Immunobiology of B Cells in Inflammatory Bowel Disease

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As B cells predominate at inflamed mucosal sites and possess multiple functions including the ability to recognize microbial TLR ligands, they are likely to play a role in the pathogenesis of inflammatory bowel disease (IBD) [1–3]. In this chapter, the impact of B cells on IBD pathogenesis is discussed in relation to the recently accumulating knowledge of B cell functions: antibody production, antigen presentation and interaction with T cells, and cytokine production (Fig. 9.1). Since most of the information regarding the immunopathogenesis of IBD in the last two decades has been obtained by the use of experimental models, the emphasis is on IBD models [4–6] rather than on human studies.

Antibody Production

B cells in the intestine are primarily located in the lymphoid follicles and as plasma cells in the lamina propria [7]. The activation of mucosal B cells occurs in the lymphoid follicles and mesenteric lymph nodes (MLN) with subsequent migration and differentiation to predominantly IgA secreting plasma cells in the lamina propria. The circulating B cells with activated phenotype ($TLR2^+$) that are capable of migrating to mucosal sites may either represent activated mucosal B cells or B cells activated in circulation, perhaps by translocated enteric bacteria/bacterial antigens [8]. The histological evidence of prominent lymphoid follicles and lymphoplasmacytic infiltrate in the inflamed intestine suggests involvement of B cells in IBD, in particular ulcerative colitis (UC). Recent studies suggest that plasma may play a pathogenic role in addition to their known function of producing antibodies. Plasma cells characterized by $CD19^+$ $CD20^-CD27^{low}$ $CXCR4^{high}$ unique immature phenotype are increased in the inflamed mucosa of UC patients, and they are capable of activating pathogenic $CD14^+$ macrophages via IgG-IC-FcγR signaling [9]. Furthermore, $CD27^+$ $CD38^{high}$ $CD20^-$ IgA^+ plasma cells, which expand in the inflamed mucosa of both UC and CD patients, could provide cytotoxicity to epithelial cells by producing granzyme B in response to IL-21 [10]. IgM^+ $CD19^+$ $CD138^+$ plasma cells are capable of producing IL-10 [11].

The frequent presence of several types of circulating antibodies reactive with both microbial antigens and self-antigens in IBD supports the notion that dysregulated immune response to normal enteric microorganisms represents the primary pathogenic event in IBD. The antibodies include anti-Saccharomyces cerevisiae antibodies (ASCA), anti-neutrophilic cytoplasmic antibodies (ANCA), and antibodies to outer membrane porin (OMP), Pseudomonas fluorescence-related sequence I2, and Cbir (see below), and anti-carbohydrate antibodies (ALCA, ACCA, AMCA) [12, 13]. However, most of the studies performed with circulating antibodies have focused on their diagnostic or prognostic utility rather than their role in IBD pathogenesis [12–15]. ASCA are detected frequently in CD, whereas seropositivity for ANCA predominates in UC.

The normal IgA dominant immune response at the mucosal sites is skewed towards IgG in chronically inflamed mucosa of IBD [16]. The isolated cells from the inflamed mucosa have been shown to secrete antibodies to bacteria to *Escherichia coli* strains [16, 17] as well as antibodies against colonic epithelial antigens [18]. Antibodies to *Escherichia coli* are more often detected in CD, whereas anti-colonic epithelial antibodies are particularly seen in UC. The colonic epithelial antigens that are reactive with antibodies include tropomyosin (40 kDa) isoforms (TM1 and TM5) and 200 kDa colon epithelial protein [19]. Studies performed in Per Brandtzaeg's laboratory have provided evidence for a pathogenic role of antibodies in UC by showing complement

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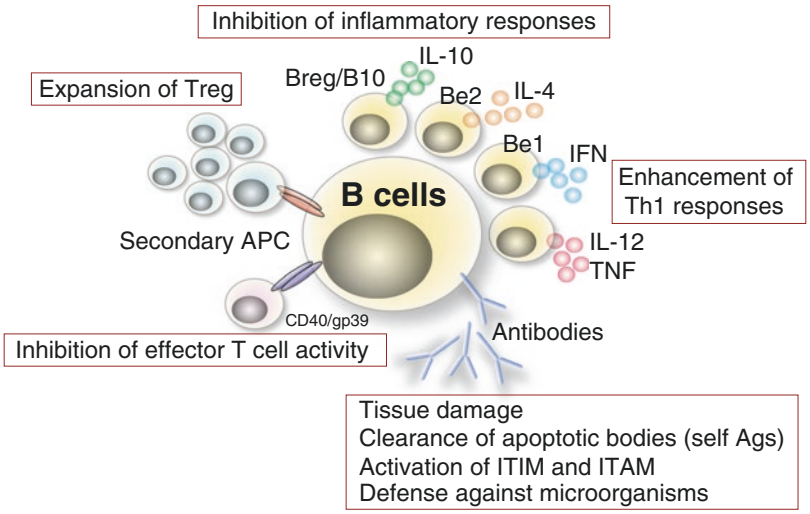

Fig. 9.1 Functional diversity of B cells in IBD: Immunoglobulins (Igs) produced by B cells may have both deleterious and protective roles in IBD. Binding of autoantibodies to colonic tissues or IgG Fc fragment-mediated ITAM-dependent activation of immune responses could result in tissue damage. Antibodies could provide protection by altering the diversity of enteric microorganisms that are required for the development of IBD and by helping the clearance of apoptotic bodies that may serve as a source of self-antigens for eliciting autoimmune responses. Furthermore, IgG may suppress immune response through the ITIM by binding to inhibitory FcγRIIb receptors expressed on immune cells. B-cell subsets could modulate inflammatory responses depending on their distinct cytokine production profiles. IL-10-producing B cells ("Breg") inhibit chronic colitis progression. B-cell subsets producing IL-12p70 or IFN-γ may have a pathogenic effect in CD, but a beneficial role in UC. B cells may also regulate immune responses by serving as a second line of APCs, by enhancing expansion of $CD4^+$ $Foxp3^+$ Tregs, and by inhibiting proliferation of effector $CD4^+$ T cells in a contact-dependent manner

activation in relation to IgG1 deposited at the apical aspect of the colonic epithelium [16, 20]. It is not clear whether above findings represent primary pathogenic events or a secondary phenomenon due to local immune response in the setting of chronic inflammation and tissue injury. In any case, the locally produced antibodies help maintain epithelial barrier, and may play a role in regulating enteric flora repertoire and excluding invading microorganisms. Decreased J-chain production with resultant reduced secretion of dimeric IgA has been reported in IBD [16, 21]. However, IgG antibodies, because of their phagocytotic enhancing properties, are likely to be more efficient than IgA in removing invading microorganisms and antigens, and could compensate for abnormality of the IgA response in IBD [16]. They could be also involved in the pathogenesis of IBD (see discussion of IgG Fcγ receptors below) [22].

Circulating autoantibodies and antimicrobial antibodies have also been reported in experimental models of IBD [5, 23–25]. The IL-4-mediated spontaneous colitis in T-cell receptor α knockout (TCRαKO) mice resembles ulcerative colitis, and is associated with expansion of MLN B cells, increased production of antibodies (ANCA, anti-nuclear, and anti-tropomyosin), and alteration of polyclonal to an oligoclonal immune response to cecal bacterial antigens [23, 24]. This raised the possibility that B cells or antibodies may be pathogenic in this model. However, B cell-deficient mice TCRαKO mice developed more severe colitis (see below) [26]. Transfer of autoantibodies or purified immunoglobulin from TCRαKO mice to B- cell-deficient TCRαKO led to attenuation of colitis and decrease in the apoptotic cells, supporting the notion that autoantibodies may have a role in clearance of self-antigens released from apoptotic cells [26]. In addition, B-1 B cells, which represent a major source of natural IgM antibodies that provide first line of defense against microorganisms, are fully activated in conventional facility as compared to specific pathogen-free facility, resulting in the inhibition of colitis in TCRαKO mice [27]. These results support a role of B cells in the "hygiene hypothesis", which is based on the observation that repeated childhood infections lead to decreased incidence of allergic diseases in adulthood [27].

The spontaneous colitis in C3H/HeJBir mice is associated with both B and T cells responses to selective enteric bacterial antigens; the colitis can be transferred with T cells. Unlike TCRαKO mice, the oligoclonal response to enteric bacteria is detected even in young C3H/HeJBir mice [25]. Serologic expression cloning of cecal bacterial antigens in C3H/HeJBir mice led to the identification of previously unknown microbial flagellins [28]. The flagellin, CBir1, was found to be the dominant antigen capable of inducing T-cell-mediated colitis. Interestingly, sera from about 50 % of patients with Crohn's disease are reactive with CBir1; the CBir1 sera reactivity identified a subset of patients with complicated CD [29].

It is well established that humoral immunity can be regulated by Fc fragments of IgG [22]. Although most receptors of IgG, FcγRs are activating receptors due to the presence of

the immunoreceptor tyrosine-based activation motif (ITAM), FcγRIIB is the only FcγR that has been shown to have inhibitory functions through immunoreceptor tyrosine-based inhibitory motif (ITIM), which includes suppression of B cells, macrophages, dendritic cells, mast cells and basophils [22]. FcγRIIB is involved in the pathogenesis of autoimmune disease, in particular lupus erythematosus. Recent studies indicate that intravenous immunoglobulin (IVIG) in autoimmune diseases and infliximab [anti-TNFα antibodies] in rheumatoid arthritis may partly act through FcγRIIB [30, 31]. Since FcγRIIB also effects antimicrobial immune responses [22], it is likely that this receptor may play an important role in the pathogenesis of IBD. FcγRIIB KO mice exhibit less distal colon inflammation during C*itrobacter rodentium* infection, probably due to increased phagocytic function of macrophages as compared to wild type mice [32]. Granulomatous inflammation developing in B cell and IL-4 deficient TCRα triple knockout mice can be suppressed by the administration of Fc fragments of IgG [33]. The importance on Fc-mediated pathway in IBD is highlighted by recent genome-wide association studies identifying FcγRIIA an UC-associated gene [34].

Antigen Presentation and Interaction with T Cells

It has become increasingly clear that B cells have functional capabilities that are not directly related to secreted immunoglobulins. B cells have been shown to serve as *a* "second line" of antigen presenting cells (secondary APCs) by conditioning the activity of effector memory T cells that have already been primed by professional antigen presenting cells such as dendritic cells [35, 36]. Indeed, B cells can suppress proliferation of effector $CD4^+$ T cells in a contact dependent manner through the interaction of CD40 on B cells and gp39 on effector T cells [37, 38]; this interaction contributes to the suppression of colitis in TCRαKO mice [37]. This observation is supported by a study showing that forced ectopic overexpression of gp39 on B cells, leading to the impairment of interaction of CD40 (B cells) and gp39 (T cells), induces the development of colitis [39]. In Gαi2 knockout mice, B cells facilitate expansion of $CD4^+CD8\alpha^+$ intraepithelial T cells and $CD3^+$ $CD4^-$ NKT cells with consequent suppression of colitis [40]. MHC class I-mediated antigen presentation is required for this B-cell-mediated induction of regulatory $CD8^+$ T cell subset capable of controlling colitis through the production of perforin [41].

Since autophagy is a cellular degradation system, which is used not only for the elimination of intracellular bacteria but is also involved in adaptive immune responses as well as MHC-dependent antigen presentation [42], it is likely that autophagy plays an important role in secondary APC function of B cells. Autophagy is also required for B cell development [43], and for B cells to induce tolerance of $CD4^+$ T cells [44]. Genome-wide association studies have identified autophagy-related gene (Atg) 16L1 as a CD susceptibility gene [1] and a deletion polymorphism upstream of IRGM, a gene essential for autophagy, is also associated with the development of CD [45].

A number of studies suggest that regulatory B cells function through interaction with regulatory $CD4^+$ $Foxp3^+$ (Treg) cells that are known to suppress a wide range of murine and human inflammatory responses [46]. B cells may enhance the expansion of Tregs either in a contact-dependent manner or a contact-independent manner through the production of IL-10 [38, 47, 48] or maximize their regulatory activity. Spontaneous colitis in mice expressing T-cell-specific dominant negative TGFβ receptor II is exacerbated when they are crossed with B-cell-deficient mice, and the B-cell deficiency is associated with a significant reduction of Tregs [49]. In addition, an acute colitis induced by dextran sulfate sodium (DSS) was exacerbated in the absence of B cells, and adoptive transfer of B cells improved it in an IL-10-independent manner [50]. These regulatory interactions of regulatory B-cells and Tregs support the findings of recent genetic studies in IBD patients, which highlight the significance of immune regulatory network to prevent the development of IBD [1].

Cytokine Production

The recent recognition of B cells as cytokine-producing cells represents a major advancement in understanding the function of B cells in inflammatory disorders. Both human and murine B cells can produce a spectrum of cytokines, especially under inflammatory conditions. The cytokines include IL-4, IFN-γ [51], IL-2, TNF-α [52], GM-CSF [53], TGF-β [54], and IL-12p70 [55]. Therefore, like $CD4^+$ T cells, B cells may be classified into functionally different subsets: IFN-γ-producing B effector 1 (Be1) and IL-4-producing B effector 2 (Be2) cells [51, 52].

Our studies in TCRαKO mice have identified a B-cell subset that regulates inflammation by the production of a regulatory cytokine IL-10; we have called these regulatory *B* cells ("Breg") [56]. As stated above, TCRαKO mice spontaneously develop a Th2-mediated chronic colitis, and B-cell-deficient TCRα double knockout mice develop much more exacerbated form of colitis as compared to TCRαKO mice indicating a protective role of B cells in this colitis model [26]. IL-10-producing B cells, which are characterized by high expression levels of CD1d, appear in the MLN of this model after, but not before, the development of colitis. Cell transfer studies conclusively showed that the inducible IL-10-producing B cells attenuate ongoing colitis [57]. A recent study using a reporter mouse system that expresses

green fluorescent protein (GFP) when IL-10 expressions are induced confirms that a major source of IL-10 in the MLN under inflammatory condition is B cells [58]. Importantly, B-cell-specific deletion of IL-10 cannot cause spontaneous colitis [58], consistent with previous reports that IL-10-producing Breg is involved in controlling the progression, but not induction, of colitis [56].

Several other studies have also identified IL-10 producing B cells to suppress diverse inflammatory diseases including IBD, graft versus host diseases (GVHD), experimental allergic encephalomyelitis and collagen-induced rheumatoid arthritis [56, 59–62]. IL-10-producing B10 cells are involved in suppressing different types of colitis, including DSS-induced acute colitis and Th1-mediated chronic colitis seen in IL-10 KO mice and CD45RB model in which colitis is induced in immunodeficient recipients by transfer of splenic $CD45RB^{high}$ $CD4^+$ T cells [63–65]. As stated above, IL-10-producing regulatory B cells exist at a very low number in normal conditions and expand under inflammatory conditions [56, 59]; these cells function primarily to suppress ongoing inflammation rather than inhibit the initiation of inflammatory process. The regulatory B cells exhibit unique phenotypic characteristics. This includes expression of both immature transitional type 2 B cells and fully matured marginal zone B cells [57, 61]. Although IL-10-producing regulatory B cells originate from B2 B cell lineage, some of these cells express a CD5, a marker associated with B1 B cells [66, 67]. Like dendritic cells, high levels of MHC class II may be expressed by some regulatory B cells [47, 48]. The development of Breg under intestinal inflammatory conditions may be induced by apoptotic cells [68].

IL-10-producing regulatory B-cell subsets may originate from either immature/naïve or activated memory B cells. Like regulatory T-cell subsets (Treg, Tr1, and Th3), it is likely that regulatory B cells also originate in the gut-associated lymphoid tissues (GALT) containing about 80 % of activated B cells [6, 7]. Our studies in TCRαKO mice indicate that "Bregs" appear in the mesenteric lymph nodes (MLNs) only under intestinal inflammatory conditions [56, 57] and are capable of expanding throughout the body [40]. Bregs, which are phenotypically characterized by high expression levels of CD1d, represents immature/naïve B cells that are polyclonally activated, presumably by stimulation with enteric microorganisms. Functionally, Bregs attenuate ongoing colitis by inhibiting proinflammatory responses such as the production of IL-1β [56, 57]. Recent studies have identified a spleen-specific IL-10-producing regulatory B cells termed "B10", which are characterized by a $CD1d^{high}$ $CD5^+$ surface phenotype [66]. The B10 cells originate from memory follicular B cell pool and develop in an antigen-dependent manner [59, 66]. B10 cells, unlike Bregs, regulate the initiation, but not progression, of inflammatory conditions such as murine lupus and experimental autoimmune encephalomyelitis by down-regulating the ability of dendritic cells to act as APCs for priming effector $CD4^+$ T cells [66, 67, 69]. Another difference between B10 cells and Breg is that B10 cells are detected in the systemic circulation, but not in lymph nodes, where Bregs develop [66].

In addition to IL-10-producing Bregs, another unique B cell population, capable of producing IL-12p70 but not IL-10, is also generated in the MLN of TCRαKO mice during colitis development and participates in the attenuation of this Th2-mediated colitis [55]. Interestingly, a unique B-cell subset, which is characterized by high expression levels of MHC class II and its ability to produce IL-12p70 in response to a bacterial product CpG (toll-like receptor 9 ligand), has been identified in the colon of these mice [70]. These unique colonic B cells are recruited from immature/transitional and recirculating naïve B2 B-cell pools. Like Bregs, they are inducible; they exist in normal colon at a very low number and expand during the recovery phase of intestinal inflammation [70]. IL-10 producing Bregs may have a wider role in inhibiting a large spectrum of inflammatory conditions, whereas IL-12p70 producing B cells may have a more limited role in suppressing Th-2-mediated colitis. Recent studies indicate that CD40L-expressing B cells suppress a $CD8^+$ T cell-induced colitis by inducing IL-10 expression in the pathogenic $CD8^+$ T cells [71]. B cells stimulated with *Hymenolepis diminuta* infection improved oxazolone colitis by producing TGF-β and cooperating with regulatory macrophages [72], and B cells expressing an ectoenzyme CD73 suppress DSS-induced colitis by producing adenosine [73].

A protective role of IL-10-producing B cells has also been demonstrated in IBD experimental models with features of human CD. These models include Gαi2 knockout mice in which the ability of regulatory B cells to produce IL-10 is impaired [74], CD45RB transfer model [75], and mice expressing T cell-specific dominant negative TGFβ receptor II in which B cells regulate colitis in an IL-10-independent manner [49]. Polyclonally activated B cells have been shown to suppress an innate immune-mediated spontaneous colitis in nuclear factor of activated T cells (NFAT) C2-deficient RAG2 double knockout mice; this suppression is not dependent on IL-10 [76]. In contrast to above studies, a pathogenic role of B cells has been reported in ileitis developing in the SAMP1/Yit congenic mouse model and in the TCRβ×TCRδ double knockout mouse model with the reconstitution of WT mouse-derived naïve $CD4^+$ T cells [77, 78]. It is possible that the function of B cells differs depending on the site of inflammation: a pathogenic role in small intestine (ileitis), but a regulatory role in large intestine (colitis). Since a recent study indicates that the development of IL-10-producing regulatory B cells is impaired in SAMP1/Yit mice [79], a pathogenic role of B may be exhibited in the absence of regulatory B cells.

Human Mucosal and Regulatory B cells

There have been a limited number of studies regarding the functional characterization of mucosal B cells in human IBD. In a recent study [8], circulating and mucosal tissue B cells (isolated from surgical resection specimens) from CD patients showed elevated levels of basal activation as indicated by TLR2 expression, spontaneous IL-8 secretion, and increased levels of phosphorylated signaling proteins. Correlation between increased expression of TLR2 and IL-8 and clinical activity was observed in CD but not in UC. Whether the hyperactivated B cells reflect a pathogenic role or merely reflect a secondary response to microbes in diseased mucosa is not clarified in the study. A more recent study [80] suggests that in IBD patients B cells could be modulated by TLR ligands towards proinflammatory or autoinflammatory activity depending on the predominance of systemic TLR ligands (LPS/endotoxin and high mobility group box 1). B cells from IBD patients also produce chemokine eotaxin in response to TLR ligands and may regulate directly or indirectly eosinophil tissue migration patterns [81].

A unique phenotype of $CD19^{+}$ $CD24^{high}$ $CD38^{high}$ $CD1d^{high}$ $CD5^{+}$ $CD27^{-}$ of human IL-10 producing regulatory B cells in peripheral blood lymphocytes (PBL) has been reported [38]. These regulatory B cells require in vitro CD40 stimulation to exhibit IL-10 production and inhibit differentiation of Th1 cells in vitro. This regulatory capacity is lacking in patients with systemic lupus erythematosus [38]. A recent study reports an increase of IL-35-producing $CD20^{+}$ regulatory B cells in the inflamed colon of CD patients as compared to UC and healthy controls [82].

A possible role of B cells in IBD has been suggested by B-cell depletion studies. In one study, UC was induced in a patient with Graves' disease after depletion of B cells through treatment with rituximab, a mouse–human chimeric anti-CD20 mAb [83, 84]. In another study, administration of this antibody in a UC patient led to the exacerbation of colitis [85]. Interestingly, the exacerbation of colitis was associated with a reduction of IL-10 production in the colon, supporting the possible protective role of IL-10-producing Bregs in UC. Recently, a clinical trial of B cell depletion by rituximab showed no significant effect of B cell depletion on inducing remission in moderately active UC [86]. However, there appeared to be increased in remission at week 4 but was not sustained.

Concluding Remarks

It is now well established that dysregulation of mucosal immune response to enteric bacteria is the underlying factor in the development of IBD. B cells form an important component of mucosal immune system for maintaining an epithelial barrier, regulation of the enteric microflora diversity, and development of adequate immune response to both enteric floral and food antigens. A compelling case for regulatory B cells has been made in IBD experimental models; however, a pathogenic role of B cells has not been excluded. The presence of circulating antibodies to self-antigens and enteric bacteria in many patients indicates B cell involvement in human IBD. Whether or not B cells play an important role in UC and CD pathogenesis has yet to be defined. Further understanding of the role of B cells in IBD would require functional characterization of human mucosal B cells in normal and diseased states. Genome-wide association studies of IBD may lead to the identification of B-cell-associated genes that may be candidate genes involved in the pathogenesis of chronic intestinal inflammation.

References

1. Abraham C, Cho JH. Inflammatory bowel disease. N Engl J Med. 2009;361(21):2066–78.
2. Xavier RJ, Podolsky DK. Unravelling the pathogenesis of inflammatory bowel disease. Nature. 2007;448(7152):427–34.
3. Strober W, Fuss I, Mannon P. The fundamental basis of inflammatory bowel disease. J Clin Invest. 2007;117(3):514–21.
4. Bhan AK, Mizoguchi E, Smith RN, Mizoguchi A. Colitis in transgenic and knockout animals as models of human inflammatory bowel disease. Immunol Rev. 1999;169:195–207.
5. Mizoguchi A, Mizoguchi E, Bhan AK. Immune networks in animal models of inflammatory bowel disease. Inflamm Bowel Dis. 2003;9(4):246–59.
6. Mizoguchi A, Mizoguchi E. Inflammatory bowel disease, past, present and future: lessons from animal models. J Gastroenterol. 2008;43(1):1–17.
7. Brandtzaeg P, Johansen FE. Mucosal B cells: phenotypic characteristics, transcriptional regulation, and homing properties. Immunol Rev. 2005;206:32–63.
8. Noronha AM, Liang Y, Hetzel JT, Hasturk H, Kantarci A, Stucchi A, et al. Hyperactivated B cells in human inflammatory bowel disease. J Leukoc Biol. 2009;86(4):1007–16.
9. Uo M, Hisamatsu T, Miyoshi J, Kaito D, Yoneno K, Kitazume MT, et al. Mucosal CXCR4+ IgG plasma cells contribute to the pathogenesis of human ulcerative colitis through FcgammaR-mediated CD14 macrophage activation. Gut. 2013;62(12):1734–44.
10. Cupi ML, Sarra M, Marafini I, Monteleone I, Franze E, Ortenzi A, et al. Plasma cells in the mucosa of patients with inflammatory bowel disease produce granzyme B and possess cytotoxic activities. J Immunol. 2014;192(12):6083–91.
11. Fillatreau S. Regulatory plasma cells. Curr Opin Pharmacol. 2015;23:1–5.
12. Peyrin-Biroulet L, Standaert-Vitse A, Branche J, Chamaillard M. IBD serological panels: facts and perspectives. Inflamm Bowel Dis. 2007;13(12):1561–6.
13. Ferrante M, Henckaerts L, Joossens M, Pierik M, Joossens S, Dotan N, et al. New serological markers in inflammatory bowel disease are associated with complicated disease behaviour. Gut. 2007;56(10):1394–403.
14. Sellin JH, Shah RR. The promise and pitfalls of serologic testing in inflammatory bowel disease. Gastroenterol Clin North Am. 2012;41(2):463–82.
15. Kuna AT. Serological markers of inflammatory bowel disease. Biochem Med. 2013;23(1):28–42.

16. Brandtzaeg P, Carlsen HS, Halstensen TS. The B-cell system in inflammatory bowel disease. Adv Exp Med Biol. 2006;579: 149–67.
17. Heddle RJ, La Brooy JT, Shearman DJ. Escherichia coli antibody-secreting cells in the human intestine. Clin Exp Immunol. 1982;48(2):469–76.
18. Hibi T, Ohara M, Toda K, Hara A, Ogata H, Iwao Y, et al. In vitro anticolon antibody production by mucosal or peripheral blood lymphocytes from patients with ulcerative colitis. Gut. 1990;31(12):1371–6.
19. Das KM. Relationship of extraintestinal involvements in inflammatory bowel disease: new insights into autoimmune pathogenesis. Dig Dis Sci. 1999;44(1):1–13.
20. Halstensen TS, Das KM, Brandtzaeg P. Epithelial deposits of immunoglobulin G1 and activated complement colocalise with the M(r) 40 kD putative autoantigen in ulcerative colitis. Gut. 1993;34(5):650–7.
21. Brandtzaeg P, Korsrud FR. Significance of different J chain profiles in human tissues: generation of IgA and IgM with binding site for secretory component is related to the J chain expressing capacity of the total local immunocyte population, including IgG and IgD producing cells, and depends on the clinical state of the tissue. Clin Exp Immunol. 1984;58(3):709–18.
22. Smith KG, Clatworthy MR. FcgammaRIIB in autoimmunity and infection: evolutionary and therapeutic implications. Nat Rev Immunol. 2010;10(5):328–43.
23. Mizoguchi A, Mizoguchi E, Chiba C, Spiekermann GM, Tonegawa S, Nagler-Anderson C, et al. Cytokine imbalance and autoantibody production in T cell receptor-alpha mutant mice with inflammatory bowel disease. J Exp Med. 1996;183(3):847–56.
24. Mizoguchi A, Mizoguchi E, Tonegawa S, Bhan AK. Alteration of a polyclonal to an oligoclonal immune response to cecal aerobic bacterial antigens in TCR alpha mutant mice with inflammatory bowel disease. Int Immunol. 1996;8(9):1387–94.
25. Brandwein SL, McCabe RP, Cong Y, Waites KB, Ridwan BU, Dean PA, et al. Spontaneously colitic C3H/HeJBir mice demonstrate selective antibody reactivity to antigens of the enteric bacterial flora. J Immunol. 1997;159(1):44–52.
26. Mizoguchi A, Mizoguchi E, Smith RN, Preffer FI, Bhan AK. Suppressive role of B cells in chronic colitis of T cell receptor alpha mutant mice. J Exp Med. 1997;186(10):1749–56.
27. Shimomura Y, Mizoguchi E, Sugimoto K, Kibe R, Benno Y, Mizoguchi A, et al. Regulatory role of B-1 B cells in chronic colitis. Int Immunol. 2008;20(6):729–37.
28. Elson CO, Cong Y, Qi F, Hershberg RM, Targan SR. Molecular approaches to the role of the microbiota in inflammatory bowel disease. Ann N Y Acad Sci. 2006;1072:39–51.
29. Targan SR, Landers CJ, Yang H, Lodes MJ, Cong Y, Papadakis KA, et al. Antibodies to CBir1 flagellin define a unique response that is associated independently with complicated Crohn's disease. Gastroenterology. 2005;128(7):2020–8.
30. Tackenberg B, Jelcic I, Baerenwaldt A, Oertel WH, Sommer N, Nimmerjahn F, et al. Impaired inhibitory Fcgamma receptor IIB expression on B cells in chronic inflammatory demyelinating polyneuropathy. Proc Natl Acad Sci U S A. 2009;106(12): 4788–92.
31. Belostocki K, Pricop L, Redecha PB, Aydin A, Leff L, Harrison MJ, et al. Infliximab treatment shifts the balance between stimulatory and inhibitory Fcgamma receptor type II isoforms on neutrophils in patients with rheumatoid arthritis. Arthritis Rheum. 2008;58(2): 384–8.
32. Masuda A, Yoshida M, Shiomi H, Ikezawa S, Takagawa T, Tanaka H, et al. Fcgamma receptor regulation of Citrobacter rodentium infection. Infect Immun. 2008;76(4):1728–37.
33. Mizoguchi A, Ogawa A, Takedatsu H, Sugimoto K, Shimomura Y, Shirane K, et al. Dependence of intestinal granuloma formation on unique myeloid DC-like cells. J Clin Invest. 2007;117(3):605–15.
34. McGovern DP, Gardet A, Torkvist L, Goyette P, Essers J, Taylor KD, et al. Genome-wide association identifies multiple ulcerative colitis susceptibility loci. Nat Genet. 2010;42(4):332–7.
35. Wolf SD, Dittel BN, Hardardottir F, Janeway Jr CA. Experimental autoimmune encephalomyelitis induction in genetically B cell-deficient mice. J Exp Med. 1996;184(6):2271–8.
36. Knoechel B, Lohr J, Kahn E, Abbas AK. The link between lymphocyte deficiency and autoimmunity: roles of endogenous T and B lymphocytes in tolerance. J Immunol. 2005;175(1):21–6.
37. Mizoguchi E, Mizoguchi A, Preffer FI, Bhan AK. Regulatory role of mature B cells in a murine model of inflammatory bowel disease. Int Immunol. 2000;12(5):597–605.
38. Blair PA, Norena LY, Flores-Borja F, Rawlings DJ, Isenberg DA, Ehrenstein MR, et al. CD19(+)CD24(hi)CD38(hi) B cells exhibit regulatory capacity in healthy individuals but are functionally impaired in systemic Lupus Erythematosus patients. Immunity. 2010;32(1):129–40.
39. Kawamura T, Kanai T, Dohi T, Uraushihara K, Totsuka T, Iiyama R, et al. Ectopic CD40 ligand expression on B cells triggers intestinal inflammation. J Immunol. 2004;172(10):6388–97.
40. 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(6):2010–5.
41. McPherson M, Wei B, Turovskaya O, Fujiwara D, Brewer S, Braun J. Colitis immunoregulation by CD8+ T cell requires T cell cytotoxicity and B cell peptide antigen presentation. Am J Physiol Gastrointest Liver Physiol. 2008;295(3):G485–92.
42. Munz C. Enhancing immunity through autophagy. Annu Rev Immunol. 2009;27:423–49.
43. Miller BC, Zhao Z, Stephenson LM, Cadwell K, Pua HH, Lee HK, et al. The autophagy gene ATG5 plays an essential role in B lymphocyte development. Autophagy. 2008;4(3):309–14.
44. Su Y, Carey G, Maric M, Scott DW. B cells induce tolerance by presenting endogenous peptide-IgG on MHC class II molecules via an IFN-gamma-inducible lysosomal thiol reductase-dependent pathway. J Immunol. 2008;181(2):1153–60.
45. McCarroll SA, Huett A, Kuballa P, Chilewski SD, Landry A, Goyette P, et al. Deletion polymorphism upstream of IRGM associated with altered IRGM expression and Crohn's disease. Nat Genet. 2008;40(9):1107–12.
46. Izcue A, Coombes JL, Powrie F. Regulatory lymphocytes and intestinal inflammation. Annu Rev Immunol. 2009;27:313–38.
47. Singh A, Carson WF, Secor Jr ER, 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(11):7318–26.
48. Rafei M, Hsieh J, Zehntner S, Li M, Forner K, Birman E, et al. A granulocyte-macrophage colony-stimulating factor and interleukin-15 fusokine induces a regulatory B cell population with immune suppressive properties. Nat Med. 2009;15(9):1038–45.
49. Moritoki Y, Lian ZX, Lindor K, Tuscano J, Tsuneyama K, Zhang W, et al. B-cell depletion with anti-CD20 ameliorates autoimmune cholangitis but exacerbates colitis in transforming growth factor-beta receptor II dominant negative mice. Hepatology. 2009;50(6):1893–903.
50. Wang L, Ray A, Jiang X, Wang JY, Basu S, Liu X, et al. T regulatory cells and B cells cooperate to form a regulatory loop that maintains gut homeostasis and suppresses dextran sulfate sodium-induced colitis. Mucosal Immunol. 2015;8(6):1297–312.
51. Harris DP, Haynes L, Sayles PC, Duso DK, Eaton SM, Lepak NM, et al. Reciprocal regulation of polarized cytokine production by effector B and T cells. Nat Immunol. 2000;1(6):475–82.
52. Wojciechowski W, Harris DP, Sprague F, Mousseau B, Makris M, Kusser K, et al. Cytokine-producing effector B cells regulate type 2 immunity to H. polygyrus. Immunity. 2009;30(3):421–33.
53. Rauch PJ, Chudnovskiy A, Robbins CS, Weber GF, Etzrodt M, Hilgendorf I, et al. Innate response activator B cells protect against microbial sepsis. Science. 2012;335(6068):597–601.

54. Olkhanud PB, Damdinsuren B, Bodogai M, Gress RE, Sen R, Wejksza K, et al. Tumor-evoked regulatory B cells promote breast cancer metastasis by converting resting CD4(+) T cells to T-regulatory cells. Cancer Res. 2011;71(10):3505–15.
55. Sugimoto K, Ogawa A, Shimomura Y, Nagahama K, Mizoguchi A, Bhan AK. Inducible IL-12-producing B cells regulate Th2-mediated intestinal inflammation. Gastroenterology. 2007;133(1): 124–36.
56. Mizoguchi A, Bhan AK. A case for regulatory B cells. J Immunol. 2006;176(2):705–10.
57. 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(2):219–30.
58. Madan R, Demircik F, Surianarayanan S, Allen JL, Divanovic S, Trompette A, et al. Nonredundant roles for B cell-derived IL-10 in immune counter-regulation. J Immunol. 2009;183(4):2312–20.
59. Bouaziz JD, Yanaba K, Tedder TF. Regulatory B cells as inhibitors of immune responses and inflammation. Immunol Rev. 2008;224: 201–14.
60. Jamin C, Morva A, Lemoine S, Daridon C, de Mendoza AR, Youinou P. Regulatory B lymphocytes in humans: a potential role in autoimmunity. Arthritis Rheum. 2008;58(7):1900–6.
61. Lund FE, Randall TD. Effector and regulatory B cells: modulators of CD4+ T cell immunity. Nat Rev Immunol. 2010;10(4):236–47.
62. Thaunat O, Morelon E, Defrance T. Am"B"valent: anti-CD20 antibodies unravel the dual role of B cells in immunopathogenesis. Blood. 2010;116(4):515–21.
63. Yanaba K, Yoshizaki A, Asano Y, Kadono T, Tedder TF, Sato S. IL-10-producing regulatory B10 cells inhibit intestinal injury in a mouse model. Am J Pathol. 2011;178(2):735–43.
64. Schmidt EG, Larsen HL, Kristensen NN, Poulsen SS, Claesson MH, Pedersen AE. B cells exposed to enterobacterial components suppress development of experimental colitis. Inflamm Bowel Dis. 2012;18(2):284–93.
65. Maseda D, Candando KM, Smith SH, Kalampokis I, Weaver CT, Plevy SE, et al. Peritoneal cavity regulatory B cells (B10 cells) modulate IFN-gamma+CD4+ T cell numbers during colitis development in mice. J Immunol. 2013;191(5):2780–95.
66. 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(5):639–50.
67. Matsushita T, Yanaba K, Bouaziz JD, Fujimoto M, Tedder TF. Regulatory B cells inhibit EAE initiation in mice while other B cells promote disease progression. J Clin Invest. 2008;118(10):3420–30.
68. Ansary MM, Ishihara S, Oka A, Kusunoki R, Oshima N, Yuki T, et al. Apoptotic cells ameliorate chronic intestinal inflammation by enhancing regulatory B-cell function. Inflamm Bowel Dis. 2014;20(12):2308–20.
69. Matsushita T, Horikawa M, Iwata Y, Tedder TF. Regulatory B cells (B10 cells) and regulatory T cells have independent roles in controlling experimental autoimmune encephalomyelitis initiation and late-phase immunopathogenesis. J Immunol. 2010;185(4):2240–52.
70. Shimomura Y, Ogawa A, Kawada M, Sugimoto K, Mizoguchi E, Shi HN, et al. A unique B2 B cell subset in the intestine. J Exp Med. 2008;205(6):1343–55.
71. Koni PA, Bolduc A, Takezaki M, Ametani Y, Huang L, Lee JR, et al. Constitutively CD40-activated B cells regulate CD8 T cell inflammatory response by IL-10 induction. J Immunol. 2013; 190(7):3189–96.
72. Reyes JL, Wang A, Fernando MR, Graepel R, Leung G, van Rooijen N, et al. Splenic B cells from Hymenolepis diminuta-infected mice ameliorate colitis independent of T cells and via cooperation with macrophages. J Immunol. 2015;194(1):364–78.
73. Kaku H, Cheng KF, Al-Abed Y, Rothstein TL. A novel mechanism of B cell-mediated immune suppression through CD73 expression and adenosine production. J Immunol. 2014;193(12):5904–13.
74. Dalwadi H, Wei B, Schrage M, Spicher K, Su TT, Birnbaumer L, et al. B cell developmental requirement for the G alpha i2 gene. J Immunol. 2003;170(4):1707–15.
75. Ostanin DV, Pavlick KP, Bharwani S, D'Souza D, Furr KL, Brown CM, et al. T cell-induced inflammation of the small and large intestine in immunodeficient mice. Am J Physiol Gastrointest Liver Physiol. 2006;290(1):G109–19.
76. Gerth AJ, Lin L, Neurath MF, Glimcher LH, Peng SL. An innate cell-mediated, murine ulcerative colitis-like syndrome in the absence of nuclear factor of activated T cells. Gastroenterology. 2004;126(4):1115–21.
77. Olson TS, Bamias G, Naganuma M, Rivera-Nieves J, Burcin TL, Ross W, et al. Expanded B cell population blocks regulatory T cells and exacerbates ileitis in a murine model of Crohn disease. J Clin Invest. 2004;114(3):389–98.
78. Dohi T, Fujihashi K, Koga T, Shirai Y, Kawamura YI, Ejima C, et al. T helper type-2 cells induce ileal villus atrophy, goblet cell metaplasia, and wasting disease in T cell-deficient mice. Gastroenterology. 2003;124(3):672–82.
79. Mishima Y, Ishihara S, Aziz MM, Oka A, Kusunoki R, Otani A, et al. Decreased production of interleukin-10 and transforming growth factor-beta in Toll-like receptor-activated intestinal B cells in SAMP1/Yit mice. Immunology. 2010;131(4):473–87.
80. McDonnell M, Liang Y, Noronha A, Coukos J, Kasper DL, Farraye FA, et al. Systemic Toll-like receptor ligands modify B-cell responses in human inflammatory bowel disease. Inflamm Bowel Dis. 2011;17(1):298–307.
81. Rehman MQ, Beal D, Liang Y, Noronha A, Winter H, Farraye FA, et al. B cells secrete eotaxin-1 in human inflammatory bowel disease. Inflamm Bowel Dis. 2013;19(5):922–33.
82. Fonseca-Camarillo G, Furuzawa-Carballeda J, Yamamoto-Furusho JK. Interleukin 35 (IL-35) and IL-37: Intestinal and peripheral expression by T and B regulatory cells in patients with Inflammatory Bowel Disease. Cytokine. 2015;75:389–402.
83. El Fassi D, Nielsen CH, Kjeldsen J, Clemmensen O, Hegedus L. Ulcerative colitis following B lymphocyte depletion with rituximab in a patient with Graves' disease. Gut. 2008;57(5):714–5.
84. El Fassi D, Nielsen CH, Junker P, Hasselbalch HC, Hegedus L. Systemic adverse events following rituximab therapy in patients with Graves' disease. J Endocrinol Invest. 2011;34(7):e163–7.
85. Goetz M, Atreya R, Ghalibafian M, Galle PR, Neurath MF. Exacerbation of ulcerative colitis after rituximab salvage therapy. Inflamm Bowel Dis. 2007;13(11):1365–8.
86. Leiper K, Martin K, Ellis A, Subramanian S, Watson AJ, Christmas SE, et al. Randomised placebo-controlled trial of rituximab (anti-CD20) in active ulcerative colitis. Gut. 2011;60(11):1520–6.