Suppressive Role of B Cells in Chronic Colitis of T Cell Receptor α Mutant Mice

By Atushi Mizoguchi, Emiko Mizoguchi, R. Neal Smith, Frederic I. Preffer, and Atul K. Bhan

Summary

The role of antibodies (Abs) in the development of chronic colitis in T cell receptor (TCR)-α−/− mice was explored by creating double mutant mice (TCR-α−/− × immunoglobulin (Ig)μ−/−), which lack B cells. TCR-α−/− × Igμ−/− mice spontaneously developed colitis at an earlier age, and the colitis was more severe than in TCR-α−/− mice. Colitis was induced in recombination-activating gene-1 (RAG-1−/−) mice by the transfer of mesenteric lymph node (MLN) cells from TCR-α−/− × Igμ−/− mice. When purified B cells from TCR-α−/− mice were mixed with MLN cells before cell transfer, colitis did not develop in RAG-1−/− mice. Administration of the purified Ig from TCR-α−/− mice and a mixture of monoclonal autoAbs reactive with colonic epithelial cells led to attenuation of colitis in TCR-α−/− × Igμ−/− mice. Apoptotic cells were increased in the colon, MLN, and spleen of TCR-α−/− × Igμ−/− mice as compared to Igμ−/− mice and TCR-α−/− mice. Administration of the purified Ig from TCR-α−/− mice into TCR-α−/− × Igμ−/− mice led to decrease in the number of apoptotic cells. These findings suggest that although B cells are not required for the initiation of colitis, B cells and Igs (autoAbs) can suppress colitis, presumably by affecting the clearance of apoptotic cells.

Although autoantibodies (autoAbs) contribute to the pathogenesis of certain autoimmune diseases such as autoimmune hemolytic anemia and Graves' disease (1-3), their role in disease such as ulcerative colitis (UC) is unknown (4-6). Recently, various animal models have been established to investigate the pathogenesis of human inflammatory bowel disease (IBD) (7-9). These animal models suggest the importance of CD4+ T cells or CD45R+B220+ CD4+ T cells and Th1 cytokines in the pathogenesis of colitis (9-14). The spontaneous chronic colitis of IL-2- and IL-10-deficient mice develops even when these mice are made deficient in B cells by crossing them with Igμ−/− mice (15, 16).

TCR-α−/− mice also spontaneously develop chronic colitis by 3-4 mo of age. The disease shares many features with human UC (17) including restriction of the inflammation to the colon and a Th2-predominant cytokine profile (18-21). Furthermore, a negative association between incidence of appendectomy and development of UC in human is supported by the lack of colitis in TCR-α−/− mice after appendectomy (resection of cecal patch; reference 22). TCR-α−/− mice harbor a unique population of peripheral T cells (TCR-α-β+) that express TCR-β chains without TCR-α or pre-T cell receptor α (pTα) chains on the cell surface (18, 19, 23-27). The lack of regulatory TCR-α-β+ T cells is associated with the presence of an expanded population of B cells (80% of mesenteric LN [MLN] cells are B cells [CD3- B220+ CD23-]) and increase in production of autoAbs including anti-neutrophil cytoplasmic antibodies (ANCA) and antitropomyosin in TCR-α−/− mice (22, 27, 28). These findings have raised the possibility that B cells, in particular autoAbs, may be involved in the pathogenesis of colitis in TCR-α−/− mice (7, 8, 17).

The present study was designed to investigate the role of B cells and autoAbs in the pathogenesis of colitis in TCR-α−/− mice by creating double mutant (TCR-α−/− × Igμ−/−) mice lacking B cells. The results suggest that although B cells are not required for the initiation of colitis, B cells and Igs (autoAbs) can contribute by suppressing colitis, presumably by affecting the clearance of apoptotic cells and the related self Ags in TCR-α−/− mice.

Materials and Methods

Mice. TCR-α−/− (23) and Igμ−/− (Igh 6 mutant) mice (29) of C57BL/6 strain (H-2b) background were purchased from The Jackson Laboratory (Bar Harbor, ME), crossed to generate the double mutant (TCR-α−/− × Igμ−/−) mice, and maintained under pathogen-free conditions at Massachusetts General Hospital.

Abbreviations used in this paper: BrdU, 5-bromo-2'-deoxyuridine; IBD, inflammatory bowel disease; MLN, mesenteric LN; RAG-1−/−, recombination-activating gene-1; Tdt, terminal deoxynucleotidyl transferase; TUNEL, terminal deoxynucleotidyltransferase-mediated dUTP-biotin nick end labeling; UC, ulcerative colitis.

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After screening by PCR, the nature of TCR-α2/8 mice was confirmed by immunophenotypic analysis of lymphocytes by FACScan® (Becton Dickinson, Mountain View, CA).

Flow cytometry. 10^6 cells obtained from MLNs and spleen were blocked by the incubation in FACS buffer (0.1% sodium azide and 0.2% BSA/PBS) containing 10% of normal rat serum and 0.1% mouse serum. After washing with FACS buffer, cells were stained with anti-CD4 (RM4-5), CD5 (57-7.3), TCR-α/β (GL3), NK-1.1 (PK136), and Mac-1 (M1/70) from PharMingen® (San Diego, CA). After blocking endogenous peroxidase activity by 0.5% H2O2, the sections were rinsed with PBS, and stained with hematoxylin and eosin. The severity of colitis in the spleen and expressing the counts as apoptotic cells per mm2. Apoptotic cells in the spleen were estimated by counting their nuclei with a fluorescence microscopy.

Results and Discussion

Agravation of Colitis in the Absence of B Cells. The colons from TCR-α2/8 mice (6-12 wk of age) and C57BL/6 (6 wk of age) were intraperitoneally transferred into RAG-1−/− mice. The sera obtained 14 d after immunization were used for ELISA and immunohistochemical analysis of colonic tissue. The sera were obtained from RAG-1−/− mice due to lack of B cells and IgG reaction (33). For ELISA, the purified colonic epithelial cells from RAG-1−/− mice were directly coated on plates by centrifugation. After fixation with EtOH for 10 min, the plates were blocked with 5% BSA and 2% rat serum/PBS, and serial dilution of sera from the immunized C57BL/6 mice was used for ELISA and immunohistochemical analysis of colonic tissue from RAG-1−/− mice (non-specific binding of secondary Ab to tissue is not present in RAG-1−/− mice due to lack of B cells and IgG reaction (33)).

For labeling of the colonic epithelial cells from RAG-1−/− mice, double mutant (TCR-α2/8−/−, IgG−/−) mice (33), the positive clones were propagated and subcloned. These hybridoma cells were injected into pristine-pretreated RAG-1−/− mice to obtain ascitic fluid containing mAb. After purification on a protein A affinity column, five autoAbs (each 400 μg) reacting with colonic tissue were cocktailed to form a combination of autoAbs. Seven weekly intraperitoneal injections of 2 mg of the purified Ig or mixture of autoAbs were administrated into TCR-α2/8−/− mice starting at 12 d of age, and the mice were killed at 8 wk of age.

Detection of circulating self Ags. To examine the presence of circulating self Ags (colonic Ags), 200 μl of sera (TCR-α2/8−/−, IgG−/−, or TCR-α2/8−/− × IgG−/− mice with or without Ig transfer) with CFA was injected into groups of five C57BL/6 mice (6 wk of age). The sera obtained 14 d after immunization were used for ELISA and immunohistochemical analysis of colonic tissue from RAG-1−/− mice (non-specific binding of secondary Ab to tissue is not present in RAG-1−/− mice due to lack of B cells and IgG reaction (33)). For ELISA, the purified colonic epithelial cells from RAG-1−/− mice (2 × 10^7/well) were directly coated on plates by centrifugation. After fixation with EtOH for 10 min, the plates were blocked with 5% BSA and 2% rat serum/PBS, and serial dilution of sera from the immunized C57BL/6 mice was added and the antibody binding was detected by incubation with alkaline-phosphatase rat anti-mouse Ig (PharMingen).
specific pathogen-free conditions. Igμ−/− mice did not develop colitis. In TCR-α−/− mice, ~70% of mice developed colitis by 20 wk of age, whereas only 17% of mice showed evidence of colitis by 12 wk of age. In contrast, all the TCR-α−/− × Igμ−/− mice developed a more severe colitis by 8 wk of age, suggesting that the disease in TCR-α−/− × Igμ−/− mice develops faster than in TCR-α−/− mice. Since TCR-α−/− × Igμ−/− mice are more immunocompromised than TCR-α−/− mice, it is possible that the severe colitis in these mice may be related to the presence of pathogens. However, enteric pathogenic organisms were not detected in the TCR-α−/− and TCR-α−/− × Igμ−/− mice.

Figure 1. (A) Mice were screened for the TCR-α and Igμ genotypes by PCR on tail DNA. In screening of Cα locus, the wild-type locus and the disrupted locus represent a 195- and a 276-bp fragment, respectively. The amplification of membrane exon of Cμ locus yields a 700- and a 900-bp fragment corresponding to the wild-type locus and the disrupted locus, respectively. The left lane indicates a molecular weight marker (bp). (B) Splenic cells (for detection of B cells) and MLN cells (for detection of T cells) from TCR-α−/−, TCR-α−/−, Igμ−/−, and TCR-α−/− × Igμ−/− mice were analyzed by FACScan. TCR-α−/− × Igμ−/− mice show no mature B cells (B220−, sIgM+) and increased percentage of T cells, comprising CD3ε+TCR-β+ cells (TCR-α−β− T cells expressing TCR-β chain without TCR-α chain on cell surface) and CD3ε+TCR-β− cells.

Figure 2. Distal segments of large intestine from TCR-α−/− and TCR-α−/− × Igμ−/− mice at 8 wk of age. The large intestine of TCR-α−/− × Igμ−/− mice is markedly thickened as compared to that of TCR-α−/− mice.
from TCR-α−/−, the role of B cells in colitis, we transferred lymphocytes RAG-1 TCR-α−/− in the pathogenesis of this colitis in TCR-α−/− mice. However, no colitis was recognized in these RAG-1 mice. However, unlike other models, mature B cells or their products may have a regulatory role in the pathogenesis of this colitis in TCR-α−/− mice.

Cell Transfer to RAG-1−/− Mice. To further investigate the role of B cells in colitis, we transferred lymphocytes from TCR-α−/− and TCR-α−/− × Igμ−/− mice to RAG-1−/− mice that lack T and B cells (33; Table 1). The transfer of MLN cells from TCR-α−/− mice of 8 or 20 wk of age did not induce colitis in RAG-1−/− mice within an 8 wk period of observation. Since 80% of cells in MLN of TCR-α−/− mice contain B cells (18), cell transfer studies were performed after B cells were depleted by panning. RAG-1−/− mice reconstituted with B cell-reduced (B220+, 10–15%) population from TCR-α−/− mice also did not show evidence of colitis. In contrast, 82% of RAG-1−/− mice reconstituted with MLN cells from TCR-α−/− × Igμ−/− mice developed colitis. However, when MLN cells from TCR-α−/− × Igμ−/− mice were mixed with equal numbers of purified MLN B cells (B220+, >98%) from TCR-α−/− mice before cell transfer, no colitis was detected in the reconstituted RAG-1−/− mice. Since our previous studies have indicated that increased colonic epithelial cell proliferation is a sensitive index of development of colitis in TCR-α−/− mice (22, 26), the results of cell transfer studies were confirmed by in vivo BrdU incorporation to detect the colonic epithelial cell proliferation. Proliferation index of colonic epithelium as detected by BrdU incorporation was markedly higher in RAG-1−/− mice reconstituted with MLN cells of TCR-α−/− × Igμ−/− mice as compared with mice reconstituted with MLN cells from TCR-α−/− mice. These findings support a suppressive role of B cells in the development of colitis.

Contribution of AutoAbs to Suppression of Colitis. B cells possess many immunological functions such as secretion of Igμ−/− mice maintained under pathogen-free conditions as confirmed by the studies performed at The Charles River Laboratories (Wilmington, MA). We also orally administered (three times) cecal contents from TCR-α−/−, Igμ−/−, and TCR-α−/− × Igμ−/− mice at 4, 8, 12, and 20 wk of age maintained under specific pathogen-free conditions.

Table 1. Cell Transfer Studies into RAG-1−/− Mice

| Donor                           | N. transferred cells | Percent of B cells | N. mice | Colitis   | BrdU index |
|---------------------------------|----------------------|--------------------|---------|-----------|------------|
| TCR-α−/−                        | 10⁷                  | 75-80              | 12      | 12        | 14.1 ± 4.9 |
| TCR-α−/− × Igμ−/−               | 10⁷                  | 10-15              | 8       | 7         | 17.6 ± 1.5 |
| TCR-α−/− × Igμ−/−               | 2 × 10⁶              | 0                  | 11      | 2         | 33.0 ± 3.7 |
| Mix                            | 4 × 10⁶              | 50                 | 10      | 0         | 13.4 ± 1.4 |
| Control                        | PBS                  | -                  | 7       | 7         | 11.7 ± 0.4 |

*Percentage of B cells in the cell population used for cell transfer.

1 In vivo proliferation of colonic epithelial cells (BrdU index) was assessed by detection of BrdU-incorporated epithelial cells.

2 B cells in MLN cell populations were depleted by panning method using Ig-coated plates before cell transfer.

3 A mixed population containing equal numbers of cells from MLN cells of TCR-α−/− × Igμ−/− mice and purified B cells from TCR α−/− mice was used for cell transfer studies.

4 As control, PBS was injected into a group of RAG-1−/− mice.
Ig, antigen presentation, and cytokine production. In TCR-α−/− mice, ANCA and autoAbs against tropomyosin (a constituent of colonic epithelial cells), small nuclear ribonucleoproteins, and DNA have been frequently detected (26, 27, 34). To define how B cells alter the pathogenesis of colitis, we passively transferred Ig into TCR-α−/− mice. Injection of purified Ig from TCR-α−/− mice clearly decreased the severity of colitis in TCR-α−/− × Igμ−/− mice (Fig. 4). TCR-α−/− × Igμ−/− mice injected with Ig from wild-type mice (TCR-α1/2 mice) also showed an improvement of disease; however, the severity of colitis in these mice seemed to be greater than that in TCR-α−/− mice injected with Ig from TCR-α−/− mice. It is possible that the suppression of colitis is due to autoAbs

Figure 5. Apoptotic cells in the colon (top) and spleen (bottom) of Igμ−/−, TCR-α−/−, and TCR-α−/− × Igμ−/− mice injected with PBS or Ig purified from sera of TCR-α−/− mice were detected by TUNEL assay (×20 objective). All mice were 8 wk of age. The numbers in the right lower corner indicate the number of apoptotic cells per mm².

Figure 6. (A) Circulating self Ags from C57BL/6 (closed triangles), Igμ−/− (closed squares), TCR-α−/− (open squares), and TCR-α−/− × Igμ−/− mice with injection of PBS (closed circles) or Ig (open circles) purified from sera of TCR-α−/− mice were quantified. 200 μl of sera (from various mice)/CFA were injected to C57BL/6 mice. On day 14 after immunization, the reactivity of sera from the immunized C57BL/6 mice against colonic epithelial antigens was determined by ELISA. (B and C) The reactivity of sera from the immunized C57BL/6 mice with sera of TCR-α−/− × Igμ−/− mice with (B) or without (C) Ig transfer was confirmed by immunohistochemical analysis using sections of colon (×40 objective) from RAG-1−/− mice.
present in TCR-α−/− mice. To confirm our hypothesis, we generated five autoAb-secreting hybridomas by using B cells of MLNs from unimmunized TCR-α−/− mice. These autoAbs showed strong reactivity against colonic tissue by immunohistochemical studies and ELISA (data not shown). The generated autoAbs were intraperitoneally injected into TCR-α−/− × Igμ−/− mice. The injection of a mixture of autoAbs generated by these hybridomas also attenuated the severity of colitis in TCR-α−/− × Igμ−/− mice (Fig. 4). These findings strongly suggest that autoAbs can contribute to suppression of colitis.

Increase of Apoptotic Cells in TCR-α−/− × Igμ−/− Mice

Apoptotic bodies comprise the major source of autoAgs and provide powerful immunogens for autoreactive T cells (35, 36). Translocation of intracytoplasmic autoantigens to cell surface during apoptosis (37) indicates that autoAbs associated with the nucleus of the cells (Fig. 6, B and C). These findings indicate that in TCR-α−/− × Igμ−/− mice, there is an increase of circulating colon-associated self Ags as compared to TCR-α−/−, Igμ−/−, and TCR-α−/− mice, and the transfer of Ig into TCR-α−/− × Igμ−/− mice leads to marked decrease of circulating self Ags. Furthermore, these findings support the hypothesis that failure of normal clearance mechanisms for apoptotic cells by lack of autoAbs leads to an increase of circulating self Ags. The increased circulating self Ags may activate self-reactive T cells and provoke organ-specific autoimmune diseases (40) such as IBD. The increase of apoptotic cells shown in lamina propria cells of colon as well as spleen and MLNs in TCR-α−/− mice is likely to reflect the activation-induced cell death (41) of effector cells caused by harmful exposure to the increased local and circulating self Ags.

In the organ-specific autoimmune disease model of experimental autoimmune encephalomyelitis (EAE), the data indicates that lack of mature B cells acting as secondary APCs may delay the recovery of the disease (42). The administration of Ig suppressed the severity of colitis, but did not completely prevent the development of colitis in TCR-α−/− × Igμ−/− mice. These findings suggest that B cells play important functions in the complex immunological network of autoimmune diseases and in the pathogenesis of colitis in TCR-α−/− mice. Since normal mice also produce natural autoAbs (43, 44), it is possible that these Abs may also contribute to the regulation of the immunological homeostasis and suppress the development of autoimmune disease such as IBD.

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Address correspondence to Dr. A. K. Bhan, Immunopathology Unit-Cox5, Massachusetts General Hospital, 100 Blossom St., Boston, MA 02114. Phone: 617-726-2588; Fax: 617-726-2365; E-mail: bhan@helix.mgh.harvard.edu

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