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Chronic Intestinal Inflammatory Condition Generates IL-10-Producing Regulatory B Cell Subset Characterized by CD1d Upregulation

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Summary

B cells possess a variety of immune functions that are involved in normal and abnormal immune responses, including autoimmune disorders. Through murine models of intestinal inflammation, we here demonstrate a B cell subset that is induced in gut-associated lymphoid tissues and is characterized by CD1d upregulation. This B cell subset appears under a chronic inflammatory environment, produces IL-10, and suppresses progression of intestinal inflammation by downregulating inflammatory cascades associated with IL-1 upregulation and STAT3 activation rather than by altering polarized T helper responses. This study indicates that B cells, by producing cytokines such as IL-10, can act as regulatory cells in immunologically mediated inflammatory reactions.

Introduction

Immune responses are regulated by the cooperative activities of cells that possess diverse immune functions. Functionally distinct subsets have been clearly defined in T cells (Abbas et al., 1996; O'Garra, 1998). Among these subsets, regulatory T cell subsets downregulate immune responses for both foreign and self-antigens and effectively participate in the suppression of autoimmune disorders (Chen et al., 1994; Asano et al., 1996; Asseman et al., 1999; Shevach, 2000). B cells possess a variety of immune functions, including the production of immunoglobulins (Igs) and cytokines, the presentation of antigens, and the regulation of dendritic cell function (Wolf et al., 1996; Moulin et al., 2000; Harris et al., 2000). Functionally distinct roles of B cells have also been reported in autoimmune diseases. B cells drive the development of several autoimmune disorders through the production of pathogenic Igs (Korganow et al., 1999; Fagarasan et al., 2000). On the other hand, B cells participate in the induction of immune tolerance (Ozaki et al., 1999; Gonnella et al., 2001) and suppression of experimental autoimmune encephalomyelitis (Wolf et al., 1996). Interestingly, even in the rheumatoid arthritis model in which Igs are required for the development of the disease, the presence of regulatory B cells has also been proposed (Korganow et al., 1999), suggesting that effector and regulatory B cells can coexist. Recently, B cell subsets (termed B effector [Be] 1 and Be 2 cells) that possess distinct cytokine production profiles have been identified (Harris et al., 2000).

One of the regulatory cytokines is IL-10. IL-10 helps preserve immune balance and suppresses the development of autoimmune diseases, including intestinal inflammation (de Waal Malefyt et al., 1992; Davidson et al., 1996). IL-10 is not only involved in the prevention of Th1 polarization (Asseman et al., 1999) but also inhibits Th2 responses (Grunig et al., 1997; Cottrez et al., 2000). In addition, IL-10 directly suppresses macrophage/monocyte-induced inflammatory responses, including the release of proinflammatory cytokines (Fiorentino et al., 1991; O'Farrell et al., 1998; Riley et al., 1999). The regulatory T cell subset producing IL-10 suppresses the development of experimental intestinal inflammation (Asseman et al., 1999). Although IL-10 is produced by numerous cell types including B cells, it is unclear whether IL-10 produced by B cells plays an important role in the suppression of inflammatory immune responses.

CD1d molecules are cell surface glycoproteins consisting of a heavy chain in noncovalent association with a β2-microglobulin light chain (Bleicher et al., 1990). The presence of two deep hydrophobic pockets in the groove of CD1d confers a unique capacity for this molecule to bind and present certain glycolipid antigens to distinct T cell subsets (Moody et al., 1999). Interestingly, the cytoplasmic tail of CD1d is linked to signaling cascades associated with the transcription of IL-10 (Colgan et al., 1999). CD1d is expressed on a wide variety of cell types, including B cells (Blumberg et al., 1991; Brossay et al., 1997). In the spleen, an increased expression of CD1d ($CD1^{high}$) has been found in certain subsets of B cells, the marginal zone B cells (Amano et al., 1998), and the recently identified $CD21^{high(hi)}IgM^{hi}$ B cell subset, termed as transitional B cells of type 2 (T2 B cells) (Loder et al., 1999). The glycolipids presented by CD1d rapidly activate natural killer T (NKT) cells (Bendelac et al., 1997), and this activation of NKT cells has been reported to be associated with suppression of insulin-dependent diabetes (Lehuen et al., 1998) and intestinal inflammation induced by dextran sodium sulfate (DSS) (Saubermann et al., 2000). In addition, an NKT cell-independent role of CD1d in immune modulation has been recently demonstrated (Dao et al., 2001). Although CD1d-mediated activation of B cells has been reported (Zeng et al., 2000), the functional role of B cells activated by CD1d-associated pathways is not well characterized.

Draining lymph nodes are important sites for modulating immune responses to foreign and self-antigens (Toh et al., 2000). Mesenteric lymph nodes (MLN), which are a part of the gut-associated lymphoid tissue (GALT),

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participate in maintaining a balance between oral tolerance and active immune responses to intestinal luminal antigens (Hershberg and Mayer, 2000). Lymphocytes sensitized in the intestine and intestine-derived dendritic cells efficiently migrate to MLN (Huang et al., 2000), and T cells from MLN of some murine colitis models are capable of transferring the disease into immunodeficient recipient mice (Hollander et al., 1995; Mizoguchi et al., 1996). An increased translocation of nonpathogenic bacteria from the intestinal lumen to MLN is associated with the development of intestinal inflammation (Dombrowicz et al., 2001). It is now generally accepted that dysregulated immune responses driven by enteric bacteria lead to the development of spontaneous intestinal inflammation in murine models of colitis (Bhan et al., 1999; Blumberg et al., 1999). The T cell receptor α knockout (TCRαKO) mouse, which spontaneously develops chronic intestinal inflammation, exhibits a marked expansion of B cells, especially in MLN. As a result, the pathogenic role of B cells was initially postulated to be responsible for intestinal inflammation observed in these mice (Mombaerts et al., 1993; Mizoguchi et al., 1996). However, subsequent studies using B cell-deficient TCRα double-knockout ($\alpha\mu$DKO) mice demonstrated a regulatory role for B cells in the pathogenesis of this intestinal inflammation (Mizoguchi et al., 1997, 2000). The intestinal inflammation also spontaneously develops in IL-2 knockout (IL2KO) mice and IL-10 knockout (IL10KO) mice. However, B cells are not required for the development of intestinal inflammation in these mice (Ma et al., 1995; Davidson et al., 1996).

In this study, we have provided evidence to indicate that upregulation of CD1d expression under chronic inflammatory conditions confers on B cells an ability to suppress the progression of chronic intestinal inflammation by enhancing IL-10 production.

Results

Upregulation of CD1d on GALT B Cells Is Associated with the Development of Intestinal Inflammation

The MLN form a part of GALT, and the enlargement of MLN is seen in TCRαKO as well as IL2KO and IL10KO mice during the development of intestinal inflammation. To examine whether there are differences in the B cell populations between these mice, MLN B cells from these different mouse models of intestinal inflammation were examined. An enlargement of MLN was observed in these mice (TCRαKO, 38 ± 5; IL10KO, 32 ± 4; IL2KO, $57 \pm 12 \times 10^6$ cells) compared to wild-type mice (WT) ($5 \pm 0.7 \times 10^6$ cells). In the MLN cells, a marked increase in the proportion of IgM^+ cells was observed in TCRαKO mice ($77.0 \pm 2.4\%$, $n = 18$), and a lesser increase of these cells was detected in IL10KO mice ($40.2 \pm 1.7\%$, $n = 10$) compared to WT mice ($30.8 \pm 4.6\%$, $n = 8$) (Figure 1A). In contrast, the IgM^+ B cells were virtually absent in MLN of IL2KO mice. Interestingly, a distinct expression pattern of CD1d was observed in MLN B cells between TCRαKO and IL10KO mice. A significant increase ($p < 0.001$) of CD1d expression on MLN B cells was detected in diseased TCRαKO mice (fluorescent mean channel [FMC] $= 210.5 \pm 22.4$, $n = 19$) as compared to WT mice (FMC $= 92.8 \pm 8.7$, $n = 16$) and diseased IL10KO mice (FMC $= 106.2 \pm 9.2$, $n = 9$) (Figure 1B). To examine whether the upregulation of CD1d on MLN B cells was associated with the development of intestinal inflammation, age-matched TCRαKO mice (6 months of age) with and without intestinal inflammation were examined. There was significant difference ($p < 0.001$) of CD1d expression of MLN B cells between TCRαKO mice with and without colitis; upregulation of CD1d was detected on MLN B cells of TCRαKO mice with colitis (FMC $= 210.5 \pm 22.4$, $n = 19$) but not in TCRαKO mice without colitis (FMC $= 108.7 \pm 6.0$, $n = 11$) (Figure 1C). Upregulation of CD1d on B cells was also detected in the colonic LP but not the Peyer's patches and appendix lymphoid follicle of diseased TCRαKO mice compared to WT mice (Figure 1D and data not shown). Since $CD1^{hi}$ B cells have been found among the T2 B cell subset (Loder et al., 1999) and marginal zone B cells (Amano et al., 1998) in the spleen, splenic B cells were also examined. There was no difference in the number and proportion of splenic $CD1^{hi}$ B cell subsets between WT mice and diseased TCRαKO mice (Figure 1E and data not shown). As previously described (Amano et al., 1998; Loder et al., 1999), the splenic marginal zone $CD1d^{hi}$ B cells were phenotypically characterized by $CD21^{hi}CD62^{hi}IgM^{hi}CD23^-$. In contrast, the MLN $CD1d^{hi}$ B cells were $CD21^{intermediate(int)}CD62^{low}IgM^{int}CD23^{hi}$ (Figure 1F and data not shown). These findings indicate that increased expression of CD1d on B cells of MLN and colonic LP, but not spleen, is associated with the development of intestinal inflammation in TCRαKO mice.

CD1d Participates in the Suppression of Intestinal Inflammatory Progression

To define the role of CD1d in the pathogenesis of intestinal inflammation, CD1d-deficient TCRα double-knockout (αCD1DKO) mice were generated. As previously described (Saubermann et al., 2000), CD1d knockout (CD1KO) mice did not spontaneously develop colitis (Figures 2A and 2C). In contrast, TCRαKO, αCD1DKO, and B cell-deficient TCRαKO ($\alpha\mu$DKO) mice spontaneously developed intestinal inflammation. The kinetics of intestinal inflammation development in αCD1DKO mice was very similar to that observed in TCRαKO mice rather than $\alpha\mu$DKO mice; $\alpha\mu$DKO mice developed colitis at an early age in comparison to αCD1DKO mice (Figure 2A). However, at 6 months of age, there was much more severe inflammation in the colon of αCD1DKO mice compared to TCRαKO mice as indicated by the increased number of inflammatory cells and more thickened colonic wall (Figures 2B–2F). Of note, ulceration characterized by loss of epithelium and fibrosis was detected in 11.5% of αCD1DKO mice with intestinal inflammation (Figures 2E and 2F). In contrast, ulceration was not detectable in the colon of TCRαKO mice. There was a marked increase in the expression of the proinflammatory cytokine, IL-1β, in the colonic mucosa of αCD1DKO mice as compared to TCRαKO mice (Figure 2G). Recently, signal transducer and activation of transcription (STAT) 3 phosphorylation has been demonstrated to participate in the perpetuation of intestinal inflammation mediated by both Th1 and Th2 pathways, and the activation of STAT3 correlates with the severity of intestinal inflammation (Suzuki et al., 2001). Increased

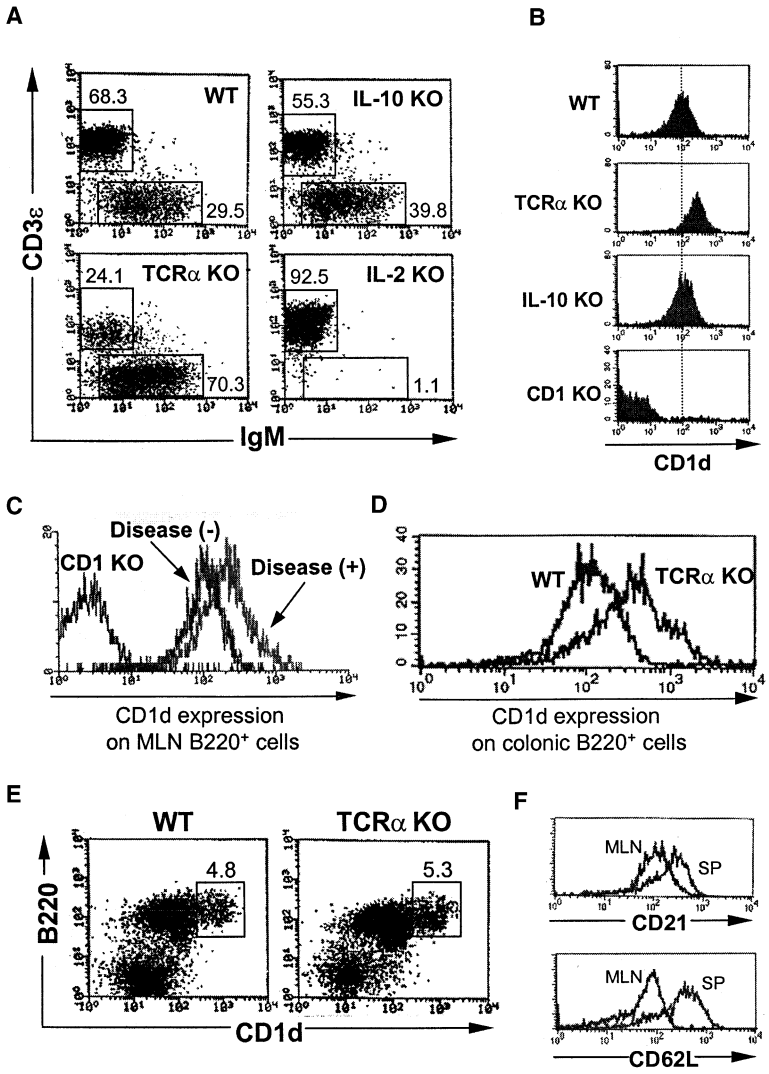

Figure 1. CD1d on Mucosal Associated B Cells Is Upregulated after the Development of Intestinal Inflammation

(A) The expression of CD3ε and IgM in MLN cells from WT, TCRαKO, IL10KO, and IL2KO mice. The TCRαKO (6 months of age), IL10KO (6 months of age), and IL2KO (4 months of age) mice exhibited intestinal inflammation.

(B) MLN cells from WT and CD1dKO mice and diseased TCRα and IL10KO mice were stained with anti-CD1d and -B220 mAbs. The figure shows CD1d expression on the gated $B220^+$ MLN cells.

(C) MLN cells from TCRαKO mice, with and without intestinal inflammation, and CD1dKO mice were stained with anti-CD1d and -B220 mAbs. The figure shows CD1d expression on the gated $B220^+$ MLN cells.

(D) Cells from colonic LP of WT and diseased TCRαKO mice were stained with anti-CD1d and -IgM mAbs. The figure shows CD1d expression on the gated IgM^+ colonic LP cells.

(E) Cells from spleen of WT and diseased TCRαKO mice were stained with anti-CD1d and -B220 mAbs.

(F) CD21 and CD62L expression on the gated $CD1d^{hi}B220^+$ B cells from the spleen (SP) and MLN of TCRαKO mice. All of the mice shown in (A)–(F) were 6 months of age.

STAT3 phosphorylation was observed in the colonic mucosa of TCRαKO mice as compared to WT mice (Figure 2H). A greater increase in STAT3 activation was reproducibly detected in αCD1DKO mice as compared to TCRαKO mice (Figure 2H); the ratio of phosphorylated STAT3/STAT3 was 0.258 ± 0.034 (n = 7) in αCD1DKO mice and 0.132 ± 0.023 (n = 7) in TCRαKO mice ($p < 0.01$). These findings suggest that, although the onset of intestinal inflammation is not changed in αCD1DKO mice, the disease progresses rapidly in αCD1DKO mice presumably due to the lack of downregulation of the inflammatory cascade.

B Cells Upregulating CD1d Suppress the Progression of Intestinal Inflammation

To examine the role of B cells with high expression of CD1d in the pathogenesis of intestinal inflammation, B cells (Figure 3A) enriched from MLN of TCRαKO or αCD1DKO mice were transferred into αμDKO mice that have no B cells and develop a more severe form of colitis with an early onset at 8 weeks of age (Mizoguchi et al., 1997). Since increased CD1d expression on B cells was found after the development of intestinal inflammation, it is likely that CD1d is required for controlling the progression rather than the initiation of intestinal inflammation. Therefore, B cells were transferred into the αμDKO mice with mild to moderate disease. The presence of intestinal inflammation was grossly confirmed by opening the abdomen and measuring the circumference of the distal colon as a marker for evaluating the progression of intestinal inflammation (Figures 3B and 3C). In αμDKO mice, the circumference of the distal colon was found to progressively increase within a 44 day period (from 12–18 weeks of age), indicating that disease in the αμDKO mice progressed rapidly during this period (Figure 3C). Transfer of B cells from TCRαKO mice was able to effectively inhibit the progression of intestinal enlargement in αμDKO mice (Figures 3B and 3C). In contrast, transfer of B cells from αCD1DKO mice failed to suppress the exacerbation of intestinal enlargement. Histological examination also confirmed the results obtained by evaluating the change of colonic circumference. The intestinal inflammation as characterized by thickening of the colonic mucosa with crypt elongation and inflammatory cell infiltration was significantly milder ($p < 0.001$) in the αμDKO mice receiving

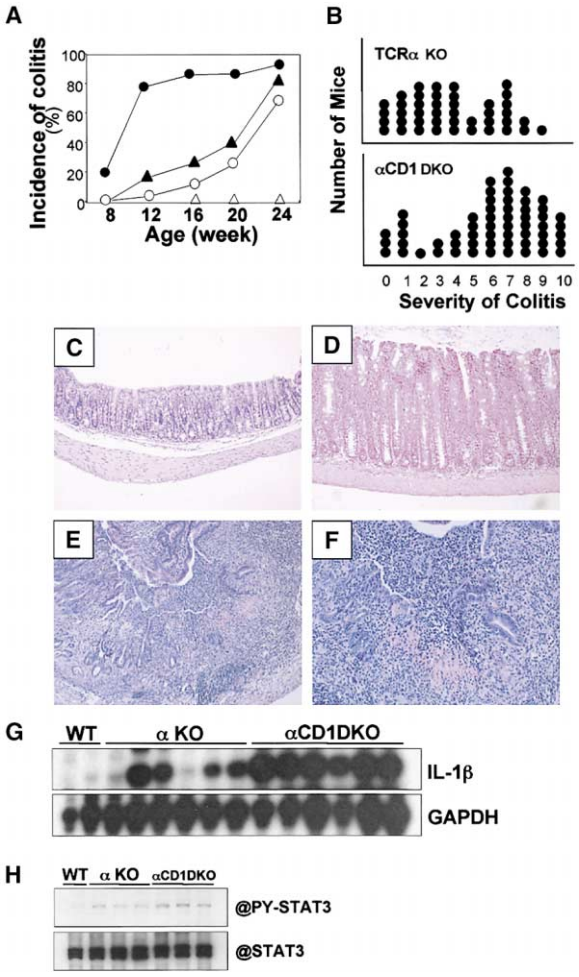

Figure 2. Absence of CD1d Leads to the Development of Severe Intestinal Inflammation in TCRαKO Mice

(A) The incidence of intestinal inflammation in TCRαKO (open circles), αμDKO (closed circles), αCD1DKO (closed triangles), and CD1KO (open triangles) mice at various ages is shown.

(B) Severity of intestinal inflammation of TCRαKO and αCD1DKO mice at 6 months of age is shown. Each dot represents an individual mouse. A significant difference ($p < 0.001$) in the disease severity is observed between TCRαKO and αCD1DKO mice.

(C–F) Severity of colonic inflammation in CD1KO (C), TCRαKO (D), and αCD1DKO (E and F) mice at 6 months of age is shown. More severe colonic inflammation including ulceration was present in αCD1DKO mice (E and F) than in TCRαKO mice (D). ([C and D] X10, [E] X4, and [F] X20 objective).

(G) Total RNA isolated from the colonic mucosa of WT, TCRαKO, and αCD1DKO mice (6 months of age) was subjected to RPA for the detection of IL-1β.

(H) Proteins extracted from the colonic mucosa of WT, TCRαKO, and αCD1DKO mice (6 months of age) were immunoblotted with anti-phospho-STAT3 (PY-STAT3) Ab. After stripping PY-STAT Ab, the membrane was reprobed with anti-STAT3 Ab.

B cells from TCRαKO mice in comparison to αμDKO mice receiving B cells from αCD1DKO mice or αμDKO mice without B cell transfer (Figures 3D and 3E). There was no significant difference between the number of reconstituted B cells within the MLNs of recipient αμDKO mice after the cell transfer from either TCRαKO or αCD1-DKO mice (Figure 3C). These findings indicate that MLN B cells with high expression of CD1d play an important role in suppressing the progression of intestinal inflammation.

Since intestinal inflammation in the TCRαKO mice is mediated by $CD4^+$ T cells (Bhan et al.,1999), the role of MHC class II-associated antigen presentation in the regulatory properties of B cells was examined by transferring B cells from MHC II-deficient TCRα double-knockout (αMHCDKO) mice into αμDKO mice. We initially observed that transfer of enriched B cells from αMHCDKO mice did not suppress the progression of intestinal inflammation in αμDKO mice (data not shown). However, it was also observed that there was a poor expansion of B cells from αMHCDKO mice in αμDKO mice, and the absolute number of reconstituted B cells within the recipient MLN was less than 3×10^5. By increasing the number of transferred B cells (3×10^7) and the frequency of injections (four times), MHC-deficient B cells were also able to inhibit the progression of intestinal enlargement in αμDKO mice (Figure 3C). In contrast, B cells from αCD1DKO mice failed to inhibit the disease progression in the recipient αμDKO mice even with the increased numbers of B cells and frequency of injections (data not shown). These findings suggest that MHC class II facilitates the expansion of B cells under chronic inflammatory conditions. CD1d, rather than MHC class II, may be effectively involved in the regulatory effect of B cells on intestinal inflammation.

CD1d Expression Is Associated with Enhanced IL-10 Production in MLN B Cells

Splenic $CD1d^{hi}$ B cells in the NZB/NZW strain, but not the C57BL/6 strain, have been reported as a source of IgM autoantibodies (Zeng et al., 2000). Since Igs participate in the suppression of the development of intestinal inflammation in TCRαKO mice (Mizoguchi et al., 1997), Ig production in αCD1DKO mice was examined. There was no significant difference in the serum Ig levels between TCRαKO and αCD1DKO mice with intestinal inflammation (Figure 4A). In addition, enzyme-linked immune spot (ELISPOT) assays also showed no significant difference in the number of MLN B cells secreting Ig and anti-tropomyosin autoantibodies between TCRαKO and αCD1DKO mice with intestinal inflammation (Figure 4B and data not shown).

Next, we screened the cytokine expression of freshly isolated MLN cells by RNase protection assay (RPA). IL-10 mRNA was reproducibly detected in the MLN cells of TCRαKO but not in αμDKO and αCD1DKO mice (Figure 4C). Furthermore, RT-PCR showed that IL-10 was detectable in the purified $B220^+$ B cells from MLN of diseased TCRαKO mice (Figure 4D). Upregulation of CD1d on these purified $B220^+$ B cells was confirmed by flow cytometric analysis (Figure 4E). In contrast, IL-10 was not detectable in the purified $B220^+$ B cells from MLN of TCRαKO mice without intestinal inflammation and diseased αCD1DKO mice (Figure 4D). These findings suggest that B cells are a source of IL-10 in the diseased TCRαKO mice, and upregulation of CD1d is associated with enhanced IL-10 production by MLN B cells.

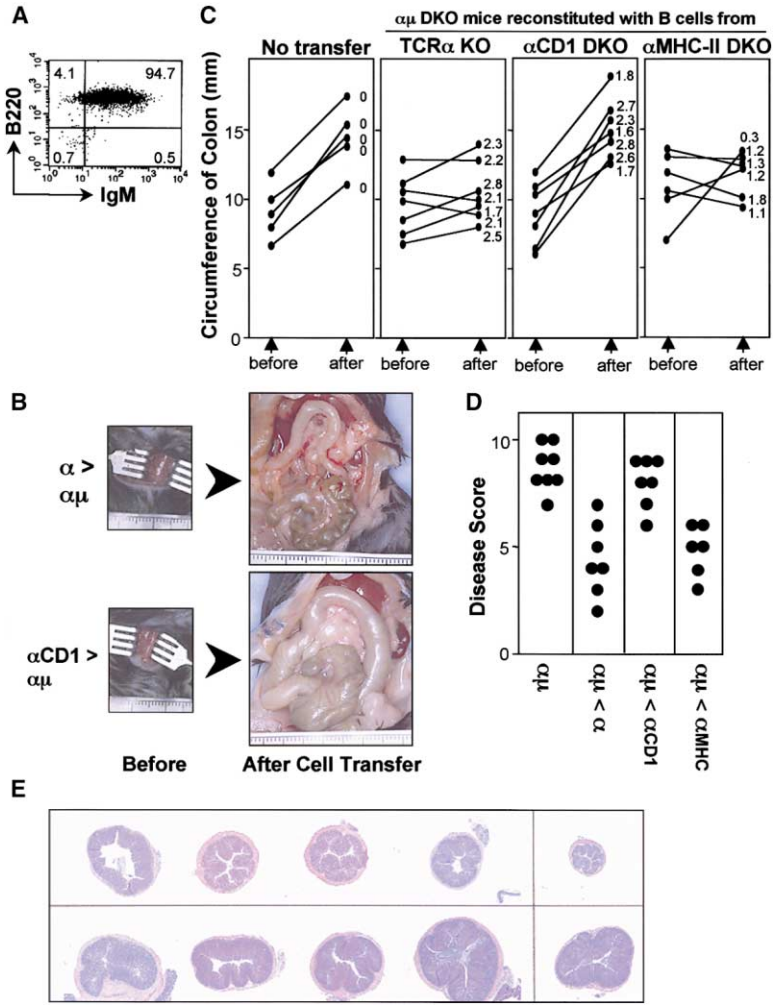

Figure 3. Transfer of B Cells Expressing CD1d Leads to Suppression of Colonic Inflammation Progression in αμDKO Mice

(A) Representative flow cytometric result of the enriched B cells used in the cell transfer studies is shown. On average, 94.3 ± 0.9% of the preparations were IgM$^+$B220$^+$ cells (range, 90.3%–96.9%). The IgM$^-$B220$^+$ cells were present at a range that varied from 2.7% to 6.9%. CD3ε$^+$ cells, and NK-1.1$^+$ cells represented less than 0.5% of the total cell preparations.

(B–E) A small abdominal incision was made in anesthetized αμDKO mice (12 weeks of age) to determine the presence of intestinal inflammation ([B] left panels). The colonic circumference was measured. MLN B cells (1.5×10^7) from TCRαKO and αCD1DKO mice were transferred into αμDKO mice 14 days after the operation. After three injections of MLN B cells (every 10 days), the recipient αμDKO mice were sacrificed 10 days after the last cell transfer, and the circumference of the distal colon was measured. For the transfer of B cells from αMHCDKO mice, 3×10^7 B cells were injected four times (every 7 days) into αμDKO mice. ([B] right panels) Gross findings of the colon of the αμDKO mice receiving the MLN B cells from TCRαKO (top panel) and αCD1DKO (bottom panel) mice are shown. (C) The figure summarizes the results of the change in the colonic circumference (before and after the cell transfer) of αμDKO mice receiving PBS alone or B cells from TCRαKO, αCD1DKO, and αMHCDKO mice. The numbers next to the lines represent the absolute numbers of reconstituted IgM$^+$B220$^+$ B cells ($\times 10^6$) in MLN of recipient αμDKO mice. (D) Severity of intestinal inflammation in αμDKO mice receiving PBS alone or B cells from TCRαKO, αCD1DKO, or αMHCDKO mice is shown. Each dot represents an individual mouse. (E) Histological findings (2.5 × objective) of the distal colon of the WT mouse (top right) and αμDKO mouse transferred with PBS alone (bottom right) and MLN B cells from TCRαKO (top left, four individual colons) and αCD1DKO (bottom left, four individual colons) mice are shown.

IL-10 Produced by MLN B Cells Regulates the Progression of Intestinal Inflammation by Directly Downregulating Inflammatory Cascades

To determine whether IL-10 produced by CD1^{hi} MLN B cells is directly involved in the suppression of intestinal inflammation, αμDKO mice that had adoptively received B cells from TCRαKO mice were treated weekly with a combination of anti-IL-10 receptor (R) and anti-IL-10 mAbs, a combination of mAbs that has been demonstrated to efficiently neutralize in vivo activity of IL-10 (Asseman et al.,1999). The in vivo neutralization of IL-10 activity by the combination of these mAbs led to progressive enlargement of the large intestine in the αμDKO mice receiving B cells from TCRαKO mice, indicating that the suppressive activity of B cells in the intestinal inflammation is mediated by IL-10 (Figures 5A and 5B). In contrast, the treatment with control Abs did not affect the inhibitory activity of the adoptively transferred B cells. Since IL-10 has been shown to promote human B cell proliferation in vitro (Rousset et al., 1992), we examined the MLN B cell populations in the recipient αμDKO mice with and without the treatment. There was no obvious difference in the number and proportion of the IgM$^+$B220$^+$ B cells in the MLN of αμDKO mice after reconstitution and treatment with the combination of mAbs (anti-IL10R plus anti-IL10) and with control Abs (Figures 5B and 5C). We also administrated these mAbs into αμDKO mice without transferring B cells. The in vivo neutralization of IL-10 activity did not exacerbate the disease in the αμDKO mice (Figure 5D). This supports our contention that IL-10 produced by B cells is involved in the regulation of intestinal inflammation in TCRαKO mice.

To further confirm these findings, IL-10-deficient TCRα double-knockout (αIL10DKO) mice were generated, and the B cells from these DKO mice were used for cell transfer studies. Unlike B cells from TCRαKO mice, the transfer of B cells from αIL10DKO mice was unable to effectively inhibit the progression of intestinal enlargement in αμDKO mice (Figure 6A). Taken together, these findings indicate that IL-10 produced by MLN B cells is involved in suppressing the progression of chronic intestinal inflammation.

Mechanistically, the intestinal inflammation of TCRαKO mice is mediated by the IL-4-dependent Th2 pathway as indicated by the suppression of intestinal

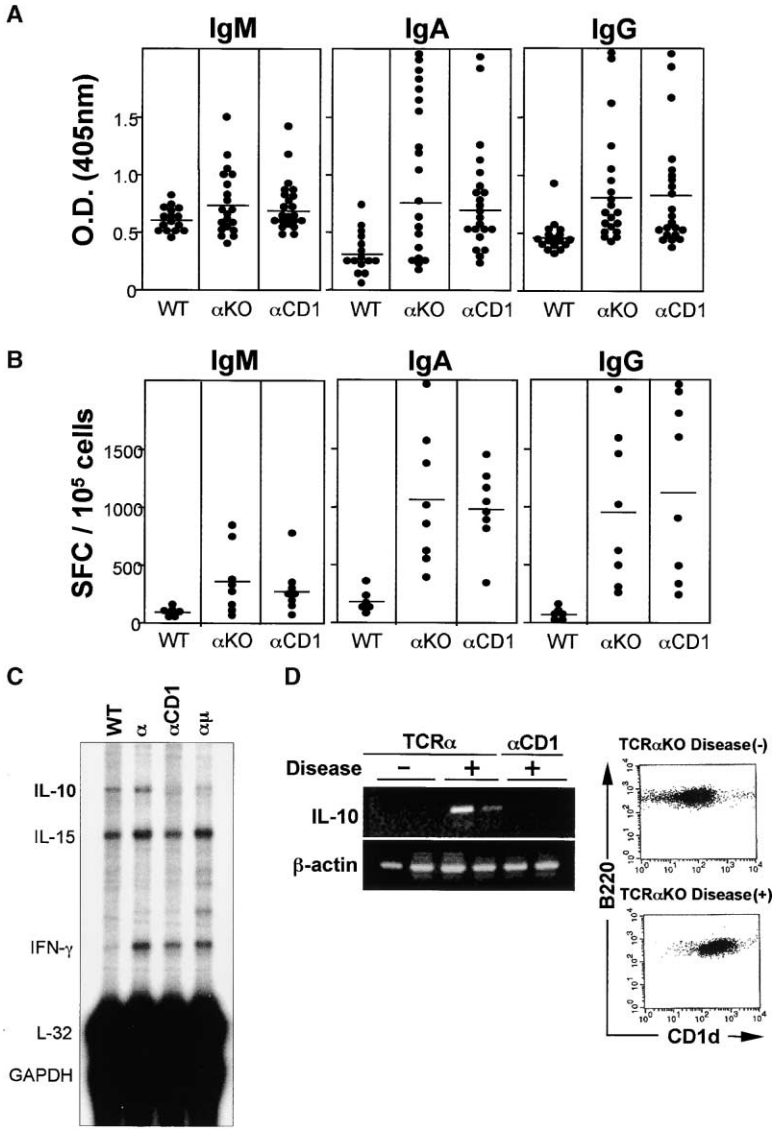

Figure 4. Absence of CD1d Affects IL-10 Production but Not Ig Secretion in TCRαKO Mice

(A) Serum samples were obtained from WT, TCRαKO, and αCD1DKO mice (6 months of age) and subjected to ELISA.

(B) MLN cells were obtained from WT, TCRαKO, and αCD1DKO mice (6 months of age). Number of Ig (IgM, IgA, and IgG)-secreting cells per 1×10^5 MLN cells was evaluated by ELISPOT assay.

(C) Total RNA extracted from single cell suspension of MLN of WT, TCRαKO, αCD1DKO, and αμDKO mice (6 months of age) was subjected to RPA for the detection of IL-10. Data are representative of three individual experiments.

(D) $B220^+$ cells were purified from MLN cells of TCRαKO mice with and without disease and αCD1DKO mice with disease (6 months of age) by using MACS. RNA extracted from 5×10^5 cells ($B220^+IgM^+ > 94.8\%$) was subjected to RT-PCR using specific primers for IL-10 and β-actin (left panel). Data are representative of four individual experiments. Flow cytometric analysis shows that upregulation of CD1d was found on the purified $B220^+$ MLN B cells of diseased TCRαKO mice but not TCRαKO mice without disease (right panel).

inflammation in IL-4-deficient TCRα double-knockout (αIL4DKO) mice (Mizoguchi et al., 1999) and in TCRαKO mice treated with anti-IL-4 mAb (Iijima et al., 1999). Since IL-10 can suppress the induction of Th2 responses (Grunig et al., 1997; Cottrez et al., 2000), it is possible that B cells regulate intestinal inflammation in TCRαKO mice by downregulating Th2 polarization of immune responses. However, there was no detectable difference in the IL-4 production by purified $CD4^+$ colonic LP and MLN T cells from αμDKO mice with and without transfer of B cells from TCRαKO and αIL10DKO mice (Figure 6B). In contrast, transfer of MLN B cells from TCRαKO, but not αIL10DKO mice, led to downregulation of IL-1β expression and STAT3 activation in the colonic LP of αμDKO mice (Figures 6C and 6D). Since IL-10 directly suppresses macrophage/monocyte-induced inflammatory responses (Fiorentino et al., 1991; O'Farrell et al., 1998; Riley et al., 1999), it is likely that once intestinal inflammation is initiated, IL-10 directly suppresses the inflammatory cascades resulting from upregulation of IL-1β and activation of STAT3.

Inflammatory Conditions Associated with Intestinal Inflammation Upregulate CD1d Expression on B Cells

As upregulation of CD1d was not found in the MLN B cells of IL10KO mice (Figure 1), it could be suggested that CD1d upregulation is mediated by IL-10. To examine this possibility, we generated IL-10-deficient TCRα double-knockout (αIL10DKO) mice. As an unexpected result, like TCRαKO mice, an upregulation of CD1d on MLN B cells was also observed in association with development of intestinal inflammation in αIL10DKO mice (Figure 7A). These findings indicate that IL-10 is not required for the upregulation of CD1d on MLN B cells under chronic inflammatory conditions. No increase of IL-4 production was observed in IL10KO mice (Davidson et al., 1996), whereas the development of intestinal inflammation in TCRαKO mice is associated with an increase of IL-4, as well as IL-6, production (Mizoguchi et al., 1999); these cytokines are known to be involved in B cell activation and Ig class switching. However, upregulation of CD1d was also detectable on MLN

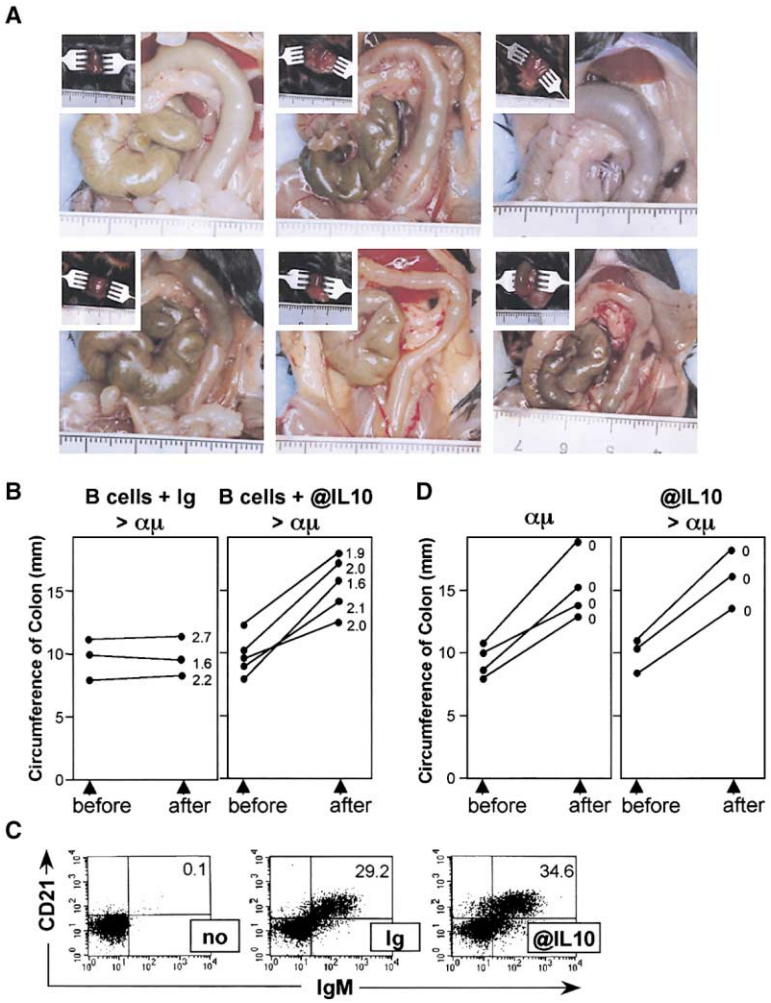

Figure 5. Regulatory B Cell Function in Intestinal Inflammation Is Inhibited by Neutralization of IL-10 Activity

(A and B) The circumference of the colon was measured by opening the abdomen of anesthetized αμDKO mice (12 weeks of age) (top left inserts in each panel). Fourteen days after the operation, the αμDKO mice were transferred with enriched MLN B cells (1.5×10^7) from TCRαKO mice. B cell transfers were performed every 10 days (three times). A combination of mAbs (@IL10) specific for IL-10 (1 mg) and IL-10R (0.5 mg) (top panels in [A]) or control rat immunoglobuins (1.5 mg) (bottom panels in [A]) were injected intraperitoneally the day after each B cell transfer. An additional dose of antibodies was administered 7 days after the final B cell transfer. The recipient mice were sacrificed on day 10 after the final B cell transfer. The gross findings of the colon of the recipient mice sacrificed on day 10 after the last cell transfer are shown as large panels in (A). The results are summarized in (B). The numbers next to the lines in (B) represent absolute number of reconstituted IgM^+B220^+ B cells ($\times 10^6$) in MLN of the recipient αμDKO mice.

(C) Cells from MLN of the αμDKO mice without (left panel) and with B cell transfer plus administration of either control immunoglobulins (middle panel) or @IL10 (right panel) were stained with anti-CD21 and -IgM and subjected to flow cytometric analysis.

(D) Change in the circumference of the colon of αμDKO mice (without B cell transfer) with and without administration of @IL10 is shown. The same protocol omitting B cell transfer as shown in (A) and (B) was used.

B cells of αIL4DKO and IL-6-deficient TCRα double-knockout (αIL6DKO) mice with intestinal inflammation (grade 4) (Figure 7A), indicating that neither IL-10, IL-4, nor IL-6 is required for the upregulation of CD1d on MLN B cells.

In our previous study, a significant decrease of intestinal epithelial proliferation, a characteristic feature of intestinal inflammation, has been described in the recipient αμDKO mice transferred with splenic B cells from WT mice (Mizoguchi et al., 2000). Therefore, we examined CD1d expression on the reconstituted B cells in the MLN of αμDKO mice receiving enriched B cells from the spleens of WT mice. Increased CD1d expression was clearly detectable on the WT-derived B cells reconstituted into the MLN of the recipient αμDKO mice with intestinal inflammation (Figure 7B). In contrast, when WT-derived splenic B cells were transferred into B cell-deficient mice that do not develop intestinal inflammation, upregulation of CD1d was not found in the reconstituted B cells in MLN of the recipient B cell-deficient mice (Figure 7B). Indeed, the transfer of splenic B cells from WT mice inhibited the disease progression of the recipient αμDKO mice (Figure 7C). To rule out the possibility that inhibition of the disease progression is not due to the few contaminating T cells, purified splenic B cells from TCRβ × TCRδ (βδ) DKO mice, which have no T cells, were also transferred into αμDKO mice. The transfer of splenic B cells from βδDKO mice also suppressed the disease progression in the recipient αμDKO mice (Figure 7C). In addition, the reconstituted B cells in the recipient αμDKO mice exhibited upregulation of CD1d compared to MLN B cells of βδDKO mice (Figure 7D). These findings indicate that chronic intestinal inflammatory conditions lead to upregulation of CD1d even on splenic B cells from WT mice, which possess an ability to inhibit the progression of intestinal inflammation in αμDKO mice.

Discussion

B cells possess diverse immune functions, and both regulatory and pathogenic roles of B cells have been demonstrated in autoimmune diseases (Wolf et al., 1996; Korganow et al., 1999; Fagarasan et al., 2000). A recent study has shown that, like Th subsets, B cell subsets can be distinguished by distinct cytokine secretion profiles (Harris et al., 2000). Here, we have defined a GALT-associated B cell subset that is characterized by upregulation of CD1d expression and enhanced production of IL-10. This B cell subset appears after the development

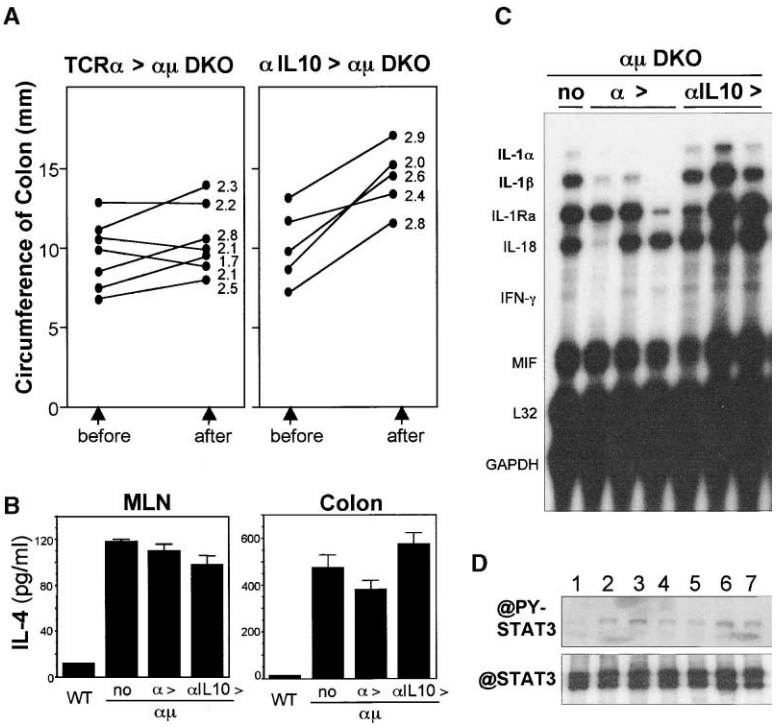

Figure 6. Transfer of MLN B Cells from αIL10DKO Mice Does Not Inhibit the Progression of Disease in αμDKO Mice

(A) Change of colonic circumference of αμDKO mice before and after MLN B cell transfer from TCRαKO or αIL10DKO mice is shown. The numbers next to the lines represent the absolute numbers of reconstituted IgM^+B220^+ B cells ($\times 10^6$) in the MLN of recipient αμDKO mice.

(B) $CD4^+$ T cells were purified from MLN of WT and αμDKO mice with and without B cell transfer from TCRαKO and αIL10DKO mice. The purified $CD4^+$ T cells were stimulated with plate-coated anti-TCRβ for 3 days. The culture supernatants were subjected to ELISA for the detection of IL-4.

(C) Total RNA was isolated from colonic mucosa of αμDKO mice with and without B cell transfer from TCRαKO and αIL10DKO mice and analyzed by RPA.

(D) Proteins extracted from the colonic mucosa of WT mice (lane 1) and αμDKO mice without (lanes 2 and 3, the average [n = 5] ratio calculated by band density of phosphorylated STAT3/density of STAT3, 0.348 ± 0.065) and with (lanes 4–7) B cell transfer from either TCRαKO (lanes 4 and 5, the average ratio [n = 5] was 0.141 ± 0.031) or αIL10DKO mice (lanes 6 and 7, the average ratio [n = 4] was 0.380 ± 0.090) were immunoblotted with anti-phospho-STAT3 (PY-STAT3) Ab. After stripping PY-STAT Ab, the membrane was reprobed with anti-STAT3 Ab.

of chronic intestinal inflammation and participates in the suppression of intestinal inflammation progression. This B cell subset is detected in TCRαKO mice where intestinal inflammation is mediated by a Th2 pathway but not in a Th1-mediated model of intestinal inflammation associated with IL-10 deficiency. The increased expression of CD1d on splenic B cells from WT mice after transfer into diseased αμDKO mice also supports this contention.

CD1d binds a variety of lipid-containing antigens (Moody et al., 1999; Burdin and Kronenberg, 1999), and the presentation of a glycolipid (α-galactosyl ceramide) by CD1d induces rapid activation of NKT cells (Kawano et al., 1997). However, NKT cells are not present in TCRαKO mice (Taniguchi et al., 1996), indicating that the regulatory function of $CD1d^{hi}$ B cell subset is not likely to be dependent on NKT cells. The presence of CD1d-restricted T cells without NK-1.1 expression has recently been identified using CD1d tetramers (Benlagha et al., 2000; Matsuda et al., 2000), and CD1d-restricted T cells that do not express an invariant TCRα chain have been described (Behar and Cardell, 2000). Therefore, it is possible that the regulatory effect of $CD1d^{hi}$ MLN B cells may be induced by the interaction of NK-1.1^- CD1-restricted T cells with $CD1d^{hi}$ B cells.

Ligation of CD1d results in a rapid and sustained production of IL-10 (Colgan et al., 1999). CD1d is capable of binding a wide variety of lipid antigens including α-galactosyl ceramide, β-galactosyl ceramide, which is unable to activate NKT cells (Naidenko et al., 1999), and phospholipids that are present in mammalian cells and considered to be potential self-antigens (Gumperz et al., 2000). Ligation of CD1d on B cells by self-phospholipids or β-anomeric glycolipid eluted from damaged cells resulting from chronic intestinal inflammation may be involved in the production of IL-10. Alternatively, the differentiation of regulatory B cells producing IL-10 may depend on an interaction with CD1d-restricted T cells, but CD1d crosslinking may not be required for IL-10 production. Increased translocation of nonpathogenic bacteria from the intestinal lumen to MLN is associated with the development of IL-4-associated intestinal inflammation (Ceponis et al., 2000; Dombrowicz et al., 2001), and a form of α-anomeric glycolipid with phytosphingosine has been detected in certain bacteria (Kawano et al., 1997). In the colitis induced by oral intake of 4% DSS, the $CD1d^{hi}$ B cells are detectable in the MLN during the healing period (3 days after cessation of DSS intake) but not in the acute phase of inflammation during DSS intake (our unpublished data). Since the translocation of enteric bacterial products into a host increases in DSS-induced colitis due to direct damage of the epithelial layer by DSS, it is possible that bacteria-derived glycolipids cause ligation of CD1d on a regulatory B cell subset and/or CD1d-dependent expansion or activation of a regulatory B cell subset.

Igs have been shown to regulate the initiation of intestinal inflammation by rapidly removing harmful self-antigens in TCRαKO mice (Mizoguchi et al., 1997). In this study, we have identified a B cell subset that can regulate intestinal inflammation through production of IL-10 after the onset of disease. The ability of B cells from TCRαKO mice to inhibit the progression of intestinal inflammation in αμDKO mice can be neutralized by the administration of a combination of anti-IL-10 and -IL-10R mAbs. Furthermore, B cell transfer from αIL10DKO mice is unable to effectively suppress the progression of intestinal inflammation in αμDKO mice. These findings

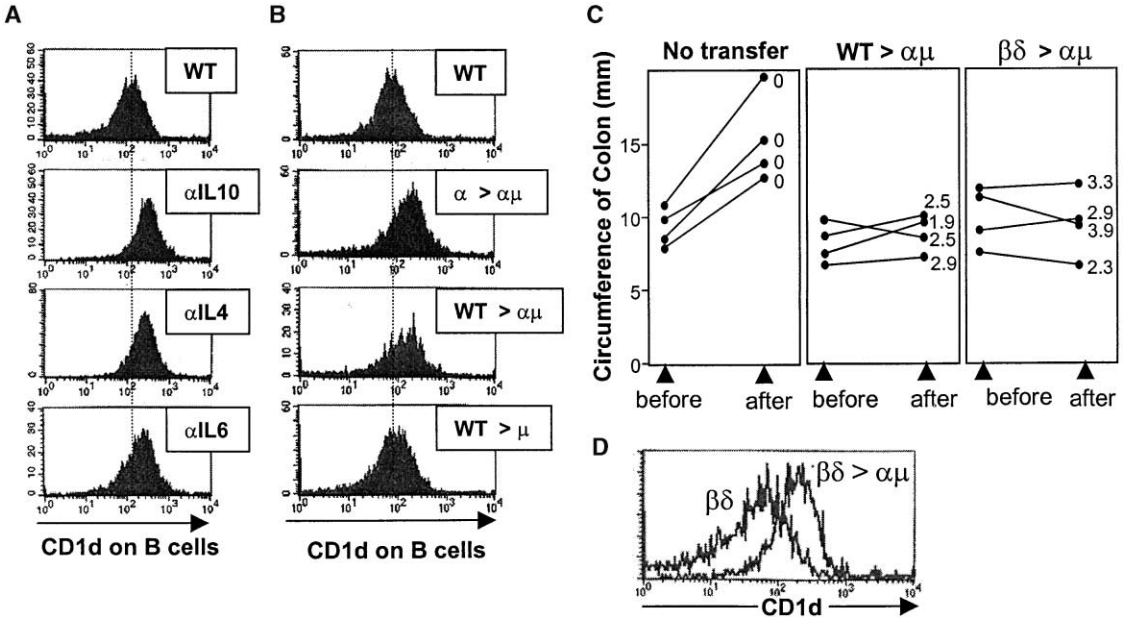

Figure 7. Chronic Inflammatory Environment Leads to Upregulation of CD1d on WT-Derived B Cells

(A) MLN cells from WT, αIL10DKO, αIL4DKO, and αIL6DKO mice (6 months of age) with mild to moderate colitis were stained with anti-CD1d and -B220 mAbs. The figure shows CD1d expression on gated $B220^+$ MLN cells.

(B) The figure shows CD1d expression on gated $B220^+$ cells of MLNs from WT mice, αμDKO mice receiving splenic B cells from TCRαKO (α > αμ) or WT (WT > αμ) mice, and B cell-deficient mice receiving splenic B cells from WT (WT > μ) mice.

(C) Change of colonic circumference of αμDKO mice receiving PBS alone (αμ) or splenic B cells from WT mice (WT > αμ) or βδDKO mice (βδ > αμ) are shown. The numbers next to the lines represent the absolute numbers of reconstituted IgM^+B220^+ B cells ($\times 10^6$) in the MLN of recipient αμDKO mice.

(D) CD1d expression on gated MLN $B220^+$ cells of αμDKO mice receiving splenic B cells from βδDKO mice (βδ > αμ) and of donor βδDKO mice (βδ) is shown.

clearly demonstrate that IL-10 produced by B cells is involved in the regulation of a Th2-mediated intestinal inflammation. Recent studies have shown that initiation of both polarized Th1 and Th2 responses can be regulated by IL-10 (Grunig et al., 1997; Cottrez et al., 2000). However, administration of IL-10 does not alter established Th1 polarization in IL10KO mice (Davidson et al., 1996). Furthermore, in vitro studies indicate that, although a high amount of IL-10 is produced by regulatory $CD4^+CD25^+$ T cells, IL-10 is not involved in the regulation of effector T cell functions (Thornton and Shevach, 1998). Similarly, the transfer of regulatory B cells from TCRαKO mice does not change an established Th2 response in αμDKO mice. In contrast, the transfer of regulatory B cells leads to downregulation of the inflammatory cascade associated with IL-1β production and STAT3 activation. Indeed, IL-10 has been demonstrated to directly suppress inflammatory responses (Fiorentino et al., 1991; O'Farrell et al., 1998; Riley et al., 1999). Therefore, it is likely that IL-10 produced by B cells plays a regulatory role in chronic inflammatory conditions, such as chronic colitis resembling human ulcerative colitis associated with the TCRαKO mice, by affecting inflammatory mediators and their effects rather than by directly affecting T cell functions.

Experimental Procedures

Mice

TCRαKO, αμDKO, αIL4DKO, and βδDKO mice of C57BL/6 background have been described previously (Mizoguchi et al., 1997, 1999). CD1dKO mice (F6 backcross to C57BL/6) (Behar et al., 1999) were obtained from Dr. M.J. Grusby, Harvard Medical School, Boston. IL-10 (F13 backcross to C57BL/6) and IL-6 (F7 backcross to C57BL/6) KO mice were purchased from Jackson Laboratory (Bar Harbor, Maine), and MHC class II KO (N5 backcross to C57BL/6) mice were purchased from Taconic Farm. To generate αCD1, αIL10, αMHC, and αIL6 DKO mice, CD1dKO, IL10KO, MHC-IIKO, and IL6KO mice were crossed with TCRαKO mice. The screening was performed by PCR using tail genomic DNA and the pairs of three primers for distinguishing homozygous and heterozygous mice as previously described (Mizoguchi et al., 1997). All mice were maintained under specific pathogen free facilities at Massachusetts General Hospital. Known pathogenic organisms including the *Helicobacter* species were not detectable in these mice.

B Cell Transfer

Cells were extracted from the pooled MLN or spleen of donor mice. For enrichment of MLN B cells, CD3ε (145-2C11), CD4 (RM4-4), CD11b (M1/70), and NK-1.1 (PK136) positive cells were depleted by using a magnetically activated cell sorting system (MACS, Miltenyi, Auburn, CA). For enrichment of splenic B cells, depletion of CD117 (2B8) and CD11c (HL3) positive cells was also carried out. After examining the purity of $B220^+IgM^+$ B cells by flow cytometric analysis, 1.5×10^7 enriched B cells from TCRαKO, αCD1DKO, αIL10DKO, βδDKO, and WT mice and 3×10^7 enriched B cells from αMHCDKO mice were used for cell transfer studies. An abdominal incision was made in anesthetized αμDKO mice (12 weeks of age) to determine the presence of intestinal inflammation. The circumference of the colon was measured. The selected αμDKO mice that had developed mild to moderate intestinal inflammation (mice with a colonic circumference ranging from 6 mm to 13 mm) were used as recipient mice. The recipient mice were injected intraperitoneally with B cells 14 days after the operation, and the B cell transfers were performed every 10 days (three times). The recipient mice were sacrificed 10 days after the last B cell transfer. Azide-free anti-IL10R (1B1.2) mAbs

were purchased from Pharmingen (San Diego, CA). Hybridoma-producing anti-IL10 (JES5-2A5) mAb was kindly provided by Dr. D.M. Rennick (DNAX), and the mAbs were purified as previously described (Asseman et al., 1999). One milligram of anti-IL-10 and 0.5 mg of anti-IL-10R mAbs were combined and injected intraperitoneally three times into $\alpha\mu$DKO mice receiving adoptively transferred B cells 1 day after the B cell transfer. In addition, an additional dose of the two antibodies was administered 7 days after the last B cell transfer.

Histological Examinations

The severity of intestinal inflammation was evaluated by using previously described criteria (Mombaerts et al., 1993) with some modifications. The disease score (0–10) was estimated by a combination of both gross and histological findings. The gross score was rated as 0, presence of normal beaded appearance; 1, absence of beaded appearance of colon; 2, focal thickened colon; and 3, marked thickness of the entire colon. The histological score was based upon the extent of intestinal wall thickening (0–3), lamina propria infiltration (0–3), and presence (0–1) of ulceration. Statistical analysis was performed using a Kruskal-Wallis test.

Flow Cytometric Analysis

Cells (3×10^5) were subjected to flow cytometric analysis. The mAbs used in the studies are FITC- or PE- conjugated anti-CD3ϵ (145-2C11), -B220 (RA3-6B2), -CD21 (7G6), and -CD1d (1B1) purchased from Pharmingen and PE-conjugated anti-Igμ from Caltag. The Mann-Whitney *U* test was used for statistical analysis.

Detection of Cytokines

MLN cells were stained with FITC-anti-B220 (RA3-6B2), and B220$^+$ B cells were positively sorted by using anti-FITC microbeads on MACS. RT-PCR was performed using total RNA extracted from 5×10^5 cells as previously described (Mizoguchi et al., 1996). Colonic LP cells were isolated as previously described (Mizoguchi et al., 1996), and CD4$^+$ T cells were purified using MACS as described above. 1×10^5 CD4$^+$ T cells in 150 μl F12/DMEM medium containing 1% FBS, 1$\times$ SITE3 (Sigma), 10 mM sodium pyruvate, and 50 μM 2-ME were stimulated with plate-coated (10 μg/ml) anti-TCRβ mAbs (H57-597) for 72 hr. The culture supernatants were subjected to OptEIA ELISA (Pharmingen) according to the manufacturer's instructions. Total RNA was extracted from the mucosal layer without the outer muscle layer of the colon as previously described (Mizoguchi et al., 1999). Total RNA was extracted as described above, and RPA was performed using 10 μg of RNA with the RiboQuant RPA system (PharMingen) according to the manufacturer's instructions.

Detection of Igs

Sera from mice were subjected to ELISA for the detection of IgM, IgA, and IgG. Mononuclear cells extracted from MLNs were subjected to ELISPOT assay for the detection of IgM-, IgA-, and IgG-secreting cells as previously described (Mizoguchi et al., 1996). Cells were cultured for 12 hr for the detection of immunoglobulin-secreting cells.

Detection of STAT3 Phosphorylation

The colonic mucosal layer obtained as described above was homogenized in a lysis buffer containing 50 mM Tris (pH 8), 0.5% NP-40, 1 mM EDTA, 150 mM NaCL, 10% glycerol, 1 mM sodium vanadate, 50 mM sodium fluoride, 10 mM sodium pyrophosphate, 1 mM phenylmethylsulfonyl fluoride, and a tablet of protease inhibitor cocktail (Roche) (Suzuki et al., 2001). The lysates (10 μg) were resolved by SDS-PAGE, and the proteins were detected by immunoblotting using ECL detection system (Amersham). Tyrosine phosphorylation of STAT3 was detected by using anti-phospho-STAT3 Ab (Cell Signaling Technology, Beverly, Massachusetts). After stripping the anti-phospho-STAT-3 specific antibody, the membranes were reprobed by using anti-STAT3 Ab (Santa Cruz Biotechnology, Santa Cruz, California). The density of bands was measured by scanning densitometry (Bio Rad), and the ratio of phosphorylated STAT3 was calculated as the density of phosphorylated STAT3 minus background density divided by the density of STAT3 minus background density.

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References

Abbas, A.K., Murphy, K.M., and Sher, A. (1996). Functional diversity of helper T lymphocytes. Nature *383*, 787–793.

Amano, M., Baumgarth, N., Dick, M.D., Brossay, L., Kronenberg, M., Herzenberg, L.A., and Strober, S. (1998). CD1 expression defines subsets of follicular and marginal zone B cells in the spleen: β2-microglobulin-dependent and independent forms. J. Immunol. *161*, 1710–1717.

Asano, M., Toda, M., Sakaguchi, N., and Sakaguchi, S. (1996). Autoimmune disease as a consequence of developmental abnormality of a T cell subpopulation. J. Exp. Med. *184*, 387–396.

Asseman, C., Mauze, S., Leach, M.W., Coffman, R.L., and Powrie, F. (1999). An essential role for interleukin 10 in the function of regulatory T cells that inhibit intestinal inflammation. J. Exp. Med. *190*, 995–1004.

Behar, S.M., and Cardell, S. (2000). Diverse CD1d-restricted T cells: diverse phenotypes and diverse functions. Semin. Immunol. *12*, 551–560.

Behar, S.M., Dascher, C.C., Grusby, M.J., Wang, C.-R., and Brenner, M.B. (1999). Susceptibility of mice deficient in CD1d or TAP1 to infection with Mycobacterium tubercrosis. J. Exp. Med. *189*, 1973–1980.

Bendelac, A., Rivera, M.N., Park, S.-H., and Roark, J.H. (1997). Mouse CD1-specific NK1 T cells. Development, specificity, and function. Annu. Rev. Immunol. *15*, 535–562.

Benlagha, K., Weiss, A., Beavis, A., Teyton, L., and Bendelac, A. (2000). In vivo identification of glycolipid antigen-specific T cells using fluorescent CD1d tetramers. J. Exp. Med. *191*, 1895–1903.

Bhan, A.K., Mizoguchi, E., Smith, R.N., and Mizoguchi, A. (1999). Colitis in transgenic and knockout animals as models of human inflammatory bowel disease. Immunol. Rev. *169*, 195–207.

Bleicher, P.A., Balk, S.P., Hagen, S.J., Blumberg, R.S., Flotte, T.J., and Terhorst, C. (1990). Expression of murine CD1 on gastrointestinal epithelium. Science *250*, 679–682.

Blumberg, R.S., Terhorst, C., Bleicher, P., McDermott, F.V., Allan, C.H., Landau, S.B., Trier, J.S., and Balk, S.P. (1991). Expression of a nonpolymorphic MHC class I-like molecule, CD1D, by human intestinal epithelial cells. J. Immunol. *147*, 2518–2524.

Blumberg, R.S., Saubermann, L.J., and Strober, W. (1999). Animal models of mucosal inflammation and their relation to human inflammatory bowel disease. Curr. Opin. Immunol. *11*, 648–656.

Brossay, L., Jullien, D., Cardell, S., Sydora, B.C., Burding, N., Modlin, R.L., and Kronenberg, M. (1997). Mouse CD1 is mainly expressed on hemopoietic-derived cells. J. Immunol. *159*, 1216–1224.

Burdin, N., and Kronenberg, M. (1999). CD1-mediated immune responses to glycolipids. Curr. Opin. Immnol. *11*, 326–331.

Ceponis, P.J.M., Botelho, F., Richards, C.D., and McKay, D.M. (2000). Interleukin 4 and 13 increase intestinal epithelial permeability by a phosphatidylinositol 3-kinase pathway. J. Biol. Chem. *275*, 29132–29137.

Chen, Y., Kuchroo, V.K., Inobe, J.-I., Hafler, D.A., and Weiner, H.L. (1994). Regulatory T cell clones induced by oral tolerance: suppression of autoimmune encephalomyelitis. Science *265*, 1237–1240.

Colgan, S.P., Hershberg, R.M., Furuta, G.T., and Blumberg, R.S.

(1999). Ligation of intestinal epithelial CD1d induces bioactive IL-10: critical role of the cytoplasmic tail in autocrine signaling. Proc. Natl. Acad. Sci. USA *96*, 13938–13943.

Cottrez, F., Hurst, S.D., Coffman, R.L., and Groux, H. (2000). T regulatory cells 1 inhibit a Th2-specific response in vivo. J. Immunol. *165*, 4843–4853.

Dao, T., Exley, M., Mehal, W.Z., Tahir, S.M.A., Snapper, S., Taniguchi, M., Balk, S.P., and Crispe, I.N. (2001). Involvement of CD1 in peripheral deletion of T lymphocytes is independent of NK T cells. J. Immunol. *166*, 3090–3097.

Davidson, N.J., Leach, M.W., Fort, M.M., Thompson-Snipes, L., Kuhn, R., Muller, W., Berg, D.J., and Rennick, D.M. (1996). T helper cell 1-type $CD4^+$ T cells, but not B cells, mediate colitis in interleukin 10-deficient mice. J. Exp. Med. *184*, 241–251.

De Waal Malefyt, R., Yssel, H., Roncarolo, M.G., Spits, H., and de Vries, J.E. (1992). Interleukin-10. Curr. Opin. Immunol. *4*, 314–318.

Dombrowicz, D., Nutten, S., Desreumaux, P., Neut, C., Torpier, G., Peeters, M., Colombel, J.-F., and Capron, M. (2001). Role of the high affinity immunoglobulin E receptor in bacterial translocation and intestinal inflammation. J. Exp. Med. *193*, 25–34.

Fagarasan, S., Watanabe, N., and Honjo, T. (2000). Generation, expansion, migration and activation of mouse B1 cells. Immunol. Rev. *176*, 205–215.

Fiorentino, D.F., Zlotnik, A., Mosmann, T.R., Howard, M., and O'Garra, A. (1991). IL-10 inhibits cytokine production by activated macrophages. J. Immunol. *147*, 3815–3822.

Gonnella, P.C., Waldner, H.P., and Weiner, H.L. (2001). B cell-deficient (mMT) mice have alterations in the cytokine microenvironment of the gut-associated lymphoid tissue (GALT) and a defect in the low dose mechanism of oral tolerance. J. Immunol. *166*, 4456–4464.

Grunig, G., Corry, D.B., Leach, M.W., Seymour, B.W., Kurup, V.P., and Rennick, D.M. (1997). Interleukin-10 is a natural suppressor of cytokine production and inflammation in a murine model of allergic bronchopulmonary aspergillosis. J. Exp. Med. *185*, 1089–1099.

Gumperz, J.E., Roy, C., Makowska, A., Lum, D., Sugita, M., Podrebarac, T., Koezuka, Y., Porcelli, S.A., Cardell, S., Brenner, M.B., and Behar, S.M. (2000). Murine CD1d-restricted T cell recognition of cellular lipids. Immunity *12*, 211–221.

Harris, D.P., Haynes, L., Sayles, P.C., Duso, D.K., Eaton, S.M., Lepak, N.M., Johnson, L.L., Swain, S.L., and Lund, F.E. (2000). Reciprocal regulation of polarized cytokine production by effector B and T cells. Nat. Immunol. *1*, 475–482.

Hershberg, R.M., and Mayer, L.F. (2000). Antigen processing and presentation by intestinal epithelial cells—polarity and complexity. Immunol. Today *21*, 123–128.

Hollander, G.A., Simpson, S.J., Mizoguchi, E., Nichogiannopoulou, A., She, J., Gutierrez-Ramos, J.C., Bhan, A.K., Burakoff, S.J., Wang, B., and Terhorst, C. (1995). Severe colitis in mice with aberrant thymic selection. Immunity *3*, 27–38.

Huang, F.P., Platt, N., Wykes, M., Major, J.R., Powell, T.J., Jenkins, C.D., and MacPherson, G.G. (2000). A discrete subpopulation of dendritic cells transports apoptotic intestinal epithelial cells to T cell areas of mesenteric lymph nodes. J. Exp. Med. *191*, 435–444.

Iijima, H., Takahashi, I., Kishi, D., Kim, J.-K., Kawano, S., Hori, M., and Kiyono, H. (1999). Alteration of interleukin 4 production results in the inhibition of T helper type 2 cell-dominated inflammatory bowel disease in T cell receptor α chain-deficient mice. J. Exp. Med. *190*, 607–616.

Kawano, T., Cui, J., Koezuka, Y., Toura, I., Kaneko, Y., Motoki, K., Ueno, H., Nakagawa, R., Sato, H., Kondo, E., et al. (1997). CD1d-restricted and TCR-mediated activation of Vα14 NKT cells by glycosylceramides. Science *278*, 1626–1629.

Korganow, A.S., Ji, H., Mangialaio, S., Duchatelle, V., Pelanda, R., Martin, T., Degott, C., Kikutani, T., Rajewsky, K., Pasquali, J.L., et al. (1999). From systemic T cell self-reactivity to organ-specific autoimmune disease via immunoglobulins. Immunity *10*, 451–461.

Lehuen, A., Lantz, O., Beaudoin, L., Laloux, V., Carnaud, C., Bendelac, A., Bach, J.-F., and Monteiro, R.C. (1998). Overexpression of natural killer T cells protects Vα14-Jα281 transgenic nonobese diabetic mice against diabetes. J. Exp. Med. *188*, 1831–1839.

Loder, F., Mutschler, B., Ray, R.J., Paige, C.J., Sideras, P., Torres, R., Lamers, M.C., and Carsetti, R. (1999). B cell development in the spleen takes place in discrete steps and is determined by the quality of B cell receptor-derived signals. J. Exp. Med. *190*, 75–89.

Ma, A., Datta, M., Margosian, E., Chen, J., and Horak, I. (1995). T cells, but not B cells, are required for bowel inflammation in interleukin 2-deficient mice. J. Exp. Med. *182*, 1567–1572.

Matsuda, J.L., Naidenko, O.V., Gapin, L., Nakayama, T., Taniguchi, M., Wang, C.-R., Koezuka, Y., and Kronenberg, M. (2000). Tracking the response of natural killer T cells to a glycolipid antigen using CD1d tetramer. J. Exp. Med. *192*, 741–753.

Mizoguchi, A., Mizoguchi, E., Chiba, C., Spiekermann, G.M., Tonegawa, S., Nagler-Anderson, C., and Bhan, A.K. (1996). Cytokine imbalance and autoantibody production in TCRα mutant mice with inflammatory bowel disease. J. Exp. Med. *183*, 847–856.

Mizoguchi, A., Mizoguchi, E., Smith, R.N., Preffer, F.I., and Bhan, A.K. (1997). Suppressive role of B cells in chronic colitis of T cell receptor α mutant mice. J. Exp. Med. *186*, 1749–1756.

Mizoguchi, A., Mizoguchi, E., and Bhan, A.K. (1999). The critical role of interleukin 4 but not interferon gamma in the pathogenesis of colitis in T-cell receptor α mutant mice. Gastroenterology *116*, 320–326.

Mizoguchi, E., Mizoguchi, A., Preffer, F.I., and Bhan, A.K. (2000). Regulatory function of mature B cells via costimulatory pathway in a murine colitis model. Int. Immunol. *12*, 597–606.

Mombaerts, P., Mizoguchi, E., Grusby, M.J., Glimcher, L.H., Bhan, A.K., and Tonegawa, S. (1993). Spontaneous development of inflammatory bowel disease in T cell receptor mutant mice. Cell *75*, 275–282.

Moody, D.B., Besra, G.S., Wilson, I.A., and Porcelli, S.A. (1999). The molecular basis of CD1-mediated presentation of lipid antigens. Immunol. Rev. *172*, 285–296.

Moulin, V., Andris, F., Thielemans, K., Maliszewski, C., Urbain, J., and Moser, M. (2000). B lymphocytes regulate dendritic cell (DC) function in vivo: interleukin 12 production by DCs from B cell-deficient mice results in T helper cell type 1 deviation. J. Exp. Med. *192*, 475–482.

Naidenko, O.V., Maher, J.K., Ernst, W.A., Sakai, T., Modlin, R.L., and Kronenberg, M. (1999). Binding and antigen presentation of ceramide-containing glycolipids by soluble mouse and human CD1d molecules. J. Exp. Med. *190*, 1069–1079.

O'Farrell, A.-M., Liu, Y., Moore, K.W., and Mui, A.L. (1998). IL-10 inhibits macrophage activation and proliferation by distinct signaling mechanisms: evidence for STAT-3 dependent and -independent pathways. EMBO J. *17*, 1006–1016.

O'Garra, A. (1998). Cytokines induce the development of functionally heterogenous T helper cell subsets. Immunity *8*, 275–283.

Ozaki, M.E., Coren, B.A., Huynh, T.N., Redondo, D.J., Kikutani, H., and Webb, S.R. (1999). $CD4^+$ T cell responses to CD40-deficient APCs: defects in proliferation and negative selection apply only with B cells as APCs. J. Immunol. *163*, 5250–5256.

Riley, J.K., Takeda, K., Akira, S., and Schreiber, R.D. (1999). Interleukin-10 receptor signaling through the JAK-STAT pathway. J. Biol. Chem. *274*, 16513–16521.

Rousset, F., Garcia, E., Defrance, T., Peronne, C., Vezzio, N., Hsu, D.H., Kastelein, R., Moore, K.W., and Banchereau, J. (1992). Interleukin10 is a potent growth and differentiation factor for activated human B lymphocytes. Proc. Natl. Acad. Sci. USA *89*, 1890–1897.

Saubermann, L.J., Beck, P., de Jong, Y.P., Pitman, R.S., Ryan, M.S., Kim, H.S., Exley, M., Snapper, S., Balk, S.P., Hagen, S.J., et al. (2000). Activation of natural killer T cells by α-galactosylceramide in the presence of CD1d provides protection against colitis in mice. Gastroenterology *119*, 119–128.

Shevach, E.M. (2000). Regulatory T cells in autoimmunity. Annu. Rev. Immunol. *18*, 423–449.

Suzuki, A., Hanada, T., Mitsuyama, K., Yoshida, T., Kamizono, S., Hoshino, T., Kubo, M., Yamashita, A., Okabe, M., Takeda, K., et al.

(2001). CIS3/SOCS3/SSI3 plays a negative regulatory role in STAT3 activation and intestinal inflammation. J. Exp. Med. *193*, 471–481.

Taniguchi, M., Koseki, H., Tokuhisa, T., Masuda, K., Sato, H., Kondo, E., Kawano, T., Cui, J., Perkes, A., Koyasu, S., and Makino, Y. (1996). Essential requirement of an invariant Vα14 T cell antigen receptor expression in the development of natural killer T cells. Proc. Natl. Acad. Sci. USA *93*, 11025–11028.

Thornton, A.M., and Shevach, E.M. (1998). $CD4^+CD25^+$ immunoregulatory T cells suppress polyclonal T cell activation in vitro by inhibiting interleukin 2 production. J. Exp. Med. *188*, 287–296.

Toh, B.H., Sentry, J.W., and Alderuccio, F. (2000). The causative H+/K+ ATPase antigen in the pathogenesis of autoimmune gastritis. Immunol. Today *21*, 348–354.

Wolf, S.D., Dittel, B.N., Hardardottir, F., and Janeway, C.A., Jr. (1996). Experimental autoimmune encephalomyelitis induction in genetically B cell-deficient mice. J. Exp. Med. *184*, 2271–2278.

Zeng, D., Lee, M.-K., Tung, J., Brendolan, A., and Strober, S. (2000). A role for CD1 in the pathogenesis of lupus in NZB/NZW mice. J. Immunol. *164*, 5000–5004.