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

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Summary

The role of antibodies (Abs) in the development of chronic colitis in T cell receptor (TCR)-$\alpha^{-/-}$ mice was explored by creating double mutant mice (TCR-$\alpha^{-/-}$ × immunoglobulin (Ig)$\mu^{-/-}$), which lack B cells. TCR-$\alpha^{-/-}$ × Ig$\mu^{-/-}$ mice spontaneously developed colitis at an earlier age, and the colitis was more severe than in TCR-$\alpha^{-/-}$ mice. Colitis was induced in recombination-activating gene-1 (RAG-$1^{-/-}$) mice by the transfer of mesenteric lymph node (MLN) cells from TCR-$\alpha^{-/-}$ × Ig$\mu^{-/-}$ mice. When purified B cells from TCR-$\alpha^{-/-}$ mice were mixed with MLN cells before cell transfer, colitis did not develop in RAG-$1^{-/-}$ mice. Administration of the purified Ig from TCR-$\alpha^{-/-}$ mice and a mixture of monoclonal autoAbs reactive with colonic epithelial cells led to attenuation of colitis in TCR-$\alpha^{-/-}$ × Ig$\mu^{-/-}$ mice. Apoptotic cells were increased in the colon, MLN, and spleen of TCR-$\alpha^{-/-}$ × Ig$\mu^{-/-}$ mice as compared to Ig$\mu^{-/-}$ mice and TCR-$\alpha^{-/-}$ mice. Administration of the purified Ig from TCR-$\alpha^{-/-}$ mice into TCR-$\alpha^{-/-}$ × Ig$\mu^{-/-}$ 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)[1] 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 $CD45RB^{high}$ $CD4^+$ 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 Ig$\mu^{-/-}$ mice (15, 16).

TCR-$\alpha^{-/-}$ 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-$\alpha^{-/-}$ mice after appendectomy (resection of cecal patch; reference 22). TCR-$\alpha^{-/-}$ mice harbor a unique population of peripheral T cells (TCR-$\alpha^-\beta^+$) 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-$\alpha^+\beta^+$ 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-$\alpha^{-/-}$ 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-$\alpha^{-/-}$ 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-$\alpha^{-/-}$ mice by creating double mutant (TCR-$\alpha^{-/-}$ × Ig$\mu^{-/-}$) 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-$\alpha^{-/-}$ mice.

Materials and Methods

Mice. TCR-$\alpha^{-/-}$ (23) and Ig$\mu^{-/-}$ (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-$\alpha^{-/-}$ × Ig$\mu^{-/-}$) mice, and maintained under pathogen-free conditions at Massachusetts General Hospital

[1] *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 d-UTP-biotin nick end labeling; UC, ulcerative colitis.

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(Boston, MA). To distinguish heterozygous from homozygous mice, pairs of three primers were used in PCR using tail DNA: KO1 (5′-TGCCTGTTCACCGACTTTGA), KO2 (5′-TGAACTGGGGTAGGTGGCGT; reference 34), and pgk-neo (5′-CACCAAAGAACGGAGCCGGTT) for screening of Cα locus, and 5′μM (5′-CTCTGTAAGGAGTCACCACC), 3′ μM (5′-AAGCCTTCCTCCTCAGCATTC), and neoTK (5′-ATTCGGGAATGACAAGACGCTGG; reference 33) for screening of Cμ locus. After screening by PCR, the nature of TCR-$\alpha^{-/-}$ × Ig$\mu^{-/-}$ mice was reconfirmed by immunophenotypic analysis of lymphocytes by FACScan" (Becton Dickinson, Mountain View, CA).

Flow Cytometry. 2×10^5 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 and hamster serum and 0.5 μg/2×10^5 cells CD16/CD32. After washing with FACS buffer, cells were stained using anti-CD3ε (145-2C11)-FITC (Boehringer Mannheim, Indianapolis, IN) or B220 (RA3-6B2)-FITC and TCR-β (H57-597), TCR-δ (GL3), or Igμ-PE (PharMingen, San Diego, CA) at 4°C for 30 min. After washing with FACS buffer, cells were analyzed on FACScan".

Histological Examination. Specimens obtained from the distal, middle, and proximal colon were fixed in 10% buffered formalin and stained with hematoxylin and eosin. The severity of colitis was determined according to the diagnostic criteria previously described (17, 26).

Detection of Proliferative and Apoptotic Cells. For labeling of the proliferative cells, 5-bromo-2′-deoxyuridine (BrdU) (Sigma Chemical Co., St. Louis, MO) was injected intraperitoneally (100 μg/g) 1 h before killing. In vivo BrdU-incorporated epithelial cells were detected by anti-BrdU mAb (Sera Lab, Crawley Down, England), followed by staining with avidin-biotinylated peroxidase complex method, and counted as previously described (22, 26).

Apoptotic cells were detected by terminal deoxynucleotidyl-transferase–mediated d-UTP-biotin nick end labeling (TUNEL) assay as described previously (31). Frozen sections were fixed in 3% buffered formalin for 10 min at room temperature. After washing with PBS, sections were fixed again in ethanol/acetic acid (2:1) for 10 min at −20°C. After blocking endogenous peroxidase activity by 0.5% H_2O_2, the sections were rinsed with PBS and immersed in terminal deoxynucleotidyl transferase (TdT) buffer (30 mM Tris [pH 7.2], 140 mM sodium cacodylate, 1 mM cobalt chloride). The sections were incubated with 2–4 mM biotinylated dUTP (Boehringer Mannheim) and 5–10 U TdT (Promega, Madison, WI) in TdT buffer at 37°C for 2 h. After terminating reactions by Tris-borate buffer, apoptotic cells were detected by staining with avidin-biotinylated peroxidase complex method. Apoptotic cells in the spleen were estimated by counting the numbers of apoptotic cells in the entire frozen tissue sections of spleen and expressing the counts as apoptotic cells per mm^2.

Cell Transfer Studies. MLN cells extracted from TCR-$\alpha^{-/-}$ mice (8 or 20 wk of age) and TCR-$\alpha^{-/-}$ × Ig$\mu^{-/-}$ mice (8 wk of age) were intraperitoneally transferred into RAG-$1^{-/-}$ mice (5–6 wk of age), which were killed 8 wk after cell transfer. In some experiments, B cells were partially depleted by the panning method using anti-Ig (30 μg/ml)–coating plates as previously described (18). Purified B cells from MLNs of TCR-$\alpha^{-/-}$ mice were obtained by negative sorting using a mixture of biotinylated mAbs (anti-CD4 [RM4-5], CD5 [57-7.3], TCR-δ [GL3], NK-1.1 [PK136], and Mac-1 [M1/70] from PharMingen [San Diego, CA]), followed by incubation with streptavidin microbeads on magnetic cell sorting system (Miltenyi Biotec Inc., Auburn, CA).

Administration of Ig or mAbs (AutoAbs) into TCR-$\alpha^{-/-}$ × Ig$\mu^{-/-}$ Mice. For Ig transfer, Ig was purified from sera pooled from 160 TCR-$\alpha^{+/-}$ mice (6–15 wk of age) or 230 TCR-$\alpha^{-/-}$ mice (6–12 wk of age) on a protein A affinity column, dialyzed, and concentrated. Monoclonal antibodies (autoAbs) capable of binding to colonic epithelial cells were generated by the fusion of NS-1 cells with B cells from MLNs of untreated TCR-$\alpha^{-/-}$ mice using polyethylene glycol (Sigma Chemical Co.) as previously described (32). After screening by immunohistochemical staining and ELISA using colonic epithelial cells of recombination-activating gene-1 (RAG-$1^{-/-}$) 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-$\alpha^{-/-}$ × Ig$\mu^{-/-}$ 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-$\alpha^{-/-}$, Ig$\mu^{-/-}$, or TCR-$\alpha^{-/-}$ × Ig$\mu^{-/-}$ mice with or without Ig transfer) with CFA were 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 (nonspecific binding of secondary Ab to tissue is not present in RAG-$1^{-/-}$ mice due to lack of B cells and Igs; reference 33). For ELISA, the purified colonic epithelial cells (2×10^5/well) 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 added and the antibody binding was detected by incubation with alkaline-phosphatase rat anti–mouse Ig (PharMingen).

Results and Discussion

Aggravation of Colitis in the Absence of B Cells. As in UC patients, autoAbs such as ANCA and antitropomyosin are frequently detectable in TCR-$\alpha^{-/-}$ mice (16, 25, 26), and B cells have been suspected to play a role in the pathogenesis of colitis in these mice (7, 8, 17). Therefore, to test the role of B cells in the development of colitis in TCR-$\alpha^{-/-}$ mice, double mutant (TCR-$\alpha^{-/-}$ × Ig$\mu^{-/-}$) mice were generated by crossing TCR-$\alpha^{-/-}$ mice with Ig$\mu^{-/-}$ mice, which lack B cells. All the mice were of inbred C57BL/6 strain. TCR-$\alpha^{-/-}$ × Ig$\mu^{-/-}$ mice were obtained from F3 parents and confirmed by PCR and FACScan" (Fig. 1). As shown in Fig. 1, TCR-$\alpha^{-/-}$ × Ig$\mu^{-/-}$ mice, as well as Ig$\mu^{-/-}$ mice lack mature B cells ($B220^+$, $sIgM^+$) and contain an increased percentage of T cells. The T cells consist of TCR-γ/δ^+ cells and the unique $CD3^+$ TCR-β^{low} cells which express TCR-β chains in the absence of TCR-α chains on the cell surface (23, 24).

Fig. 2 shows the gross appearance of the distal part of colons from TCR-$\alpha^{-/-}$ and TCR-$\alpha^{-/-}$ × Ig$\mu^{-/-}$ mice at 8 wk of age. The colons from TCR-$\alpha^{-/-}$ mice have a normal beaded appearance due to the presence of firm stools in the lumen. In contrast, the colons from TCR-$\alpha^{-/-}$ × Ig$\mu^{-/-}$ mice have thickened wall with presence of loose stools. Fig. 3 shows the severity of colitis in TCR-$\alpha^{-/-}$, Ig$\mu^{-/-}$, and TCR-$\alpha^{-/-}$ × Ig$\mu^{-/-}$ mice maintained under

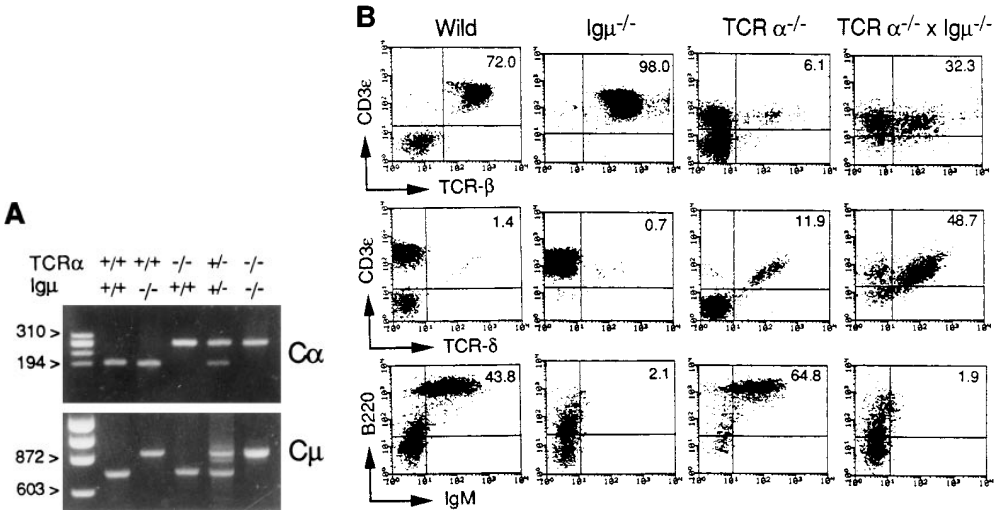

Figure 1. (*A*) Mice were screened for the TCR-α and Igμ genotypes by PCR on tail DNA. In screening of Cα locus, the wild-type locus and the disrupted locus represent a 195- and a 276-bp fragment, respectively. The amplification of membrane exon of Cμ locus yields a 700- and a 900-bp fragment corresponding 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-$\alpha^{+/-}$, TCR-$\alpha^{-/-}$, Ig$\mu^{-/-}$, and TCR-$\alpha^{-/-}$ × Ig$\mu^{-/-}$ mice were analyzed by FACScan". TCR-$\alpha^{-/-}$ × Ig$\mu^{-/-}$ mice show no mature B cells (B220$^+$,sIgM$^+$) and increased percentage of T cells, comprising CD3ϵ^+TCR-β^{low} cells (TCR-$\alpha^-\beta^+$ T cells expressing TCR-β chain without TCR-α chain on cell surface) and CD3ϵ^+TCR-δ^+ cells.

specific pathogen-free conditions. Ig$\mu^{-/-}$ mice did not develop colitis. In TCR-$\alpha^{-/-}$ 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-$\alpha^{-/-}$ × Ig$\mu^{-/-}$ mice developed a more severe colitis by 8 wk of age, suggesting that the disease in TCR-$\alpha^{-/-}$ × Ig$\mu^{-/-}$ mice develops faster than in TCR-$\alpha^{-/-}$ mice. Since TCR-$\alpha^{-/-}$ × Ig$\mu^{-/-}$ mice are more immunocompromised than TCR-$\alpha^{-/-}$ 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-$\alpha^{-/-}$ and TCR-$\alpha^{-/-}$ ×

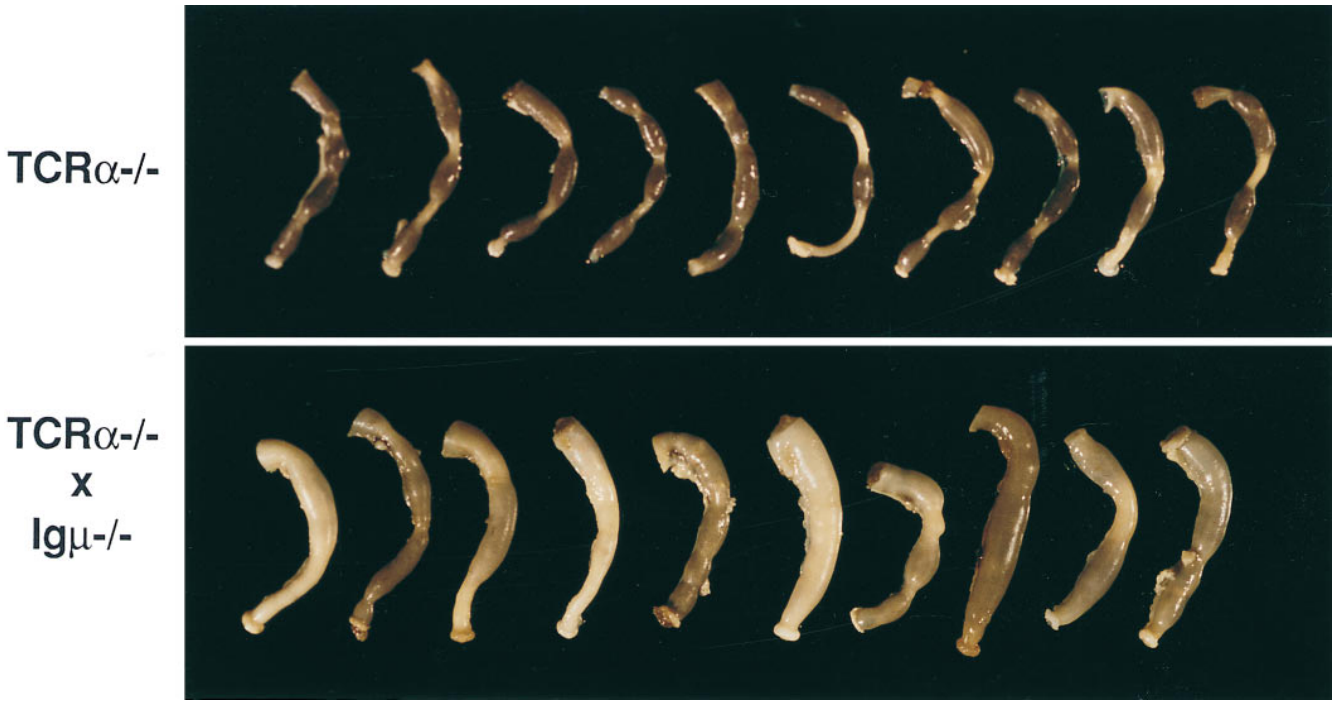

Figure 2. Distal segments of large intestine from TCR-$\alpha^{-/-}$ and TCR-$\alpha^{-/-}$ × Ig$\mu^{-/-}$ mice at 8 wk of age. The large intestine of TCR-$\alpha^{-/-}$ × Ig$\mu^{-/-}$ mice is markedly thickened as compared to that of TCR-$\alpha^{-/-}$ mice.

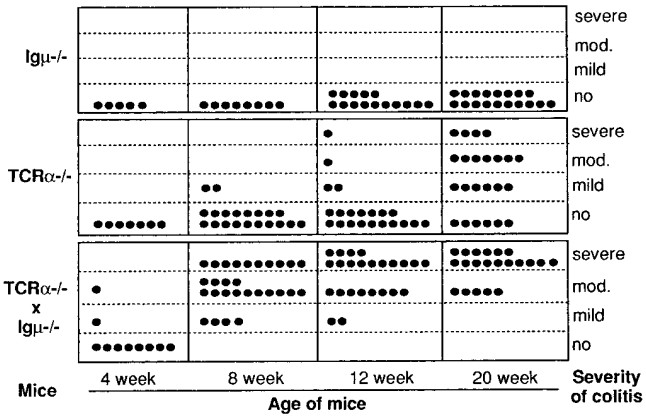

Figure 3. The severity of colitis determined by histological examinations in TCR-$\alpha^{-/-}$, Ig$\mu^{-/-}$, and TCR-$\alpha^{-/-}$ × Ig$\mu^{-/-}$ mice at 4, 8, 12, and 20 wk of age maintained under specific pathogen-free conditions.

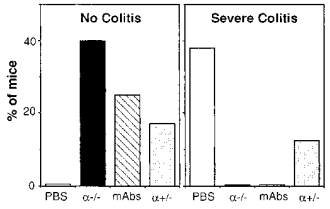

Figure 4. TCR-$\alpha^{-/-}$ × Ig$\mu^{-/-}$ mice were intraperitoneally injected with PBS (*open bar*), purified Ig (2 mg, six times) from TCR-$\alpha^{-/-}$ (*solid bar*), TCR-$\alpha^{+/-}$ (*dotted bar*) mice, or a mixture of mAbs (autoAbs) reactive with colonic epithelial cells (*hatched bar*); and killed at 8 wk of age. The percentage of mice revealing no (normal) or severe colitis is shown. The results were obtained from groups of 10–16 mice.

Ig$\mu^{-/-}$ 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-$\alpha^{-/-}$ × Ig$\mu^{-/-}$ mice with colitis into immunodeficient RAG-$1^{-/-}$ and SCID mice to investigate the possibility that an unknown pathogen may be present in TCR-$\alpha^{-/-}$ × Ig$\mu^{-/-}$ mice. However, no colitis was recognized in these RAG-$1^{-/-}$ and SCID mice 8 wk after oral administration (data not shown). These findings taken together indicate that, like the other murine models of human IBD (15, 16), B cells are not necessary for the development of spontaneous colitis in TCR-$\alpha^{-/-}$ mice. However, unlike other models, mature B cells or their products may have a regulatory role in the pathogenesis of this colitis in TCR-$\alpha^{-/-}$ mice.

Cell Transfer to RAG-$1^{-/-}$ Mice. To further investigate the role of B cells in colitis, we transferred lymphocytes from TCR-$\alpha^{-/-}$ and TCR-$\alpha^{-/-}$ × Ig$\mu^{-/-}$ mice to RAG-$1^{-/-}$ mice that lack T and B cells (33; Table 1). The transfer of MLN cells from TCR-$\alpha^{-/-}$ 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-$\alpha^{-/-}$ 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-$\alpha^{-/-}$ mice also did not show evidence of colitis. In contrast, 82% of RAG-$1^{-/-}$ mice reconstituted with MLN cells from TCR-$\alpha^{-/-}$ × Ig$\mu^{-/-}$ mice developed colitis. However, when MLN cells from TCR-$\alpha^{-/-}$ × Ig$\mu^{-/-}$ mice were mixed with equal numbers of purified MLN B cells ($B220^+$, >98%) from TCR-$\alpha^{-/-}$ 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-$\alpha^{-/-}$ 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-$\alpha^{-/-}$ × Ig$\mu^{-/-}$ mice as compared with mice reconstituted with MLN cells from TCR-$\alpha^{-/-}$ 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

Table 1. *Cell Transfer Studies into RAG-$1^{-/-}$ Mice*

Donor	Transferred cells		No. mice	Colitis		BrdU index‡
	No.	Percent of B cells*		−	+	
TCR-$\alpha^{-/-}$	10^7	75–80	12	12	0	14.1 ± 4.9
TCR-$\alpha^{-/-}$§	10^7	10–15	8	7	1	17.6 ± 1.5
TCR-$\alpha^{-/-}$ × Ig$\mu^{-/-}$	2×10^6	0	11	2	9	33.0 ± 3.7
Mix‖	4×10^6	50	10	10	0	13.4 ± 1.4
Control¶	PBS	–	7	7	0	11.7 ± 0.4

*Percentage of B cells in the cell population used for cell transfer.
‡In vivo proliferation of colonic epithelial cells (BrdU index) was assessed by detection of BrdU-incorporated epithelial cells.
§B cells in MLN cell populations were depleted by panning method using Ig-coated plates before cell transfer.
‖A mixed population containing equal numbers of cells from MLN cells of TCR-$\alpha^{-/-}$ × Ig$\mu^{-/-}$ mice and purified B cells from TCR$\alpha^{-/-}$ mice was used for cell transfer studies.
¶As control, PBS was injected into a group of RAG-$1^{-/-}$ mice.

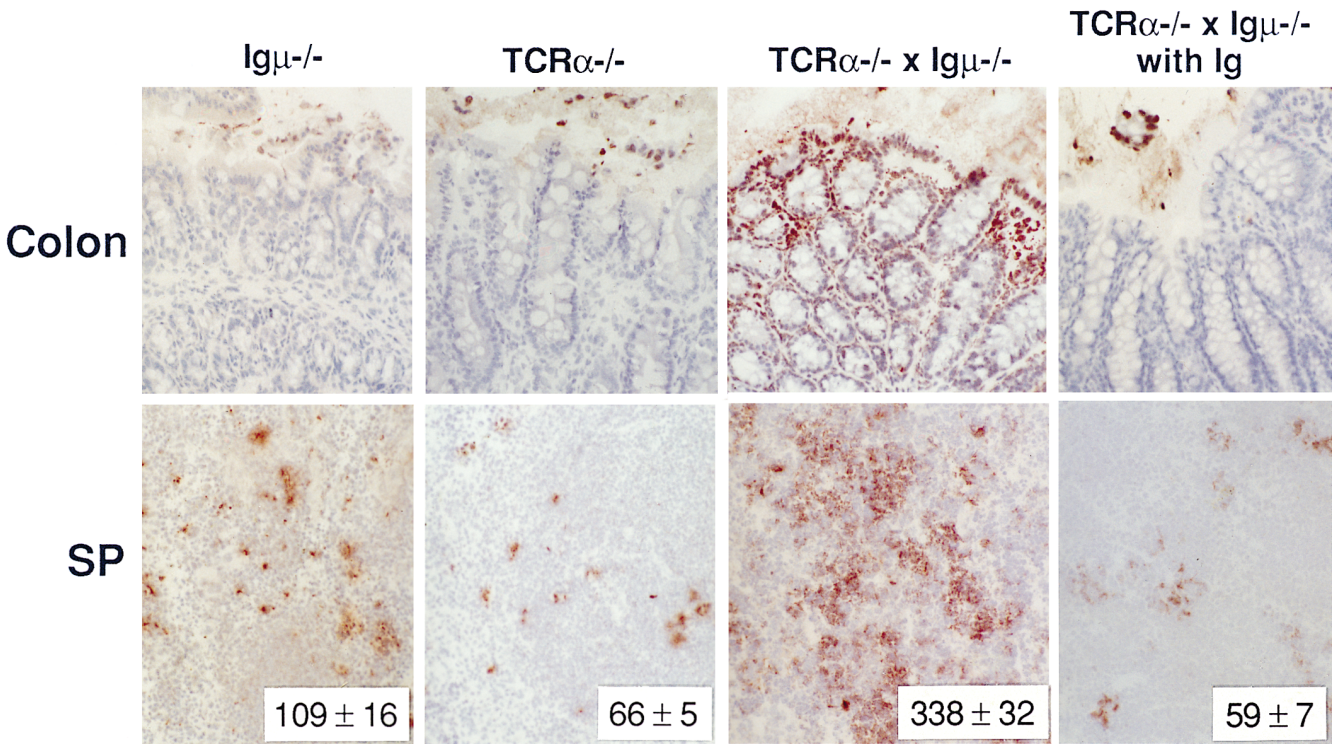

Figure 5. Apoptotic cells in the colon (*top*) and spleen (*bottom*) of Ig$\mu^{-/-}$, TCR-$\alpha^{-/-}$, and TCR-$\alpha^{-/-}$ × Ig$\mu^{-/-}$ mice injected with PBS or Ig purified from sera of TCR-$\alpha^{-/-}$ mice were detected by TUNEL assay (×20 objective). All mice were 8 wk of age. The numbers in the right lower corner indicate the number of apoptotic cells per mm^2.

Ig, antigen presentation, and cytokine production. In TCR-$\alpha^{-/-}$ 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-$\alpha^{-/-}$ × Ig$\mu^{-/-}$ mice. Injection of purified Ig from TCR-$\alpha^{-/-}$ mice clearly decreased the severity of colitis in TCR-$\alpha^{-/-}$ × Ig$\mu^{-/-}$ mice (Fig. 4). TCR-$\alpha^{-/-}$ × Ig$\mu^{-/-}$ mice injected with Ig from wild-type mice (TCR-$\alpha^{+}/^{-}$ mice) also showed an improvement of disease; however, the severity of colitis in these mice seemed to be greater than that in TCR-$\alpha^{-/-}$ × Ig$\mu^{-/-}$ mice injected with Ig from TCR-$\alpha^{-/-}$ mice. It is possible that the suppression of colitis is due to autoAbs

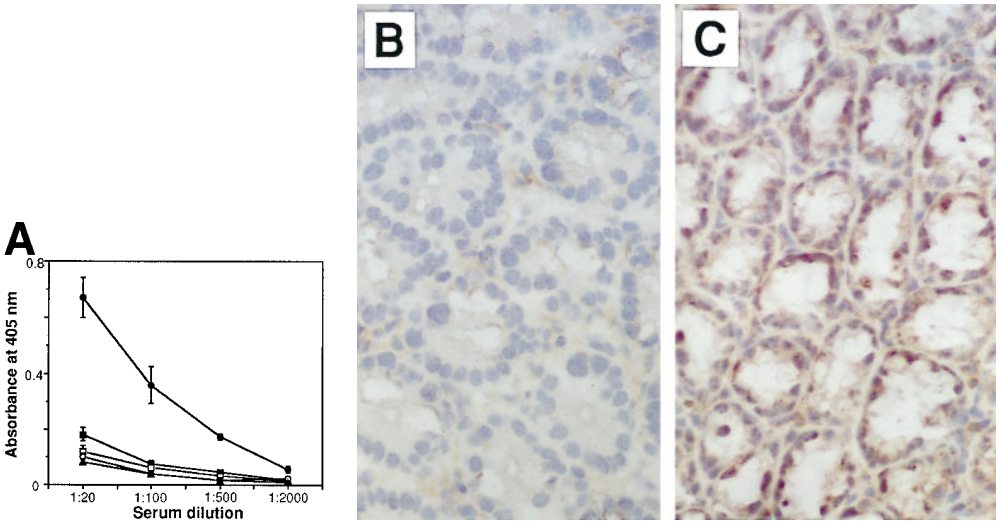

Figure 6. (*A*) Circulating self Ags from C57BL/6 (*closed triangles*), Ig$\mu^{-/-}$ (*closed squares*), TCR-$\alpha^{-/-}$ (*open squares*), and TCR-$\alpha^{-/-}$ × Ig$\mu^{-/-}$ mice with injection of PBS (*closed circles*) or Ig (*open circles*) purified from sera of TCR-$\alpha^{-/-}$ mice are 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-$\alpha^{-/-}$ × Ig$\mu^{-/-}$ 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-$\alpha^{-/-}$ mice. To confirm our hypothesis, we generated five autoAb-secreting hybridomas by using B cells of MLNs from unimmunized TCR-$\alpha^{-/-}$ 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-$\alpha^{-/-} \times$ Ig$\mu^{-/-}$ mice. The injection of a mixture of autoAbs generated by these hybridomas also attenuated the severity of colitis in TCR-$\alpha^{-/-} \times$ Ig$\mu^{-/-}$ mice (Fig. 4). These findings strongly suggest that autoAbs can contribute to suppression of colitis.

Increase of Apoptotic Cells in TCR-$\alpha^{-/-} \times$ Ig$\mu^{-/-}$ 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 (ANCA) can bind intracytoplasmic Ags in the apoptotic process. It has been postulated that rapid clearance of apoptotic bodies by macrophages can prevent tissue damage caused by the harmful exposure to self Ags (38, 39). Therefore, to investigate the suppressive role of autoAbs in spontaneous colitis, apoptotic cells were enumerated by TUNEL assay (Fig. 5). TUNEL assay revealed marked increase in the number of apoptotic cells in the colon (epithelial cells and lamina propria cells), MLN, and spleen of TCR-$\alpha^{-/-} \times$ Ig$\mu^{-/-}$ mice compared to TCR-$\alpha^{-/-}$ and Ig$\mu^{-/-}$ mice. However, when TCR-$\alpha^{-/-} \times$ Ig$\mu^{-/-}$ mice received passively transferred Ig from TCR-$\alpha^{-/-}$ mice, the number of detectable apoptotic cells strikingly decreased. We also examined the expression of Fas, Fas ligand, bcl-2, and IL-1β converting enzymes (ICE) in the colon by immunohistochemical analysis, FACScan", and/or reverse transcriptase PCR. There was no detectable difference in the expression of the molecules involved in apoptosis between the TCR-$\alpha^{-/-} \times$ Ig$\mu^{-/-}$ mice and TCR-$\alpha^{-/-}$ mice (data not shown). This suggests that the increase in the number of apoptotic cells in TCR-$\alpha^{-/-} \times$ Ig$\mu^{-/-}$ mice is caused by alteration in the clearance of apoptotic cells rather than due to increase in apoptosis.

Increase of Circulating Self Ags in TCR-$\alpha^{-/-} \times$ Ig$\mu^{-/-}$ Mice. Since apoptotic cells are the major source of self Ags (35, 36), the presence of circulating colonic Ags (self Ags) was examined. C57BL/6 mice were immunized with sera from TCR-$\alpha^{-/-}$, Ig$\mu^{-/-}$, or TCR-$\alpha^{-/-} \times$ Ig$\mu^{-/-}$ mice (8 wk of age), and the reactivity of sera from the immunized C57BL/6 mice (14 d after immunization) to colonic epithelial Ags was analyzed by ELISA. The sera from C57BL/6 mice immunized with sera of TCR-$\alpha^{-/-} \times$ Ig$\mu^{-/-}$ mice showed significantly higher ($P < 0.001$) reactivity to colonic Ags compared to the other groups including the sera from the C57BL/6 mice immunized with sera from Ig-transferred TCR-$\alpha^{-/-} \times$ Ig$\mu^{-/-}$ mice (Fig. 6 *A*). Immunohistochemical analysis confirmed these results; the sera from C57BL/6 mice immunized with sera from TCR-$\alpha^{-/-} \times$ Ig$\mu^{-/-}$ mice, but not from other groups, strongly reacted with colonic epithelial cells. The reactivity was mostly associated with the nucleus of the cells (Fig. 6, *B* and *C*). These findings indicate that in TCR-$\alpha^{-/-} \times$ Ig$\mu^{-/-}$ mice, there is an increase of circulating colon-associated self Ags as compared to TCR-$\alpha^{+/-}$, Ig$\mu^{-/-}$, and TCR-$\alpha^{-/-}$ mice, and the transfer of Ig into TCR-$\alpha^{-/-} \times$ Ig$\mu^{-/-}$ 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-$\alpha^{-/-} \times$ Ig$\mu^{-/-}$ 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-$\alpha^{-/-} \times$ Ig$\mu^{-/-}$ 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-$\alpha^{-/-}$ 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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