected mice: a mechanism for regulation of CD4⁺ T-cell subsets. *Proc Natl Acad Sci U S A.* 1994; 91:18–22. [PubMed: 7904066]
97. Villunger A, Egle A, Marschitz I, Kos M, Bock G, Ludwig H, et al. Constitutive expression of Fas (Apo-1/CD95) ligand on multiple myeloma cells: a potential mechanism of tumor-induced suppression of immune surveillance. *Blood.* 1997; 90:12–20. [PubMed: 9207432]
98. Watt V, Ronchese F, Ritchie D. Resting B cells suppress tumor immunity via an MHC class-II dependent mechanism. *J Immunother.* 2007; 30:323–32. [PubMed: 17414323]
99. Wei B, Velazquez P, Turovskaya O, Spricher K, Aranda R, Kronenberg M, et al. Mesenteric B cells centrally inhibit CD4⁺ T cell colitis through interaction with regulatory T cell subsets. *Proc Natl Acad Sci U S A.* 2005; 102:2010–5. [PubMed: 15684084]
100. Weinstock JV, Summers RW, Elliott DE, Qadir K, Urban JF Jr, Thompson R. The possible link between de-worming and the emergence of immunological disease. *J Lab Clin Med.* 2002; 139:334–8. [PubMed: 12066130]
101. Werner-Klein M, Dresch C, Marconi P, Brocker T. Transcriptional targeting of B cells for induction of peripheral CD8 T cell tolerance. *J Immunol.* 2007; 178:7738–46. [PubMed: 17548611]
102. Xiu Y, Wong CP, Bouaziz JD, Hamaguchi Y, Wang Y, Pop SM, et al. B lymphocyte depletion by CD20 monoclonal antibody prevents diabetes in nonobese diabetic mice despite isotype-specific differences in Fc{gamma}R effector functions. *J Immunol.* 2008; 180:2863–75. [PubMed: 18292508]
103. Yanaba K, Bouaziz JD, Haas KM, Poe JC, Fujimoto M, Tedder TF. A regulatory B cell subset with a unique CD1dhiCD5⁺ phenotype controls T cell-dependent inflammatory responses. *Immunity.* 2008; 28:639–50. [PubMed: 18482568]
104. Yanaba K, Hamaguchi Y, Venturi GM, Steeber DA, St Clair EW, Tedder TF. B cell depletion delays collagen-induced arthritis in mice: arthritis induction requires synergy between humoral and cell-mediated immunity. *J Immunol.* 2007; 179:1369–80. [PubMed: 17617630]
105. Zhan HG, Mountz JD, Fleck M, Zhou T, Hsu HC. Specific deletion of autoreactive T cells by adenovirus-transfected, Fas ligand-producing antigen-presenting cells. *Immunol Res.* 2002; 26:235–46. [PubMed: 12403361]
106. Zhang HG, Liu D, Heike Y, Yang P, Wang Z, Wang X, et al. Induction of specific T-cell tolerance by adenovirus-transfected, Fas ligand-producing antigen presenting cells. *Nat Biotechnol.* 1998; 16:1045–9. [PubMed: 9831033]
107. Zhang X, Deriaud E, Jiao X, Braun D, Leclerc C, Lo-Man R. Type I interferons protect neonates from acute inflammation through interleukin 10-producing B cells. *J Exp Med.* 2007; 204:1107–18. [PubMed: 17485512]
108. Zhong X, Tumang JR, Gao W, Bai C, Rothstein TL. PD-L2 expression extends beyond dendritic cells/macrophages to B1 cells enriched for V(H)11/V(H)12 and phosphatidylcholine binding. *Eur J Immunol.* 2007; 37:2405–10. [PubMed: 17683117]
109. Zimecki M, Whiteley PJ, Pierce CW, Kapp JA. Presentation of antigen by B cells subsets. I. Lyb-5⁺ and Lyb-5[−] B cells differ in ability to stimulate antigen specific T cells. *Arch Immunol Ther Exp (Warsz).* 1994; 42:115–23. [PubMed: 7503644]
110. Zoi-Toli O, Meijer CJ, Oudejans JJ, de Vries E, van Beek P, Willemze R. Expression of Fas and Fas ligand in cutaneous B-cell lymphomas. *J Pathol.* 1999; 189:533–8. [PubMed: 10629554]
111. Zuniga E, Motran CC, Montes CL, Yagita H, Gruppi A. *Trypanosoma cruzi* infection selectively renders parasite-specific IgG⁺ B lymphocytes susceptible to Fas/Fas ligand-mediated fratricide. *J Immunol.* 2002; 168:3965–73. [PubMed: 11937553]

Fig. 1.

A model showing the possible duality in roles of a single autoreactive B cell during the regulation or promotion of autoimmunity. Self antigen recognition by an autoreactive B cell receptor leads to activation of a killer/regulatory or stimulatory B cell depending on the secondary signals received concurrently. LPS and factors promoting a Th2 effector phenotype may favor production of killer/regulatory B cells, while factors that promote a Th1 or Th17 phenotype may favor a stimulatory B cell phenotype. Killer/regulatory B cells producing IL-10, TGF β , FasL, and/or other death-inducing ligands may interact with an autoreactive T cell leading to induction of tolerance. Stimulatory B cells producing IL-6, TGF β , upregulated B7 molecules, and/or decreased death ligand expression may interact with autoreactive T cells leading to induction of IL-17 and promotion of autoimmunity. It remains unclear whether a single autoreactive B cell can be forced into either one of these roles, or whether regulatory and stimulatory autoreactive B cells come from separate B cell compartments.

Table 1**Regulators of FasL expression by B lymphocytes**

Stimulators of FasL on B cells	References
Phorbol myristate acetate/ionomycin	Hahne et al. [30]
Lipopolysaccharide	Hahne et al. [30], Tian et al. [91]
Schistosome egg antigen (SEA)	Lundy et al. [48,49]
SEA + IL-10 and/or IL-4	Lundy et al. [48]
<i>Trypanosoma cruzi</i> infection	Zuniga et al. [111]
HIV or SIV infection	Samuelsson et al. [75], Sasaki [77]
MuLV infection	Rich et al. [73]
Epstein-Barr virus infection	Tanner and Alfieri [90]
Ligation of CD54 (Burkitt lymphoma)	Kim et al. [38]
B7-H4 ligation (EBV lines)	Song et al. [85]
Cockroach allergen	Lundy et al. [47]
Toxic mistletoe lectin	Bussing et al. [11]
Inhibitors of FasL on B cells	
MHC Class II-transactivator	Gourley et al. [28]
<i>T. cruzi</i> infection + IL-4	Acosta Rodriguez et al. [1]

Table 2

Evidence of killer B cells in disease

Disease/model	Species	B cell FasL or TRAIL	Lymphocyte apoptosis	References
B cell lymphoma	Human	Both	ND	Mariani and Krammer [53,54], Zoi-Toli et al. [110]
Diffuse large B cell lymphoma	Human	FasL	ND	Mullauer et al. [66], Kojima et al. [39]
B-Chronic lymphocytic leukemia	Human	FasL	T cells	Tinhofer et al. [92], Sampalo et al. [74]
Multiple myeloma	Human	Both	ND ^a	Villunger et al. [97], Silvestris et al. [82,83]
Schistosome infection	Mouse	FasL	Th cells	Lundy et al. [49], Lundy and Boros [48]
<i>Trypanosoma cruzi</i> infection	Mouse	FasL	B cells	Zuniga et al. [111]
Epstein-Barr virus infection	Human	FasL	Th cells	Tanner and Alfieri [90]
Immunodeficiency viruses	Human, simian, mouse	FasL	Th cells	Samuelsson et al. [75], Sasaki et al. [77], Rich et al. [73]
Cockroach allergen asthma	Mouse	FasL (CD5 ⁺)	Th cells	Lundy et al. [47]
MRL lpr/lpr (lupus-prone)	Mouse	FasL	T cells	Bonardelle et al. [8]
HY incompatible transplant	Mouse	FasL	ND	Minagawa et al. [61]

ND not determined

^aApoptosis of erythroblasts and osteoblasts was demonstrated

Table 3

Phenotypic differences between B-1a and B-2 cells in mice

Functional property	B-1a Cells	B-2 Cells
Surface phenotype	CD19 ⁺ /CD11b ⁺ /CD5 ⁺	CD19 ⁺ /CD11b ⁻ /CD5 ⁻
Tissue of origin	Fetal liver	Bone marrow
Peripheral localization	Peritoneal and pleural cavity	Lymphoid organs
Percentage of total splenocytes-mice	1–5%	~50%
V-D-J rearrangement	Restricted	Unrestricted
Class switch recombination	Very low	High
Somatic hypermutation	Very low	High
Antibody specificities	Primitive: CHO and lipids	Proteins
T cell dependent	No	Yes
Frequency of autoantibody producers	Moderate to high	Low
Secreted antibodies	Low to none (IgM and IgA)	High (all isotypes)
Develop into plasma cells	Rarely	Subset upon activation
Available deficient mice	Btk mutant (Xid)	RAG ^{-/-} (B-2 and T deficient)
Cytokine production ^a	High IL-10	Be1 and Be2 subsets
Fas ligand expression ^a	High, constitutive, inducible	Low, inducible

CHO carbohydrate, Ig immunoglobulin, Btk Bruton's tyrosine kinase, Xid X-linked immunodeficiency, Be B effector cell

^aFasL and IL-10 expression attributable to CD5⁺/CD19⁺ B cells but not proven to be from *bona fide* B-1a cells