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Staphylococcal Enterotoxin B Stimulates Expansion of Autoreactive T Cells That Induce Apoptosis in Intestinal Epithelial Cells: Regulation of Autoreactive Responses by IL-10

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T cell responses to self Ags and normal microbial flora are carefully regulated to prevent autoreactivity. Because IL-10-deficient mice develop colitis, and this response is triggered by luminal flora, we investigated whether IL-10 regulates the ability of microbial Ags to induce autoreactive T cells that could contribute to intestinal inflammation. T cells from wild-type mice were primed with staphylococcal enterotoxin B (SEB) in vitro, which induced an autoreactive proliferative response to syngeneic feeder cells. The cells were predominately CD3+ and CD4+. T cells from IL-10-deficient mice were constitutively autoreactive, and SEB priming enhanced this further. The autoreactive, proliferative response of T cells from wild-type mice was suppressed by IL-10 in the primary or secondary culture, and this effect was inhibited by neutralizing Abs to the IL-10R. To confirm that an autoreactive repertoire was expanded after SEB priming, we used CBA/J mice (Mls-1a) in which autoreactive T cells recognizing the endogenous viral superantigen are depleted (Vβ6, 7, 8.1 TCR-bearing cells). However, SEB rescued these autoreactive T cell repertoires. Adding anti-MHC class II Ab blocked the autoreactive response. SEB-primed splenic or colonic T cells also induced apoptosis in syngeneic intestinal epithelial cells that was blocked significantly by IL-10. Thus, microbial Ags have the potential to abrogate self tolerance by stimulating autoreactive T cells that become cytolytic to target cells. IL-10 plays a protective role in maintaining self tolerance after microbial stimulation by preventing the activation of T cells that contribute to epithelial cell damage. The Journal of Immunology, 2000, 164: 2994–3001.

The intestinal immune system is charged with the task of maintaining immunological tolerance to the host’s tissue as well as to bacterial and dietary Ags persistently encountered in the lumen. One mechanism by which oral tolerance ameliorates autoimmune disease is through the production of Th2-type cytokines (IL-4, IL-10, TGF-β) by CD4+ T cells, which, in turn, inhibit activation of Th1 cells that produce IFN-γ and mediate cell-mediated immunity (1). Because the relative proportions of Th1 and Th2 cells have such an impact on the host response, the factors controlling T cell differentiation are of interest.

IL-10 is a potent suppressor of macrophage function and leads to an inhibition of Th1-type cytokine production from T cells (2). This immunoregulatory effect is associated with inhibiting the expression of class II MHC (3), reducing the production of proinflammatory cytokines such as IL-1, IL-6, and TNF-α (4), and down-regulating the expression of costimulatory molecules such as B7 (5) or ICAM-1 (6) on macrophages. IL-10 also plays a role in the maintenance of anergy in T cells activated by a primary mixed lymphocyte reaction (7). The failure to develop tolerance to otherwise benign Ags that persist in the lumen may contribute to chronic inflammatory bowel disease, such as Crohn’s disease or ulcerative colitis, in humans (8). In fact, tolerance against autologous intestinal flora is broken in lamina propria lymphocytes isolated from active lesions of inflammatory bowel disease patients (9) or in mononuclear cells from spleen or intestine from mice with experimental colitis (10, 11). Interestingly, tolerance toward autologous flora was restored by treatment with IL-10 or Abs to IL-12 in the murine model (10).

Recent advances in genetic engineering have revealed several experimental models of colitis. For example, spontaneous colitis develops in mice in which there is a disruption of genes encoding IL-2 (12), TCR β-chain (13), TGF-β (14), or IL-10 (15). Moreover, the intestinal flora that is present in wild-type mice without colitis is sufficient to trigger enteric disease in the animals with these genetic disruptions (16–19). The role for flora as a trigger of colitis was also established in SCID mice reconstituted with CD45R+B+CD4+ lymphocytes (20). Furthermore, disease in these animals was prevented when they were treated with exogenous IL-10 (21) or the transfer of T cells that express high levels of IL-10 (22, 23). Because colitis is also induced after the transfer of CD4+ T cells from IL-10-deficient mice to recombinase-activating gene-2-deficient mouse recipients exposed to a normal flora (24), it is reasonable to suggest that IL-10 plays a key role in controlling the excessive immune responses to luminal flora.

The present study investigated the hypothesis that potentially destructive autoreactive responses can be induced by microbial Ags and that these responses are inhibited by IL-10. The results

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suggest that highly immunogenic stimuli, such as staphylococcal enterotoxin B (SEB), are capable of inducing autoreactive T cells that express a repertoire that recognizes self Ags, proliferate in response to syngeneic cells, and are capable of injuring syngeneic cells through cell-mediated immunity. In addition, these responses are regulated by IL-10. The implications for the roles of these responses in immune-mediated disease of the intestine are discussed.

Materials and Methods

Mice

IL-10−/−/129/SvEv mice (H-2b, I-Eb) were provided by DNAX Research Institute (Palo Alto, CA) and were bred in the Animal Resource Center of the University of Texas Medical Branch (Galveston, TX). Wild-type 129/SvEv mice were purchased from Taconic Farms (Germantown, NY) and were bred under the same conditions. C57BL/10 IL-10−/− mice (H-2d, I-Ab, I-Eb) and wild-type controls, CBA/J mice (H-2b, Mls-1a), as well as C3H/HeJ mice were purchased from The Jackson Laboratory (Bar Harbor, ME). The animal research committee of the University of Texas Medical Branch approved all procedures used in these studies.

Cytokines and Abs

Recombinant murine IL-10 was provided by Schering-Plough (Kenilworth, NJ). Hybridomas producing anti-mouse CD8 (53-6.72, TIB-105) or anti-mouse I-Aa (10.2.16, TIB-93) were purchased from American Type Culture Collection (Manassas, VA) and grown according to the instructions provided. Supernatants were used as a source of Ab. Anti-mouse I-Eb, I-Ab Ab (MS/114) were produced from ascites and were concentrated using ammonium sulfate sedimentation by Dr. Victor Reyes (University of Texas Medical Branch). Anti-IL-10R Ab (1B1.2) was provided by Dr. K. W. Moore (DNAX, Palo Alto, CA). The activity of rIL-10 and the neutralizing activity of this Ab were confirmed as previously described (25) using the IL-10-dependent mouse mast cell line (MC/9, American Type Culture Collection CRKL 8306), in which 0.1 μg/ml Ab inhibited the proliferation of 2 × 105 MC/9 cells in the presence of 1 ng/ml mouse rIL-10 and 5 pg/ml mouse rIL-3 (data not shown). Isotype controls for mouse IgG (ChromPure Mouse IgG) and rat IgG (ChromPure Rat IgG) were purchased from Jackson ImmunoResearch Laboratories (West Grove, PA). PE- or FITC-conjugated anti-mouse CD3, CD4, or CD8 was purchased from Caltag (Burlingame, CA). FITC-conjugated anti-mouse Vβ3, Vβ6, Vβ7, Vβ8.1, Vβ8.2, biotin-conjugated anti-mouse TCR-αβ, PE-conjugated anti-mouse CD8, biotinylated-human IL-10 (26), and isotype controls for the corresponding Abs were obtained from PharMingen (San Diego, CA). Streptavidin-PE was purchased from Sigma (St. Louis, MO).

Cell isolation

Single-cell preparations were made by mincing spleen and lysing RBC in ammonium chloride (0.15 M), potassium carbonate (1 mM), and EDTA (0.1 mM) buffer (pH 7.3) lysing buffer; using standard techniques (27). Colonic mucosal T cells were isolated using a modification of a previously described procedure (28). Briefly, large intestines from five C3H/HeJ mice were isolated and flushed, lymphoid nodules were removed, and tissue was opened longitudinally in HBSS (Life Technologies, Grand Island, NY). Tissue was cut into 2- to 5-mm segments and stirred at 37°C for 45 min in DMEM containing 50 U/ml of type VIII collagenase (Sigma), 0.5 mg/ml of dispase grade II (Roche, Indianapolis, IN), 0.01% gentamicin (Life Technologies), and 50 mM of l-glutamine. After collecting the cell suspension in the supernatant, the enzymatic treatment was repeated again. Pooled supernatants were filtered through nylon wool, and enriched lymphocytes were centrifuged through a discontinuous 44/70% Percoll (Pharmacia, Alameda, CA) gradient for 18 min at 800 × g at 4°C. Cells at the interface between the 70 and 44% layers were collected and washed. Approximately 95% of the lymphocytes were viable, as measured by trypan blue exclusion.

T cell stimulation

T cells prepared from spleen or colon (2.5 × 106 cells/ml) were stimulated with 10 μg/ml SEB (Sigma) in RPMI 1640 (Life Technologies) containing 10% FCS (HyClone, Logan, UT) as a primary stimulation. After 72 h of incubation the cells were washed three times to remove the residual SEB and resuspended in 10 μg/ml SEB in RPMI 1640. After 4 days of resting, cells were washed once, and 2 × 105 viable responder cells were cultured with 2.5 × 105 syngeneic feeder cells in 96-well flat-bottom plates. Feeder cells were prepared by incubating fresh splenocytes with 100 μg/ml of mitomycin C (Sigma) at 37°C for 30 min and washing three times. In some experiments recombinant mouse IL-10 and/or anti-IL-10R Ab (1B1.2) was added in either the priming or secondary stimulation. T cells were also activated with anti-CD-3 or PMA (5 ng/ml) and ionomycin (500 ng/ml; Sigma) as previously described (29) to compare their autoreactivity with that of SEB-primed cells. To detect proliferative responses, responder cells were incubated for 72 h, and [3H]thymidine (1 μCi/well; ICN, Irvine, CA) was added for the last 16 h. The cells were harvested using an automatic cell harvester (Skatron, Sterling, VA), and [3H]thymidine incorporation was determined by liquid scintillation counting using a Liquid Scintillator (Beckman, Fullerton, CA). All experiments were performed in triplicate.

Purification of T cells

In some experiments MHC class II+ cells and/or CD8+ cells were depleted to obtain highly enriched CD4+ T cells (30). Spleen cells or mesenteric lymph node cells (1 × 106) were incubated in DMEM containing 10% FCS with MS/114 (anti-MHC class II) and/or 53-6.72 (anti-CD8) Abs for 30 min on ice. After being washed three times, cells were incubated at 4°C with sheep anti-rat IgG Ab-coated magnetic beads (Dynabeads M-450; Dynal, Oslo, Norway) in DMEM at one bead per cell for 20 min while rotating. Magnetic bead-binding cells were removed using a magnetic separator (PerSeptive Biosystems, Framingham, MA). Flow cytometric analysis always revealed the residual MHC class II+ or CD8+ cells to be <1%. The recovered cells were resuspended in RPMI and used for culture.

Flow cytometry of surface Ag expression

One million cells were incubated in PBS-0.1% azide containing fluorescence- or biotin-conjugated Abs on ice for 30 min. When using biotin-conjugated Abs, cells were washed once and incubated with streptavidin-PE for 20 min on ice. After being washed three times, cells were fixed in 1% paraformaldehyde and analyzed by FACSScan (Becton Dickinson, San Jose, CA).

Cytokine assay

The IL-2 concentration of culture supernatant was detected by bioassay using the CTL-2/2 hybridoma (27). Cells (5000/50 μl medium) were incubated with 50 μl of samples or standards (recombinant human IL-2, Genzyme, Cambridge, MA) in triplicate for 24 h. [3H]Thymidine (1 μCi/well) was added during the last 8 h of incubation, and [3H]Thymidine incorporation was detected as described above. Specific IL-2 activity (units per milliliter) was calculated according to the standard curve of the identical assay.

Cytotoxicity assay

The cytotoxic activity of SEB-primed effector cells (splenocytes or colonic mucosal lymphocytes) was detected by the JAM assay (31) using Mode-K cells (duodenal epithelial cell line from C3H/HeJ origin (32)) as target cells. Effector cells from C3H/HeJ mice were stimulated with 10 μg/ml of SEB for 18 h, washed three times, and used as effector cells. In some experiments cells were pretreated with recombinant mouse IL-10 and/or anti-IL-10R Ab (1B1.2) for 2 h before addition of SEB. Mode-K cells were labeled with 10 μCi/ml of [3H]thymidine for 4 h, washed three times, and used as target cells. Labeled Mode-K cells (1 × 105) were added to each well of a 96-well flat-bottom plate and incubated with increasing numbers of effector cells up to a 40:1 E:T cell ratio. After 24 h cultures were treated with trypsin/EDTA for 3 min and harvested onto a glass-fiber filter. The amount of tritium, which was retained only in the live cells, was counted by beta counter. Data are expressed as the percent survival and are calculated by dividing the counts per minute of samples with that of Mode-K cells only (average of 18 wells). In some experiments, supernatant from SEB-primed responder cells was checked for cytotoxicity. Culture inserts 0.4 mm pore size; Transwell, Corning Costar, Cambridge, MA) were also used between effector and target cells.

Statistical analysis

Paired Student’s t test (two tailed) was performed to compare the mean values of two corresponding datasets.

Results

Induction of autoreactive T cells by SEB

To determine whether activation of splenic T cells by a microbial Ag could induce autoreactive responses, splenocytes from IL-10
KO (C57BL/10) or wild-type mice were primed with SEB in vitro (Fig. 1). The primary proliferative response to 10 μg/ml SEB of splenocytes from IL-10 KO mice was comparable to that of wild-type splenocytes. SEB-primed splenocytes from C57BL/10 mice showed a significant proliferative response after secondary culture with mitomycin C-treated syngeneic feeder cells. The magnitude of the secondary response in IL-10-deficient mice of the same strain was comparable to that of wild-type mice. In contrast, medium-primed splenocytes from wild-type mice did not respond to the secondary stimulation with the syngeneic feeders, whereas those from IL-10-deficient mice underwent a significant proliferation in response to the syngeneic feeders, albeit less than that observed after priming with SEB. Interestingly, 67.6 ± 5.2 U of IL-2 were produced in the supernatant of SEB-primed splenocytes from IL-10-deficient mice, which was higher than that in wild-type (46.1 ± 3.1). Adding recombinant mouse IL-10 (10–100 ng/ml) to the culture suppressed the IL-2 production of both IL-10 KO and wild-type splenocytes, whereas the constitutive proliferative response observed in IL-10 KO mice was not affected. These data indicate that priming with SEB induced autoreactive proliferative responses, and IL-10-deficient cells tend to be autoreactive without priming (Fig. 1).

**IL-10 inhibits the autologous response in both induction and effector phases**

We examined the possibility that IL-10 regulates the induction of the autoreactive response by SEB in several strains of mice. Splenocytes from three different strains of mice were primed with SEB in the presence or the absence of recombinant mouse IL-10, and secondary autoreactive responses to the syngeneic feeder cells were detected. Although the magnitude of the secondary response varied among strains, IL-10 in the primary culture significantly inhibited the autoreactive response in the 129 and CBA/J mice (Fig. 2A). We also examined whether IL-10 inhibited the autoreactive response of SEB-primed cells (Fig. 2B). Adding IL-10 to the secondary culture of SEB-primed cells suppressed the autoreactive response in all strains. This inhibition was confirmed to be IL-10 specific, since the effect of IL-10 was completely abrogated by addition of anti-IL-10R Ab, but not by isotype control, in C57BL/10 and 129/SvEv strain. Interestingly, this Ab was not effective in the CBA/J strain, suggesting heterogeneity of the structure of IL-10R in CBA/J mice.

To confirm that IL-10 was mediating as specific effect through the IL-10R, responder cells were assayed for binding of IL-10 by flow cytometry. Resting or SEB-stimulated T cells from spleen or colon expressed IL-10R, as evidenced by IL-10 binding (Fig. 2C), or mRNA, detected by RT-PCR (data not shown). Binding of IL-10 did not change with stimulation.

**Polyclonal stimuli induce autoreactivity**

As SEB, which stimulates about 20% of T cells, induced autoreactivity, we examined the hypothesis that polyclonal stimuli in general have a potential to induce autoreactivity. Splenocytes from CBA/J mice were primed with SEB as well as anti-CD3 or PMA plus ionomycin, and secondary responses to the feeder cells were detected. All these polyclonal stimuli induced the secondary autoreactive responses, and these responses were inhibited by IL-10 in the secondary cultures (Fig. 3). Ag-specific stimulation with OVA did not induce the secondary response (data not shown).

**SEB expands T cell populations expressing an autoreactive repertoire in Mls-1a+ mice**

To determine the expansion of autoreactive T cells, we employed the model involving endogenous viral superantigen in mice. Mice that have the integration of Mtv-7 genome derived from mouse mammary tumor virus are designated Mls-1a+ (33, 34). Mls-1a mice, such as CBA/J, have only a marginal number of T cells bearing TCR Vβ6,-7, and -8.1 chains, because these populations are depleted in the thymus due to the endogenous viral superantigen Mls-1a (33, 35). Expansion of these T cell populations directly indicates the expansion of autoreactive T cells in Mls-1a mice. SEB-primed autoreactive T cell lines from CBA/J splenocytes were established by growing cells with feeder cells in medium containing IL-2. After several weeks of culture, TCR Vβ-chain usage was determined by flow cytometry analysis. More than 95% of the cells expressed CD4 and TCR α/β (P. Ernst et al., unpublished observations). Although freshly isolated splenocytes from CBA/J mice contained only a marginal number of Vβ7+ T cells (2% of CD3+ cells), this population increased to 12% in the T cell lines. T cells recognizing the endogenous viral superantigen and bearing the Vβ6 TCR were also expanded in the T cell lines (Fig. 4). Splenocytes from C3H/HeJ mice (Mls-1b) also had more Vβ6+ or Vβ7+ T cells after expansion in response to SEB. Vβ8.1+ T cells are depleted in Mls-1b strains; however, we detected 9% of Vβ8 cells in the freshly isolated splenocytes from both CBA/J and C3H/HeJ strains, because the Ab also recognizes Vβ8.2+ cells. Expansion of this population was also observed in the T cell lines. In other experiments anti-Mls-1a Ab (33) partially inhibited the secondary response of SEB-primed CBA/J cells, suggesting that part of the autoreactive response was directed toward the Mls-1a Ag itself (data not shown). These data provide direct evidence that priming with superantigen abrogated self tolerance and expanded autoreactive T cells in vitro.

**Autoreactive T cell responses are mediated by CD4+ T cell responses**

Because Th cells are capable of transferring disease in several models of colitis, the role of CD4+ Th cells in the secondary autoreactive response was determined. CD8-depleted cells showed...
a comparable level of secondary response as the splenocyte preparation (Fig. 5A). Moreover, addition of anti-MHC class II Ab to the secondary culture, but not an isotype control Ab, significantly blocked the autoreactive response in all three strains examined (Fig. 5B). These observations suggest that CD4+ T cells mediated the autoreactive response. Adding IL-10 in the primary or secondary culture of CD8-depleted cells suppressed the secondary response, indicating that the regulatory effect of IL-10 was independent of the presence of CD8+ T cells (Fig. 5A).

**Priming conditions for autoreactive T cells stimulate cell-mediated immunity to epithelial targets**

To address the possibility that autoreactive T cells may be able to contribute to epithelial cell damage and inflammation in the digestive tract, Mode-K cells were used as a target for cell-mediated immunity. Splenocytes from C3H/HeJ mice (syngeneic to Mode-K) were stimulated with SEB for 18 h and checked for a cytotoxic effect by JAM assay. Fig. 6A shows that SEB-stimulated splenocytes as well as CD8-depleted cells mediated cytotoxic activity against the Mode-K target cells. Supernatant from SEB-stimulated splenocytes did not kill the target (data not shown). Furthermore, the cytotoxic effect was abolished when a culture insert was used to separate effector and target cells, indicating that this cytotoxicity was facilitated by direct contact with the target cells. Because most of the cytotoxic activity resided in the CD4+ T cells, colonic mucosal T cells, which include intestinal intraepithelial lymphocytes (IEL) and lamina propria lymphocytes (LPL) that contain increased proportions of CD4+ T cells treated with IL-10 compared with those in untreated cells.

![FIGURE 2. IL-10 inhibits autoreactive response induced by SEB. A. Splenocytes were primed with SEB in the presence or the absence of IL-10 (100 ng/ml), and secondary autoreactive responses were detected. Adding IL-10 to the primary culture suppressed the secondary autoreactive responses. Data are expressed as the stimulation index, i.e., counts per minute from culture with feeders/counts per minute from culture with medium only. The ranges of counts per minute for the different strains were: C57BL/10, 3,000–4,500; 129/SvEv, 11,000–12,000; and CBA/J, 8,000–16,000. **, p < 0.05 for inhibition by cells treated with IL-10 compared with that in untreated cells. B. SEB-primed splenocytes were given a secondary stimulation with syngeneic feeders. IL-10 (100 ng/ml), anti-mouse IL-10R Ab (1B1.2), or rat IgG (isotype control) was added as indicated. Adding IL-10 to the secondary culture suppressed the autoreactive response. The responses were restored by anti-IL-10R Ab, but not by isotype controls, in C57BL/10 and 129/SvEv strains. This Ab, however, was not effective in the CBA/J strain. **, p < 0.05 for inhibition of the responses in cells treated with IL-10 compared with those in untreated cells. Data are expressed as the mean ± SEM for six observations from two independent experiments. C. Mononuclear cells isolated from the spleen or colon of C3H/HeJ mice were incubated in medium or primed with SEB as described in Materials and Methods. Subsequently, cells were probed for the surface expression of CD3 and IL-10 receptors. After gating on the lymphocyte population, two-color analysis was performed to detect IL-10 binding by CD3-positive T cells. This figure shows that both splenic and colonic T cells expressed IL-10R, but the level did not change after stimulation.**
notype detected by FACS analysis. Depletion of CD8+ T cells, because this reaction was inhibited by anti-MHC class II Abs. These findings support the idea that the autoreactive cells are CD4+ T cell feeder cells, because this reaction was inhibited by anti-MHC class II Abs (36, 37). As shown in Fig. 6A, SEB-activated colonic mucosal T cells kill the syngeneic epithelial cell target. Moreover, this cytotoxicity was inhibited by the addition of IL-10, and the Ab recognizing the IL-10R (1B1.2) blocked the inhibition.

Discussion

Microbial Ags are quite capable of activating intestinal T cells in humans (9) and mice (10, 11). Recently, SEB has been shown to cross the intestinal epithelial cell barrier (38) and stimulate T cells (39–41), leading to the activation of T cells producing IFN-γ and IL-2 (42). Based on the results presented in this report, any stimulation that activates a relatively large subset of T cells generates autoreactive T cells that proliferate when re-exposed to self Ags expressing class II MHC. Moreover, these autoreactive T cells are cytolytic to epithelial cells in vitro.

The autoreactive cells induced by SEB included CD4+ T cells, because most of the primed cells contained CD4+ TCRαβ+ phenotypes detected by FACS analysis. Depletion of CD8+ cells before priming did not attenuate the autoreactivity. Moreover, this autoreactivity was mediated by MHC class II molecules on the feeder cells, because this reaction was inhibited by anti-MHC class II Abs. These findings support the idea that the autoreactive cells are CD4+ T cells. As activated T cells may express only modest amounts of class II MHC, the T cells are not capable of long term expansion in the absence of feeder cells. Moreover, because SEB induces the expression of PsL on T cells, it is likely that, in time, activation will lead to a negative selection of the T cells as they die due to Fas/FasL interactions (43). The magnitude of the proliferative responses varied among strains of mice, but they were present in all strains tested. In addition, autoreactive T cells could be generated from spleen or colon, which supports our previous preliminary studies suggesting that these responses occur in gut-associated lymphoid tissue, including the mesenteric lymph nodes or intestinal Peyer’s patches (44).

CD4+ autoreactive T cells prepared from the spleens of IL-10-deficient mice displayed a level of spontaneous autoreactivity without prior priming with SEB. Despite this intrinsic autoreactivity, stimulation with SEB did not increase the response to levels greater than those observed in control mice. This somewhat unanticipated finding may reflect a ceiling for autoreactivity that is

![FIGURE 4.](http://www.jimmunol.org/)

T cells bearing autoreactive TCR repertoires are expanded after priming with SEB. SEB-primed autoreactive T cell lines from CBA/J (Mls-1a; □) or C3H/HeJ (Mls-1b; □) mice were established and maintained in growth medium supplemented with IL-2 and feeder cells. Cells were double stained with CD3 and TCR Vβ-specific Abs and were analyzed by FACS. The percentage of specific Vβ-chain usage in CD3+ cells was calculated. Freshly isolated splenic T cells (left end of each line) recognizing SEB (Vβ3, Vβ8) in both strains of mice as well as Vβ7 and Vβ6 TCR+ cells in C3H/HeJ mice underwent expansion (right end of each line). Vβ7 and Vβ6 TCR+ cells in freshly isolated splenic T cells from CBA/J were depleted due to the expression of the endogenous viral superantigen. However, the percentage of T cells bearing these Vβ-chains increased to 14 and 6%, respectively, after stimulation. This observation provided direct evidence that priming with SEB expanded autoreactive T cell populations.

![FIGURE 5.](http://www.jimmunol.org/)

Secondary autoreactive responses were mediated by CD4+ T cells. A. CD8+ T cells were depleted from CBA/J splenocytes using anti-CD8 Abs (53-672) followed by sheep anti-rat IgG-coated magnet beads, yielding highly enriched preparations of CD4+ T cells. Total spleen cells and equal numbers of CD8-depleted cells were primed with SEB followed by resting and secondary culture with feeders. CD8-depleted cells showed comparable secondary response as the total spleen cells, suggesting that CD4+ T cells were autoreactive. Adding IL-10 to the primary or secondary culture of CD8-depleted cells inhibited the secondary autoreactive response. *, p < 0.05 for SEB-primed cells exposed to feeders vs medium. **, p < 0.05 for inhibition of the responses in cells treated with IL-10 compared with those in untreated cells. B, SEB-primed splenocytes from C57BL/10 (I-Ak, I-Ek), 129/SvEv (I-Ak, I-Ek), or CBA/J (I-Ak, I-Ek) mice were cultured with syngeneic feeder cells in the presence of anti-MHC class II Abs (M5/114 for I-Ab or I-Ek, 10.2–16 for I-Ak) or isotype controls. Proliferative responses were measured after 3 days. Secondary responses were significantly inhibited by anti-MHC class II Abs (**, p < 0.05 for inhibition by anti-class II Abs vs the isotype control). Data are expressed as the mean ± SEM for nine observations from three independent experiments.
defined by the number of autoreactive precursors and the magnitude of their expansion. It should be remembered that normally, autoreactive cells are almost nonexistent, so any increase may be quite significant. This is supported by the observation that adoptive transfer of these T cells into T cell-deficient mice can induce colitis in the recipient (24). These observations suggest that IL-10 confers a protective effect against the activation of autoreactive T cells induced by normal flora. Although there was no difference between IL-10-deficient and wild-type mice in the level of secondary autoreactive responses after priming with SEB, exogenous IL-10 suppressed the autoreactivity, including both proliferative and cytolytic effects, when delivered during the priming or secondary stimulation. IL-10R were expressed on both T cells and cells of other lineages, so the inhibitory activity of IL-10 may have been mediated at different levels.

The specificity of the effect of IL-10 was confirmed by blocking the IL-10R with anti-IL-10R Ab. This Ab was effective in the

129/SvEv and C57BL/10 strains, but not in the CBA/J strain. Interestingly, IL-10 inhibited IL-2 production in the C57BL/10 and 129SvEv strains after stimulation with SEB, but not in CBA/J mice (data not shown). Heterogeneity of cell surface molecule among mouse strains has been reported for CD8 (45), IL-4R (46), or FasL (47), which affects not only the recognition by mAbs but also the function of the molecules. Our observations suggest that there is heterogeneity in the structure and function of the IL-10R; however, further experimentation is necessary to precisely evaluate the basis for the inability of the Ab to inhibit IL-10 responses in CBA/J mice.

Regulation of autoreactivity in the priming phase by IL-10 is consistent with the finding that supplementation of IL-10 prevented the development of colitis in IL-10-deficient mice (48) or in the CD45RB<sup>high</sup> transfer model (22, 23). IL-10 also suppressed autoreactivity in the secondary culture. Endogenous IL-10 produced from feeder cells seemed to be insufficient to inhibit the autoreactivity, because secondary proliferative responses were similar whether the feeder cells were obtained from IL-10-deficient or control mice.

Although proliferation in response to syngeneic feeders provides evidence that SEB can abrogate tolerance, it is possible that the proliferative response was not directed to self Ags, but, rather, to residual SEB or some factor in the growth medium. The possibility that residual SEB in the primed cells was restimulating the responder cells was discounted for several reasons: 1) the responder cells contained only a marginal number of APC, which might carry over SEB into the secondary stimulation, because SEB induced only a limited proliferation to the responder cells without adding fresh feeder cells; 2) responder cells could be maintained for up to 12 wk after repeated washing and restimulation with syngeneic feeder cells, suggesting that little residual SEB was carried over; and 3) the autologous response was significantly inhibited by IL-10, but IL-10 did not inhibit the proliferative response caused by SEB. The fact that T cells from IL-10-deficient mice were capable of modest proliferative responses upon stimulation with syngeneic Ags without priming suggests that the responses were indeed autoreactive. Although it is possible that xenogenic proteins in the growth medium, such as those provided by FCS, may be presented by feeders, small amounts of proliferation were still observed when cultures were maintained in mouse serum (data not shown). However, the most direct evidence for the expansion of autoreactive cells was the expression of autoreactive T cell repertoires.

To address the affect of SEB on T cells that bear autoreactive repertoires, the well-characterized murine model of TCR repertoire to endogenous viral superantigens was employed. Mls-1 has been known to act as a minor lymphocyte-stimulating Ag. Subsequently, the Mls-1 Ag was shown to be encoded by the mtn-7 gene, which is a murine mammary tumor viral gene that has integrated into their genome of some strains of mice (34). These mice are referred to as Mls-1<sup>+</sup> mice, while mice lacking this Ag are Mls-1<sup>-</sup>. It is known that specific T cells in Mls-1<sup>+</sup> mice bearing specific TCR β-chains (i.e., V<sup>ß6</sup> and V<sup>ß7</sup>) proliferate in response to this Ag. Because the Mls-1 Ag is expressed on host cells, these T cells are deleted in Mls-1<sup>-</sup> mice. The results described in this report show that SEB could expand the number of T cells from Mls-1<sup>+</sup> mice that bear this autoreactive repertoire. Although most of these autoreactive T cells are deleted during T cell development, this process is imperfect, leading to the escape of potentially autoreactive clones. The intestinal intraepithelial lymphocyte compartment is particularly enriched with T cells expressing these autoreactive TCRs (49, 50). Because Mls-1 Ag
can be expressed on intestinal epithelial cells (51), autoreactivity of T cells recognizing this Ag must be prevented by a state of anergy. However, cytokines, such as IL-2, can abrogate anergy in autoreactive IEL (29, 52). Zinkernagel and colleagues (53) have also shown that transgenic T cells expressing a receptor recognizing a transgenic viral peptide that is expressed on pancreatic β islet cells remain anergic. However, upon stimulation with virus infection, the anergic T cells react to viral Ag and induce diabetes. Thus, presentation of a microbial Ag in an immunogenic fashion can induce autoreactivity that can contribute to disease. This may be due to antigenic mimicry between the pathogen and the host Ag or the activation of autoreactive T cells due to the burst of cytokines, such as IL-2, that are released during the infection.

Having established that autoreactive T cells can expand after exposure to SEB, we showed that these cells were capable of inducing damage to host cells. As described in Fig. 6, T cells from the spleen or colon that were activated with SEB could induce death and DNA degradation in Mod-K cells. Because SEB induces the expression of FasL on T cells (43), it is possible that apoptosis of epithelial cells was mediated by interactions between Fas and FasL. This model is supported by the results of other studies documenting the expression of Fas on epithelial cells and FasL by intestinal T cells in graft-versus-host disease (54, 55). Previous reports have suggested that changes in epithelial cell structure and function can occur after exposing tissue to SEB (41, 56). The ability of SEB to induce cytokines (42) as well as apoptosis in epithelial cells following exposure to SEB may contribute to the T cell-mediated pathology.

As described above, colitis in many animal models is dependent on normal flora, and T cells recognizing luminal microbial Ags become activated during colitis. Moreover, adoptive transfer of T cells from colitic mice will lead to colitis in T cell-deficient recipients. These studies suggest that microbial triggers activate a deleterious T cell response; however, a role for autoreactive T cells has not been implicated previously. In this report we show that a microbial superantigen, SEB, is capable of inducing autoreactive T cells in systemic and colonic tissue and that this response is present in experimental colitis and restored by treatment with interleukin-10 or antibodies to interleukin-12. Eur. J. Immunol. 26:934.


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