Regulatory B cells in human inflammatory and autoimmune diseases: from mouse models to clinical research

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Abstract

B cells have been generally considered to be positive regulators of immune responses because of their ability to produce antigen-specific antibodies and to activate T cells through antigen presentation. Impairment of B cell development and function may cause inflammatory and autoimmune diseases. Recently, specific B cell subsets that can negatively regulate immune responses have been described in mouse models of a wide variety of inflammatory and autoimmune diseases. The concept of those B cells, termed regulatory B cells, is now recognized as important in the murine immune system. Among several regulatory B cell subsets, IL-10-producing regulatory B cells are the most widely investigated. On the basis of discoveries from studies of such mice, human regulatory B cells that produce IL-10 in most cases are becoming an active area of research. There have been emerging data suggesting the importance of human regulatory B cells in various diseases. Revealing the immune regulation mechanisms of human regulatory B cells in human inflammatory and autoimmune diseases could lead to the development of novel B cell targeted therapies. This review highlights the current knowledge on regulatory B cells, mainly IL-10-producing regulatory B cells, in animal models of inflammatory and autoimmune diseases and in clinical research using human samples.

Keywords: IL-10, multiple sclerosis, regulatory B cells, rheumatoid arthritis, systemic lupus erythematosus

Introduction

The immune system of mammals including mice and humans not only protects the host from a broad range of pathogenic micro-organisms but also avoids misguided or exaggerated responses that would be harmful to the host. The balance between activating and inhibitory subsets is important for the immune system to work optimally. This is most clearly understood for T cells, in which effector T cells and regulatory T cells have opposing roles in maintaining this delicate balance. Several regulatory T cell subsets have been identified that are specialized for immune suppression, including naturally arising CD4+CD25+FoxP3+ regulatory T cells (1) and T-regulatory type 1 cells (2), both of which produce high amounts of IL-10.

Historically, B cells have been considered to be positive regulators of humoral immune responses because of their ability to terminally differentiate into plasma cells and produce antigen-specific antibodies (3). B cells can also serve as antigen-presenting cells, leading to optimal antigen-specific CD4+ T cell expansion, memory formation and cytokine production (4–6). B cells may also positively regulate CD8+ T cell responses in mouse models of autoimmune diseases (7, 8). Furthermore, co-stimulatory molecules, such as CD80, CD86 and OX40L, expressed on B cells are also important for optimal T cell activation (9, 10). Thus, in addition to producing antibodies, B cells can positively regulate cellular immune responses.

Specific B cell subsets, however, negatively regulate immune responses and have been termed regulatory B cells (11–13). There is accumulating evidence demonstrating that regulatory B cells play an important role in a variety of mouse models of inflammation and autoimmunity (14–19). Although the identification of regulatory B cells and the definition of their mechanisms of action are recent events, regulatory B cells are now broadly recognized as an important new component of the immune system (11, 20). Several regulatory B cell subsets have been described in mice; IL-10-producing regulatory B cells are the most widely studied regulatory B cells (19–21).
Human regulatory B cells, also predominantly identified based on their production of IL-10, exhibit a phenotypic and functional heterogeneity similar to that of mouse IL-10-producing regulatory B cells (22–24). Human regulatory B cells were enriched in both transitional (CD24hiCD38hi) and memory (CD24hiCD27+ B cells (23, 24). CD19+CD24hiCD38hi B cells inhibited pro-inflammatory cytokine production by CD4+ T cells, dependent on IL-10, CD80 and CD86, but not transforming growth factor beta (TGF-β) (23). IL-10 production by CD24hiCD27+ B cells regulated tumor necrosis factor alpha (TNF-α) production from monocytes (24). Human CD19+CD25+CD86hiCD1d+ B cells could also suppress the proliferation of CD4+ T cells and enhance Forkhead box protein 3 and cytotoxic T-lymphocyte antigen 4 expression in regulatory T cells by producing IL-10 and TGF-β (23). In addition, IL-10 production was enriched in CD27hiCD38hi plasmablast B-cell compartments (26). Thus, human regulatory B cells did not belong to a single B-cell subset (Fig. 1). Regardless of the different markers used to identify human regulatory B cells, the majority of protective effects of regulatory B cells are dependent on IL-10. Although studies of human regulatory B cells are limited, there have been emerging data proposing the importance and potential future therapeutic application of peripheral blood regulatory B cells in human diseases (Tables 1–4).

The present review will focus on the role of regulatory B cells in inflammatory and autoimmune diseases, in animal models of human diseases and in clinical research using human samples.

**Systemic lupus erythematosus**

Systemic lupus erythematosus (SLE) is a heterogeneous, inflammatory, multisystem autoimmune disease characterized by the production of antinuclear antibodies, abnormalities in T cell and B cell function, and the involvement of internal organ systems. Renal involvement is one of the most serious complications of SLE.

Several strains of mice spontaneously develop a disease that closely resembles human SLE. In 2005, enhanced IL-10 production by the CpG-stimulated B cells from Palmerston North mice was first reported (45), which develop an SLE-like disease accompanied by antinuclear antibody production, LE cells, glomerulonephritis and arteritis (46). The roles of regulatory B cells in a spontaneous lupus model have been closely investigated in two other models: New Zealand Black

**Table 1.** Examples of clinical studies on human regulatory B cells in patients with systemic lupus erythematosus

<table>
<thead>
<tr>
<th>Regulatory B cell subsets (compared with healthy controls)</th>
<th>Supernatant IL-10 levels from B cells (compared with healthy controls)</th>
<th>Function (compared with healthy controls)</th>
<th>Stimulus for IL-10 assay</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Increased CD5+IL-10+ B cells</td>
<td>ND</td>
<td>ND</td>
<td>LPS + PI</td>
<td>(27)</td>
</tr>
<tr>
<td>Increased CD154+IL-10+ B cells</td>
<td>ND</td>
<td>ND</td>
<td>SAC</td>
<td>(28)</td>
</tr>
<tr>
<td>Similar CD24hiCD38hi B cells</td>
<td>ND</td>
<td>Impaired</td>
<td>CD40L + PI</td>
<td>(23)</td>
</tr>
<tr>
<td>Decreased IL-10+ B cells</td>
<td>Increased</td>
<td>ND</td>
<td>LPS + PI</td>
<td>(29)</td>
</tr>
<tr>
<td>Increased CD1dhiCD5+ B cells</td>
<td>Increased</td>
<td>ND</td>
<td>IL-2 + SAC</td>
<td>(30)</td>
</tr>
<tr>
<td>Increased IL-10+ B cells</td>
<td>Similar</td>
<td>Impaired</td>
<td>CpG or LPS + CD40L + PI</td>
<td>(24)</td>
</tr>
</tbody>
</table>

ND, not done; PI, PMA+ ionomycin; SAC, *Staphylococcus aureus* Cowan 1; CD40L, CD40 ligand.
CD40L, CD40 ligand; PI, PMA+ ionomycin; ND, not done.

Table 3. Examples of clinical studies on human regulatory B cells in patients with multiple sclerosis

<table>
<thead>
<tr>
<th>Regulatory B cell subsets (compared with healthy controls)</th>
<th>Supernatant IL-10 levels from B cells (compared with healthy controls)</th>
<th>Function (compared with healthy controls)</th>
<th>Stimulus for IL-10 assay</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>ND</td>
<td>Decreased</td>
<td>Impaired</td>
<td>CD40L + PI</td>
<td>(31)</td>
</tr>
<tr>
<td>Decreased IL-10+ B cells</td>
<td>Decreased</td>
<td>ND</td>
<td>LPS + PI</td>
<td>(33)</td>
</tr>
<tr>
<td>Increased IL-10+ B cells</td>
<td>Decreased</td>
<td>ND</td>
<td>CD40L + anti-IgM/IgG</td>
<td>(36)</td>
</tr>
<tr>
<td>Decreased IL-10+ B cells</td>
<td>Decreased</td>
<td>ND</td>
<td>CpG</td>
<td>(37)</td>
</tr>
<tr>
<td>Decreased IL-10+ B cells</td>
<td>Decreased</td>
<td>Impaired</td>
<td>Cd40L + PI</td>
<td>(38)</td>
</tr>
<tr>
<td>Increased IL-10+ B cells</td>
<td>Decreased</td>
<td>ND</td>
<td>CpG or LPS + Cd40L</td>
<td>(39)</td>
</tr>
<tr>
<td>ND</td>
<td>Decreased</td>
<td>ND</td>
<td>IL-21 + CpG + LPS + PI</td>
<td>(40)</td>
</tr>
<tr>
<td>Decreased IL-10+ B cells</td>
<td>Decreased</td>
<td>ND</td>
<td>LPS + Cd40L</td>
<td>(41)</td>
</tr>
<tr>
<td>Decreased IL-10+ B cells</td>
<td>Decreased</td>
<td>Impaired</td>
<td>Cd40L</td>
<td>(42)</td>
</tr>
<tr>
<td>Increased IL-10+ B cells</td>
<td>Decreased</td>
<td>Impaired</td>
<td>LPS + PI</td>
<td>(43)</td>
</tr>
</tbody>
</table>

ND, not done; CD40L, CD40 ligand; PI, PMA+ ionomycin.

Table 4. Examples of clinical studies on human regulatory B cells in patients with other inflammatory and autoimmune diseases

<table>
<thead>
<tr>
<th>Disease</th>
<th>Regulatory B cell subsets (compared with healthy controls)</th>
<th>Supernatant IL-10 levels from B cells (compared with healthy controls)</th>
<th>Function (compared with healthy controls)</th>
<th>Stimulus for IL-10 assay</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pemphigus</td>
<td>Decreased IL-10+ B cells</td>
<td>Decreased</td>
<td>ND</td>
<td>Cd40L + PI</td>
<td>(31)</td>
</tr>
<tr>
<td>Asthma</td>
<td>Decreased IL-10+ B cells</td>
<td>Decreased</td>
<td>ND</td>
<td>LPS + PI</td>
<td>(33)</td>
</tr>
<tr>
<td>Allergic rhinitis</td>
<td>Decreased IL-10+ B cells</td>
<td>Decreased</td>
<td>ND</td>
<td>CD40L</td>
<td>(37)</td>
</tr>
</tbody>
</table>

ND, not done; PI, PMA+ ionomycin; CD40L, CD40 ligand.

(NZB) x New Zealand White (NZW) F1 hybrid (NZB/W) mice and MRL/lpr mice.

NZB/W mice spontaneously develop an SLE-like disease accompanied by production of anti-double strand DNA antibodies and immune complex-mediated nephritis (47). Depletion of mature B cells initiated in 4-week-old NZB/W mice accelerated the spontaneous disease, which paralleled the decrease in regulatory B cells (48). On the contrary, mature B cell depletion in 12 to 28-week old NZB/W mice significantly delayed disease onset. These data were interpreted to suggest that there are two distinct B cell populations that have either protective or disease-promoting roles during disease progression. Especially, regulatory B cells seem to play a vital role during disease initiation of SLE rather than disease progression. Although the phenotype of regulatory B cells is similar between NZB/W mice and C57BL/6 mice, young NZB/W mice have expanded regulatory B cells compared with age-matched C57BL/6 mice (48), which is similar to the report in which young Palmerston North mice have expanded regulatory B cells. The CD1d+CD5+B220+ B cell subset, which is enriched in IL-10-producing regulatory B cells, is increased 2.5-fold during the disease course in wild-type (WT) NZB/W mice, while CD19−/−NZB/W mice, which also develop SLE, lack this CD1d+CD5+B220+ regulatory B cell subset (49). Finally, the potential therapeutic effect of regulatory B cells in SLE is
highlighted by the extended survival of CD19−/−N2ZB/W mice following the adoptive transfer of splenic CD1d−/−CD5+ regulatory B cells from WT N2ZB/W mice (49).

MRL/+/−mice, which have a mutation in the Fas gene, also spontaneously develop an SLE-like disease characterized by autoimmune production, arthritis, vasculitis, glomerulonephritis and sialadenitis (50). In a previous report, B cell specific depletion of IL-10 did not affect spontaneous autoimmune in MRL/+/−mice (51). On the other hand, Mauri et al. reported that CD24hiCD23hi-T2-marginal zone precursor (MZP) B cells were enriched in IL-10-producing regulatory B cells from MRL/+/−mice and that the transfer of anti-CD40 antibody-treated T2-MZP B cells significantly improved nephritis and prolonged survival of MRL/+/−mice in an IL-10 dependent manner (52). Moreover, anti-CD40 antibody-treated T2-MZP B cells induced the differentiation of IL-10-producing CD4+ T cells (52). Although there are some discrepancies as to phenotypes and roles of regulatory B cell subset between NZB/W mice and MRL/+/− mice, these studies using mouse models of spontaneous lupus suggest the importance of the protective role and potential therapeutic effects of regulatory B cells.

The first clinical study using peripheral blood B cells from SLE patients showed that the frequencies of CD5+ B cells producing IL-10 were significantly higher in SLE patients than those in normal controls, when they were cultured in the presence or absence of phorbol-12-myristate-13-acetate (PMA) and ionomycin, or lipopolysaccharide (LPS) (27). Another study showed that IL-10 production was closely associated with CD154 expression on B cells, suggesting importance of cellular activation for IL-10 production, and that the IL-10-producing CD154hi B cells increased aberrantly when peripheral blood mononuclear cells (PBMC) from SLE patients were stimulated with Staphylococcus aureus Cowan 1 (SAC) (28).

Consistent with these studies, Tedder et al. also reported that IL-10-producing B cell frequencies in the peripheral blood were significantly higher in patients with several autoimmune diseases including SLE, rheumatoid arthritis (RA), Sjögren syndrome, autoimmune bullous diseases and multiple sclerosis (MS), compared with those in normal controls when stimulated with LPS or CpG in the presence of CD40 ligation for 48 h with additional PMA and ionomycin (PI) (24). Yang et al. (29) also showed an increase in CD19hiIL-10+ B cells in the blood of SLE patients when the cells were stimulated with LPS for 24 h with the additional PI.

Recently, the concept of regulatory B cell dysfunction in autoimmune diseases has been introduced by demonstrating functional impairment of CD24hiCD38hi regulatory B cells in SLE patients (23). After CD40 stimulation, CD24hiCD38hi regulatory B cells suppressed IFN-γ and TNF-α production from CD4+ T cells in normal controls, whereas they did not have the inhibitory effect in SLE patients. This effect was partially dependent on IL-10. CD24hiCD38hi regulatory B cells isolated from peripheral blood of SLE patients were unresponsive to CD40 stimulation and produced less IL-10 than those from normal controls. Another study showed that in SLE patients, FSChi fractions of B cells stimulated with IL-2 and SAC for 72 h had less suppressive effects on T cell proliferation compared with those from normal controls (30). Collectively, although regulatory B cells are increased in the peripheral blood of SLE patients, the function of those cells is impaired.

**Rheumatoid arthritis**

RA is characterized by chronic inflammation of the joint capsule and synovial membrane, resulting in synovial proliferation, cartilage injury, and bone erosion, with eventual joint destruction (53). It is often associated with systemic multorgan vasculitis, pulmonary fibrosis and extra-articular rheumatoid nodulosis.

B cells are considered harmful in RA, because B cell depletion therapy using rituximab has been shown to be effective. Studies using mouse RA models have shown that B cells are important for initiation of inflammation. Collagen-induced arthritis (CIA) is a model for RA that develops in susceptible mouse strains immunized with heterogeneous type II collagen emulsified in complete Freund’s adjuvant (54, 55). Mature B cell depletion prior to CIA induction significantly reduced the disease severity, while B cell depletion after the establishment of disease did not affect the disease severity (56).

On the other hand, several reports using CIA have demonstrated a protective function and therapeutic potential of regulatory B cells. When B cells from arthritogenic mice were stimulated with collagen and an anti-CD40 monoclonal antibody (mAb), they produced high levels of IL-10 and low levels of IFN-γ (57). Induction of CIA was inhibited and the established disease was improved by the adoptive transfer of these B cells into immunized recipient mice, which is accompanied by inhibition of T11 cell differentiation. This regulatory effect of B cells depends on IL-10 production, since the absence of these B cells into immunized recipient mice, which is accompanied by inhibition of T1 cell differentiation (58).

In addition, T2-MZP B cells played a key role in controlling autoimmunity and inflammation in an antigen-induced arthritis model (59), in which immunization with methylated bovine serum albumin in complete Freund’s adjuvant with Bordetella pertussis toxin is used (60). T2-MZP B cells had the ability to support regulatory T cell differentiation at the expense of T1 and T17 cell differentiation and the adoptive transfer of these cells inhibited inflammation in this antigen-induced arthritis model (59).

Other than T2-MZP B cells, ex vivo expanded CD19hiCD5+ regulatory B cells also have the potential to delay CIA onset and ameliorate disease severity through inhibition of T17 cell differentiation, when adoptively transferred (61). In a different study, administrating apoptotic thymocytes to mice before the onset of CIA resulted in protection from severe joint inflammation and bone destruction (62). Activated splenic B cells increased IL-10 secretion through a direct response to apoptotic cells in vitro, and inhibition of IL-10 in vivo reversed the beneficial effects of apoptotic cell treatment (62). Thus, these studies have suggested that regulatory B cells can regulate the disease course and outcome in mouse RA models.

In contrast to SLE, analysis of peripheral blood regulatory B cells in RA patients had not been done fairly until recently. Mauri et al. compared the function and the numbers of peripheral blood regulatory B cells from RA patients with those from normal controls (31). In this study, they showed that CD24hiCD38+ regulatory B cells from normal controls...
inhibited CD4+CD25+ T cell differentiation into T\(_{\text{H}1}\) and T\(_{\text{H}17}\) cells and promoted their conversion into regulatory T cells, partially through IL-10 production. By contrast, CD24hiCD38hi regulatory B cells from RA patients failed to suppress T\(_{\text{H}17}\) differentiation and convert naive T cells into functional regulatory T cells. In addition, both the frequency and absolute number of CD24hiCD38hi regulatory B cells were significantly decreased in peripheral blood from RA patients compared with those from normal controls. The frequency of CD24hiCD38hi regulatory B cells in RA patients negatively correlated with the disease activity.

Recently four reports on regulatory B cells in RA patients were published (32–35). Among them, three different groups reported a decreased number or frequency of IL-10-producing B cells or regulatory B cell subsets in RA patients compared with normal controls: decreased frequency of IL-10+ B cells after in vitro stimulation with CpG for 24 h with the additional PI (32), decreased number of CD19-TIM-1+IL-10+ B cells and CD19+CD5+CD1d+IL-10+ B cells when PBMC were stimulated with LPS and PI (33), and decreased frequency of CD19+CD5+CD1dhi B cells (34). On the other hand, one group showed increased frequencies of IL-10+ B cells in RA patients when PBMC were stimulated with CpG in the presence of CD40 ligation for 48 h with the additional PI (35). The discrepancy may be due to the difference in stimuli used for IL-10 production from B cells. Of interest, in all four reports, each number or frequency of regulatory B cells negatively correlated with disease activity score in 28 joints reflecting the disease severity (63).

On the basis of the multiple reports describing a negative correlation between regulatory B cell number or frequency and disease severity, regulatory B cells may contribute to suppression of the disease in RA patients, although their function may be attenuated.

**Multiple sclerosis**

As a representative organ-specific autoimmune disease, MS is characterized by multifocal inflammation, demyelination, gliosis and axonal loss in the central nervous system (CNS) and is one of the foremost causes of non-traumatic neurological disability in young adults (64, 65).

Experimental autoimmune encephalomyelitis (EAE) is a T\(_{\text{H}1}\)-mediated autoimmune disease of the CNS induced by immunization with myelin proteins or peptides, such as myelin oligodendrocyte glycoprotein (MOG) (66, 67). It is an established MS model that is characterized by acute CNS inflammation, demyelination and paralysis. The regulatory role of B cells in EAE was first evident in 1996 (68). When μMT mice, which genetically lack B cells, were immunized with myelin basic protein peptide, they developed a more severe form of EAE than WT mice, suggesting a regulatory role for B cells in EAE (68). A regulatory role for B cells was also demonstrated in another model of EAE using proteolipid protein peptide (69). Furthermore, B cells regulated EAE induced by immunization with MOG peptide through IL-10 production because the adoptive transfer of WT B cells, but not IL-10−/− B cells, normalized EAE severity in μMT mice (70).

In agreement with this, Tedder et al. showed that B cell depletion by anti-CD20 mAb treatment 7 days before MOG peptide immunization in WT mice exacerbated the EAE symptoms (71). This effect is associated with regulatory B cells, as similar effects were observed with selective CD1d+CD5+ regulatory B cell depletion using anti-CD22 mAb (72). Adoptive transfer of CD20−/− CD1d+CD5+ regulatory B cells before EAE induction completely normalized EAE exacerbated by treatment with anti-CD20 mAb, indicating the therapeutic potency of regulatory B cells in a mouse MS model (71). Interestingly, B cell depletion using anti-CD20 mAb after the establishment of the disease (on day 14) ameliorated the disease symptoms in contrast to depletion before EAE induction, and the adoptive transfer of regulatory B cells did not suppress established EAE (71, 72). By contrast, regulatory T cell depletion after onset of EAE worsened late-phase disease. Thereby, regulatory B cells regulate the effector subsets during disease initiation, while regulatory T cells are mainly involved in the regulation of the late phase of the disease.

Using an EAE model, it has been clarified that diverse molecules are involved in regulatory B cell function. Chimeric mice lacking B cell-specific CD40 expression developed a severe form of EAE (70), suggesting that CD40 expression on B cells was needed for regulatory function of B cells. A strong linkage between CD40 expression and IL-10 production was also indicated in a MRL/lpr model and a CIA model (52, 57). A different study revealed that B cells required B7.1 (CD80) and B7.2 (CD86). B7 family members of costimulatory molecules, for the resolution of EAE (73).

Apart from costimulatory molecules, Toll-like receptors (TLRs) were also shown to be involved in regulatory B cell function, since B cells activated with TLR2 and TLR4 ligands produced IL-10 and suppressed EAE (74). In addition, store-operated Ca\(^{2+}\) influx induced by the endoplasmic reticulum calcium sensors STIM1 and STIM2 was critical for IL-10 production by regulatory B cells (75). Furthermore, in vivo regulatory B cell function required IL-21 along with cognate interactions with CD4+ T cells through CD40 and major histocompatibility complex class II (MHC-II) since the adoptive transfer of CD1d+CD5+ regulatory B cells from IL-21 receptor−/−, CD40−/−, or MHC-II−/− mice had no effect on the disease course of EAE (76). In summary, regulatory B cells can control and resolve EAE through complex mechanisms involving diverse surface molecules.

The first study about IL-10 production by peripheral blood B cells from MS patients was reported in 2007. Duddy et al. (36) showed that IL-10 production from B cells in both relapsing–remitting MS and secondary progressive MS patients was significantly decreased compared with those in normal controls when PBMC were cultured with CD40 ligand (CD40L) in the presence or absence of anti-human IgG and IgM polyclonal antibody for 48 h, which was corrected by mitoxantrone treatment. Another study also showed reduction of IL-10 production from B cells in MS patients when incubated with CpG for 24 h (37). A different study demonstrated that frequency of IL-10+ B cells in patients with relapsing–remitting MS significantly decreased compared with those in normal controls, when PBMC were cultured with CpG for 72 h with the additional PI (38).
Meanwhile, Correale and Farez found that helminth-infected MS patients had a significantly milder course compared with uninfected MS patients (77). They demonstrated that IL-10 production from B cells in helminth-uninfected MS patients was significantly reduced compared with those in normal controls, whereas IL-10 production levels in helminth-infected MS patients were apparently increased to the same extent as normal controls (22). They also showed that B cells from helminth-infected MS patients, but not uninfected MS patients, suppressed proliferation and IFN-γ production by myeloid basic protein-reactive T cell lines (22). In addition, they revealed that TLR2 stimulation by helminth molecules was important for exerting the regulatory effects of B cells (78).

Collectively, IL-10 production and regulatory function of peripheral blood B cells are impaired in MS patients. Manipulating regulatory function of B cells can be a novel therapeutic approach for the treatment of MS.

Inflammatory bowel disease

Inflammatory bowel disease (IBD) is a chronic relapsing intestinal inflammatory disease classified into two major forms, Crohn's disease and ulcerative colitis (UC), which are mediated by both common and distinct mechanisms with different clinical features (79–81). The prevalence and incidence of IBD are increasing with time and in different regions around the world (82).

B cells have been generally considered to play a pathogenic role in IBD (83) since increased production of anti-goblet cells autoantibodies was associated with IBD, especially UC (84). However, Mizoguchi et al. (85) showed a protective role of B cells and autoantibodies in T cell receptor (TCR) α chain-deficient (TCRα−/−) mice, which spontaneously developed chronic colitis. They revealed that the disease onset was accelerated and the symptoms became more severe in B cell-deficient TCRα−/− mice. They subsequently demonstrated that CD1d+ B cells were induced in gut-associated lymphoid tissues of mice with intestinal inflammation and dampened the disease symptoms via IL-10 production in TCRα−/− mice (86). In addition, in TCRα−/− mice, IL-10-producing B cells also suppressed T cell-mediated intestinal inflammation through induction of IL-12-producing B cells (87).

The efficacy of adoptive transfer of B cells from mesenteric lymph nodes was demonstrated in not only TCRα−/− mice but also G protein α inhibitory subunit 2-deficient mice, another spontaneous model of IBD (87, 88). CD1d+CD5− regulatory B cells from spleen or peritoneal cavity have also been demonstrated to have suppressive activity in different mouse colitis models such as dextran sodium sulfate-induced acute colitis (89–91). CD1d+CD5− regulatory B cells control the intestinal inflammation by decreasing IFN-γ-producing T cells and increasing regulatory T cells (90, 91). Recently IL-10-producing B cells with remarkable phenotypes (CD19+CD25−CD1d+IgM+CD5−CD23−TIM-1−) were identified in IL-33-treated mice, and their adoptive transfer blocked the development of colitis in IL-10−/− mice (92). Furthermore, impaired development of IL-10-producing regulatory B cells was shown in G protein α inhibitory subunit 2-deficient mice and SAMP/YitFc mice which spontaneously developed intestinal inflammation in ileum and caecum, resembling Crohn's disease (88, 93).

Collectively, regulatory B cells play an important role in the suppression of intestinal inflammation and ameliorate disease manifestations in IBD mouse models in an IL-10-dependent manner.

Studies of regulatory B cells in patients with IBD are extremely limited compared with those in SLE, RA, or MS patients. There is only one report on regulatory B cells from IBD patients, in which IL-10 production was significantly decreased in B cells from patients with Crohn's disease and UC when incubated with CpG for 72h, compared with those from normal controls (39). Meanwhile, a double-blinded randomized controlled trial of rituximab therapy for steroid-resistant moderately active UC showed no significant effect on inducing remission (94). Moreover, an exacerbated case of UC with decreased IL-10 expression after rituximab therapy was reported (95). There are also some case reports with autoimmune disease such as RA, Graves' disease, nephritic syndrome or bullous LE, where rituximab therapy was a trigger of UC (96–99). Taken together, regulatory B cells could contribute to protection against IBD.

Type 1 diabetes

Type 1 diabetes (T1D) is an autoimmune disease in which insulin-producing β-cells in the pancreatic islets are destroyed at an early age by an immune process that involves both CD4+ and CD8+ T cells recognizing islet autoantigen, resulting in hyperglycemia and associated complications (100–102).

The role of regulatory B cells has been studied using non-obese diabetic (NOD) mice. Although destruction of islet β-cells is primarily mediated by T cells in NOD mice (103), B cells also play a pathogenic role in the initiation phase of the disease since B cell depletion by anti-CD20 mAb in prediabetic littermates protected NOD mice against diabetes (104).

Some studies, however, indicated a regulatory function of B cells in NOD mice. The adoptive transfer of LPS-activated B cells starting at 4 weeks of age decreased the diabetes incidence (105). In addition, the adoptive transfer of B cell receptor-stimulated B cells starting at 5–6 weeks of age both delayed onset and reduced the incidence of diabetes (106). This effect was dependent on IL-10 as the adoptive transfer of IL-10−/− NOD B cells did not suppress the diabetes (106). A recent study showed that the tolerogenic dendritic cells prevented and reversed diabetes in NOD mice partially through proliferation of IL-10+ B cells and conversion of CD19+ B cells into IL-10-producing B cells (107). These data suggest that regulatory B cells have the potential to control diabetes in NOD mice.

There are very few reports on regulatory B cells in T1D patients. Thompson et al. (40) reported that no difference was observed in the frequency of IL-10+ B cells in peripheral blood between T1D patients and normal controls when PBMC were cultured with IL-21 for 72h with the additional CpG, LPS, and PI. On the other hand, Kleffel et al. (41) reported that T1D patients showed decreased frequencies of IL-10+ B cells compared with normal controls when cultured with LPS and CD40L for 96h. The discrepancy between these two studies may have come from the difference in ways used...
to stimulate B cells. Further studies are needed to understand the involvement of regulatory B cells in pathogenesis of human T1D.

Pemphigus
Pemphigus is another organ-specific autoimmune disease, mediated by IgG autoantibodies to desmoglein 1 and/or desmoglein 3. These autoantibodies block keratinocyte adhesion, resulting in intraepidermal blistering of mucosa membranes and skin (108, 109). In pemphigus patients, activated B cells are generally considered as a positive regulator via secretion of autoantibodies.

Regulatory B cell involvement in pemphigus has not been investigated in a mouse model of pemphigus. In humans, Zhu et al. (42) revealed the up-regulated frequency of CD24hiCD38hi regulatory B cells in peripheral blood of pemphigus patients. Regulatory B cells from pemphigus patients had a lower capacity to produce IL-10 after 48 h of stimulation with CpG, LPS and CD40L compared with those from normal controls (42). In addition, as was the case with SLE (23), they failed to suppress IFN-γ production from CD4+ T cells (42).

On the other hand, as B cell depletion therapy is effective for pemphigus, regulatory B cells in patients with pemphigus treated with rituximab were also investigated. Pemphigus patients who experienced complete remission 79 months after rituximab therapy had higher frequencies of both CD24hiCD38hi regulatory B cells and IL-10+ B cells than either untreated patients or patients who experienced incomplete remission after rituximab therapy (110). Thus, regulatory B cell function may be impaired in pemphigus patients, and recovery of functional regulatory B cells after B cell depletion may contribute to remission of the disease.

Allergic diseases
Allergic diseases include many heterogenous pathologies with distinct clinical manifestations. These pathologies generally result from an uncontrolled inflammatory response to allergens and can lead to a number of disorders such as asthma, allergic rhinitis, and atopic dermatitis. Mechanisms promoting allergic inflammation are characterized by a T2-polarized immune response. One of the significant causes of allergic inflammation development is an alteration in the immune regulatory processes (111).

The first experiments demonstrating the regulatory function of B cells in allergic disease model were performed in 1974 (112). Treatment with cyclophosphamide before sensitization enhanced inflammatory responses in a guinea pig model of skin hypersensitivity, suggesting a regulatory role for B cells in this model. Since then, very few studies have demonstrated the existence of regulatory B cells in allergic disease models. However, observations that CD19 deficiency results in increased and prolonged contact hypersensitivity (CHS) reactions were reported recently and have pointed to the regulatory function of B cells in skin allergic diseases (113). In addition, adoptive transfer of splenic CD19−/−CD5− B cells from mice with CHS inhibited CHS responses (113, 114). Other than the CHS model, regulatory B cell involvement has been investigated in several allergic diseases. CpG injections reduce inflammation in a late-phase experimental allergic conjunctivitis model through an increased proportion of IL-10-producing B cells with a follicular phenotype (115). Adoptive transfer of splenocytes from CpG-treated mice suppressed allergen-induced inflammatory responses in recipient mice (115). Other studies have shown that B cells isolated from helminth-infected mice have a protective function in allergy by controlling fatal anaphylaxis or ovalbumin-induced airway inflammation via IL-10 production (116–119). Augmentation of IL-10 production from B cells by helminth infection was also demonstrated in humans (22, 120).

Among a variety of human allergic diseases, food allergy has been the most investigated, using human samples, in association with regulatory B cells. Specific in vitro re-stimulation with casein induced a decrease of the frequencies of IL-10-producing CD5− B cells in the milk allergy group but unchanged or increased them in the milk-tolerant group (121, 122). These reports suggest a role for regulatory B cells in the establishment of milk tolerance. Interestingly, milk intake associated with IFN-γ injections completely suppressed the milk allergy by increasing regulatory B cells, but the ingestion of milk alone could not (123). Moreover, in the context of bee-venom allergy, beekeepers who spontaneously develop tolerance toward the allergen and patients undergoing immunotherapy exhibited a higher level of antigen-specific regulatory B cells after 72 h culture with CpG, compared with patients before immunotherapy (124). Similar to the study on milk allergy, this study also suggests the importance of regulatory B cells in establishment of allergen tolerance.

The frequency and function of IL-10-producing regulatory B cells in patients with asthma was recently examined. Lower numbers of IL-10-producing CD24hiCD27hi regulatory B cells were found in asthma patients when B cells were cultured with LPS for 48 h with additional PI (43). When CD4+ T cells activated with the dust-mite allergen were co-cultured with LPS-stimulated B cells, they produced less IL-10 in asthma patients compared with those in healthy controls, suggesting defective function of regulatory B cells in patients with asthma (43). Another group also showed the decreased frequency of CD24hiCD27hi B cells in allergic rhinitis patients (44).

Taken together, regulatory B cells may contribute to suppression of allergic diseases and establishment of allergen tolerance, but the function may be impaired. Primary human B cells transfected with IL-10 secreted less IgE which plays an important role in allergy (125). Thus, manipulating regulatory B cell function can be a safe therapeutic strategy for allergic diseases.

Conclusion
In this article, studies on regulatory B cells in a wide variety of inflammatory and autoimmune diseases and their mouse models have been reviewed. On the basis of a large amount of data using mouse models, regulatory B cells play an important role in preventing the disease onset or suppressing the disease symptoms. In clinical research, several reports show regulatory B cell dysfunction in human diseases. In addition, the therapeutic potency of regulatory B cells is also indicated. Clarifying more details of the immune regulation by human
regulatory B cells could provide a basis for the development of novel B cell-mediated therapeutic strategies.

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