IL-6–dependent spontaneous proliferation is required for the induction of colitogenic IL-17–producing CD8⁺ T cells

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We propose a novel role for interleukin (IL) 6 in inducing rapid spontaneous proliferation (SP) of naive CD8⁺ T cells, which is a crucial step in the differentiation of colitogenic CD8⁺ T cells. Homeostasis of T cells is regulated by two distinct modes of cell proliferation: major histocompatibility complex/antigen–driven rapid SP and IL-7/IL-15–dependent slow homeostatic proliferation. Using our novel model of CD8⁺ T cell–dependent colitis, we found that SP of naive CD8⁺ T cells is essential for inducing pathogenic cytokine–producing effector T cells. The rapid SP was predominantly induced in mesenteric lymph nodes (LNs) but not in peripheral LNs under the influence of intestinal flora and IL-6. Indeed, this SP was markedly inhibited by treatment with anti–IL-6 receptor monoclonal antibody (IL-6R mAb) or antibiotic-induced flora depletion, but not by anti–IL-7R mAb and/or in IL-15–deficient conditions. Concomitantly with the inhibition of SP, anti–IL-6R mAb significantly inhibited the induction of CD8⁺ T cell–dependent autoimmune colitis. Notably, the transfer of naive CD8⁺ T cells derived from IL-17⁻/⁻ mice did not induce autoimmune colitis. Thus, we conclude that IL-6 signaling is crucial for SP under lymphopenic conditions, which subsequently caused severe IL-17–producing CD8⁺ T cell–mediated autoimmune colitis. We suggest that anti–IL-6R mAb may become a promising strategy for the therapy of colitis.
Although studies have addressed the involvement of CD4+ T cells in animal models of IBD (18), there is little information about the possible contribution of CD8+ T cells to the pathogenesis. In this report, we established a novel Tc17 cell–dependent IBD model and initially demonstrated that IL-6–dependent SP of naive CD8+ T cells was essential for the expansion of colitogenic Tc17 cells. Thus, we propose that the control of SP of naive CD8+ T cells by anti–IL-6R mAb will become a novel strategy for developing the therapy for autoimmune diseases.

RESULTS AND DISCUSSION
Adoptive transfer of naive CD8+ T cells causes severe autoimmune colitis
Severe colitis was induced by a single adoptive transfer of CD44+CD62L+ naive CD8+ T cells into syngeneic RAG2-/- mice. Transfer of naive CD8+ T cells or CD4+ T cells caused severe weight loss and thickening of the large intestinal wall compared with untreated control mice within 6 wk (Fig. 1, A and B). Hematoxylin-eosin (HE) staining of colon tissue sections revealed large numbers of infiltrating cells, in addition to hemorrhagic necrosis, neangiogenesis, and depletion of goblet cells, in the CD8+ T cell–transfered mice, which were also assigned histological scores (Fig. 1 C). In addition, elevation of quantitative markers for enteropathy such as serum KC, serum amyloid A (SAA), and ICAM-1 (Fig. 1 D and not depicted) (19, 20) suggested that some inflammatory responses would be induced in the colon. However, it still remained unclear whether the CD8+ T cell–induced pathogenesis was specific to the large intestine. To make this point clear, we examined the induction of pathogenic inflammatory responses in various organs. The pathogenic responses were observed only in the large intestine, and not in other organs such as the liver, kidney, lung, heart, and brain (unpublished data). These pathological events strongly indicated that naive CD8+ T cells could be a critical population for the induction of autoimmune colitis under lymphopenic conditions.

A kinetics study revealed that IFN-γ, IL-17, and TNF-α were produced by CD8+ T cells, and these cytokine-producing cells preferentially expanded at the mesenteric LNs (mLNs) rather than the peripheral LNs (pLNs) within 1 wk of cell transfer (Fig. 1 E and not depicted). Concomitantly with the increase of cytokine–producing CD8+ T cells in mLNs, CD8+ T cells were infiltrated into colon tissues accompanied with inflammatory CD11b+ cells (Fig. 1 F). Almost all of the CD11b+ cells coexpressed F4/80 macrophage markers (unpublished data). These findings suggest that transferred naive CD8+ T cells would initially be stimulated in the mLNs and then migrate into the peripheral tissues at subsequent pathogenic stages. Thus, it appeared that the rapid expansion of naive CD8+ T cells to generate cytokine–producing effector CD8+ T cells would be closely related with the pathogenesis of autoimmune colitis.

Kinetics of SP essential for inducing pathogenic effector memory CD8+ T cells in mLNs
Recent studies have demonstrated that adoptive transfer of naive T cells into immunodeficient mice resulted in two distinct types of proliferation (1). HP is driven by low-affinity self-MHC/peptide ligands and homeostatic cytokines such as IL-7 and IL-15 (21, 22), whereas SP exhibits rapid and massive proliferation induced by antigen recognition via the TCR (23). After transfer of CFSE–labeled naive CD8+ T cells into RAG2-/- mice, dilution of the cellular fluorescence intensity was analyzed as an index of the cell proliferation at early stages. From a kinetics study (Fig. 2 A), we found that SP of naive CD8+ T cells occurred preferentially in the mLNs rather than in the pLNs within 5 d after the adoptive transfer. This rapid SP was detected neither in pLNs or mLNs at day 3. SP was not detectable in pLNs even at 7 d after CD8+ T cell transfer. At day 7, the numbers of CD8+ T cells collected from the mLNs were much larger than those from the pLNs (Fig. S1, available at http://www.jem.org/cgi/content/full/jem.20071133/DC1). CD8+ T cells expanded by SP in mLNs but not pLNs, and rapidly acquired more characteristics of a “memory-type” phenotype, such as CD44 markers and a cytokine–producing ability, in the early stages (Fig. 2 B and not depicted). Therefore, these cytokine–producing effector memory cells might be critical for the initiation of colitis. SP in mLNs was not detectable in the following two cases: (a) when CD8+ T cells derived from OT-1–TCR transgenic mice were transferred into RAG2-/- mice (Fig. 2 C), and (b) when RAG2-/- mice were treated with antibiotics to deplete intestinal flora (Fig. 2 D). Moreover, it was demonstrated that SP was different from HP of CD8+ T cells judging from the evidence that naive CD8+ T cells transferred into OT-1/RAG2-/- mice exhibited SP in mLNs, whereas HP was completely blocked in both pLNs and mLNs by endogenously existing OT-1 T cells (Fig. 2 E). Thus, these data clarified that SP, which was triggered with antibiotic–sensitive flora in mLNs via TCR, was distinct from HP induced under the influence of IL-7 and/or IL-15. Alternatively, SP was not an HP–related end stage of T cell proliferation. A previous report had indicated that adoptive transfer of naive CD8+ T cells into lymphopenic hosts elicited no pathology, including colitis (24). This might be because of the strain differences between C57BL/6 and BALB/c mice, which have genetically different predispositions in controlling their susceptibility to immune diseases (25), including our established colitis model (unpublished data).

Critical role of IL–6 for SP while inducing pathogenic effector T cells
In contrast to HP (26), it remains unclear which factors are involved in SP of naive CD8+ T cells in addition to antigen stimulation. We found that mRNA expression levels of IL–6 and TGF–β were significantly higher in the mLNs compared with pLNs (Fig. 3 A). To evaluate the role for IL–6 and TGF–β, as well as the homeostatic cytokines IL–7 and IL–15, in SP and HP, we performed the following experiments: (a) CD8+ T cells were transferred into RAG2-/- mice treated with anti–IL-7R mAb; (b) IL-15-/- mouse–derived CD8+ T cells were transferred into IL-15/RAG2 double–knockout mice; (c) IL-15-/- CD8+ T cells were transferred into IL-15/RAG2 double–knockout mice treated with anti–IL-7R mAb;
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IL-7 and/or IL-15 were involved in HP (Fig. 3B). It was also demonstrated that TGF-β/H9252 had no effect on HP (Fig. S2, available at http://www.jem.org/cgi/content/full/jem.20071133/DC1).

Unexpectedly, however, SP was completely blocked by the (d) CD8+ T cells were transferred into RAG2−/− mice treated with anti-IL-6R mAb; and (e) CD8+ T cells were transferred into RAG2−/− mice treated with anti-TGF-β mAb. Neither IL-7, IL-15, nor TGF-β contributed to SP, whereas IL-7 and/or IL-15 were involved in HP (Fig. 3B). It was also demonstrated that TGF-β had no effect on HP (Fig. S2, available at http://www.jem.org/cgi/content/full/jem.20071133/DC1).

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Figure 2. Kinetics of SP essential for inducing pathogenic effector memory T cells in mLNs. 5 × 10^5 cells naive CD8+ T cells from C57BL/6 mice were labeled with CFSE and intravenously injected into RAG2−/− mice. (A) Proliferation of CD8+ T cells was monitored by flow cytometry 3, 5, and 7 d after the injection. The representative FACS profiles are indicated in the figure. (B) CD44 expression and cytokine production levels of CD8+ T cells from pLNs and mLNs in the adoptively transferred mice were examined by staining with mAbs against CD44, IFN-γ, or IL-17 at day 5. The representative FACS profiles are shown. Percentages are indicated. (C) CFSE-labeled CD8+ T cells from OT-1 TCR transgenic mice were intravenously injected into RAG2−/− mice. Proliferation of the CD8+ T cells in mLNs was analyzed at day 7. The representative FACS profiles are indicated. (D) CFSE-labeled CD8+ T cells from C57BL/6 mice were intravenously injected into untreated and antibiotic-treated RAG2−/− mice. Proliferation of the CD8+ T cells in the mLNs of the untreated and antibiotic-treated mice was analyzed by FACS. (E) CFSE-labeled CD8+ T cells from C57BL/6-background Ly5.1 mice were intravenously injected into RAG2−/− or OT-1/RAG2−/− mice. Proliferation of the CD8+ T cells in the pLNs or mLNs of OT-1/RAG2−/− mice was analyzed by FACS.
administration of anti–IL-6R mAb (Fig. 3 C). In contrast, HP was not affected by the anti–IL-6R mAb at all (Fig. 3 C). As shown in Fig. 3 D, both IFN-γ and IL-17A production were markedly inhibited by treatment with anti–IL-6R mAb (Fig. 3 D). From these findings, IL-6 signaling, but not IL-7, IL-15, or TGF-β, was considered to be critical for SP in the present model. It has been demonstrated that IL-6 was closely related to the survival of T cells (27, 28). However, there was no significant difference in CD8+ T cell apoptosis between control and anti–IL-6R mAb–treated mice (Fig. S3).

As described in Fig. 2, we clearly demonstrated that HP and SP of CD8+ T cells were totally distinct in T cell homeostasis, and that SP was not the end stage of HP. We also demonstrated that SP appeared to be triggered with antibiotic-sensitive flora antigen via TCR, consistent with a previous report (29). Therefore, at present, we consider that anti–IL-6R mAb does not affect the apoptosis of HP-related T cells but instead inhibits the SP-related generation of flora-triggered pathogenic CD8+ T cells, which is accelerated in an IL-6–dependent manner. Indeed, IL-6 was demonstrated to promote TCR-mediated T cell proliferation directly (unpublished data).

**Anti–IL-6R mAb inhibits CD8+ T cell–dependent autoimmune colitis**

In vivo injection of anti–IL-6R mAb markedly suppressed the weight loss and abnormal thickening of the large intestine wall, which are associated with CD8+ T cell–induced colitis (Fig. 4 A). HE staining revealed that blockade of IL-6 signaling significantly suppressed cell infiltration into the large intestine (Fig. 4, B and C). Although the mRNA levels of various inflammatory cytokines, including TNF-α, IL-1β, IFN-γ, and IL-17, were up-regulated in the tissues from the colitis
mice, the expression levels in the anti–IL-6R mAb–treated mice were almost the same as those in the untreated normal mice (Fig. 4 D). This evidence demonstrated that IL-6 signaling was not only involved in the up-regulation of inflammatory cytokine levels in the colon tissues but was also related to the pathogenesis of CD8+ T cell–dependent colitis.

Requirement of Tc17 cells in autoimmune colitis
To define the role of IL-17 (11–14) on the pathogenesis of the CD8+ T cell–mediated autoimmune colitis, we performed adoptive transfer of naive CD8+ T cells derived from IL-17−/− mice. Deficiency of IL-17 in CD8+ T cells caused a remarkable suppression of pathology, including severe weight loss, thickening of the large intestinal wall, and colitogenic responses (Fig. 5, A–C). Thus, we propose that Tc17 cells would be critical effectors in the pathogenesis of autoimmune diseases.

Figure 4. Anti–IL-6R mAb inhibits CD8+ T cell–dependent autoimmune colitis. 5 × 10^5 naive CD8+ T cells from C57BL/6 mice were intravenously injected into RAG2−/− mice. (A) Body weight of untreated (n = 4), naive CD8+ T cell–transferred (n = 3), or naive CD8+ T cell–transferred/anti–IL-6R mAb–treated (n = 3) mice were monitored for 9 wk. Body weight percentages against the respective preinjection values were calculated every week. The means and SDs are indicated. (B) Colon tissues were obtained from untreated, control, or anti–IL-6R mAb–treated mice 9 wk after the cell transfer. Morphology of the representative colon tissues is shown. Bar, 1 cm. (C) HE staining was performed on the colon tissues from the untreated, control, or anti–IL-6R mAb–treated mice. The representative histological micrographs are shown at two different magnifications. Means and SDs of the colitis score are indicated in the bar graph. Bars: (left) 1 mm; (right) 200 μm. (D) TNF-α, IL-1β, IL-17A, and IFN-γ mRNA expression levels in the colon tissues from the untreated, control, and anti–IL-6R mAb–treated mice were determined by real-time PCR. The means and SDs are indicated.
demonstrated that several cytokines, including IL-1β, TNF-α, IFN-γ, and IL-17, were involved in the induction of inflammation during colitis (30). However, little has been investigated about the physiological role of IL-17 production by CD8+ T cells in contrast to CD4+ T cells. Our results initially demonstrated a pivotal role of Tc17 cells in the pathogenesis of autoimmune colitis.

Adoptive transfer of naive CD8+ T cells underwent two distinct types of proliferation, SP and HP. We found that IL-6 signaling was critical for only SP, which was triggered by recognition of antibiotic-sensitive microbe antigens in the gut. The important role of antibiotic-sensitive flora–induced SP was demonstrated from the finding that intestinal flora depletion by antibiotics treatment caused the prevention of weight loss and colitis (unpublished data). Therefore, IL-17 was required for the development of the CD8+ T cell–dependent colitis, but IL-17 might synergistically act with IFN-γ to induce the final colitogenic responses, including migration of effector CD8+ T cells and other inflammatory cells into the colon tissues. We are now investigating the critical role of both IL-17 and IFN-γ in colitis. As shown in Fig. 3 D, IL-17 and IFN-γ double-positive CD8+ T cells are rapidly proliferated in mLNs. Therefore, there is a good possibility that SP-induced IL-17 and IFN-γ double-producing Tc17 cells play a critical role in autoimmune colitis.

We have established a novel model of colitis by transfer of naive CD8+ T cells into syngeneic RAG2−/− mice that mimicked the symptoms of IBD, such as weight loss and excess inflammatory cytokine production. So far, it has been demonstrated that several cytokines, including IL-1β, TNF-α, IFN-γ, and IL-17, were involved in the induction of inflammation during colitis (30). However, little has been investigated about the physiological role of IL-17 production by CD8+ T cells in contrast to CD4+ T cells. Our results initially demonstrated a pivotal role of Tc17 cells in the pathogenesis of autoimmune colitis.

Adoptive transfer of naive CD8+ T cells underwent two distinct types of proliferation, SP and HP. We found that IL-6 signaling was critical for only SP, which was triggered by recognition of antibiotic-sensitive microbe antigens in the gut. The important role of antibiotic-sensitive flora–induced SP was demonstrated from the finding that intestinal flora depletion by antibiotics treatment caused the prevention of weight loss and colitis (unpublished data), in parallel with the blocking of SP, which is essential for pathogenic CD8+ T cell induction (Fig. 2 D). Moreover, IL-6–dependent SP was demonstrated to be critical for the differentiation of the final effector cells, Tc17 cells involved in autoimmune colitis.
This report initially proposes a novel mechanism for maintaining the homeostasis of CD8+ T cells, which is triggered under lymphopenic conditions in an IL-6–dependent manner. We designated this phenomenon as SP of CD8+ T cells, which also can be dissociated from IL-6–independent SP of CD4+ T cells (1, 18). Our results also indicate a novel application of anti–IL-6R mAb for the immunotherapy of Tc17 cell–mediated colitis, and propose that the IL-6–dependent SP could be a promising therapeutic target in autoimmune diseases such as IBD. We are now investigating the general role of SP in other CD8+ T cell–mediated immune diseases.

MATERIALS AND METHODS

Mice. WT C57BL/6 mice were purchased from Charles River Laboratories. C57BL/6-background RAG2-/- mice were provided by M. Ito (Central Institute for Experimental Animals, Kanagawa, Japan). OT-1/TcR transgenic mice were purchased from Taconic. OT-1/RAG2-/- mice were recovered from the spleens and pLNs of the mouse strains from Taconic. OT-1/RAG2-/- mice were bred in specific pathogen-free conditions according to the guidelines for animal care of our institute. All mice were used in accordance with the guidance of an institutional committee at Hokkaido University.

Preparation and adoptive transfer of naive CD8+ T cells. Total lymphoid cells were recovered from the spleens and pLNs of the mouse strains indicated in the figures and resuspended in RPMI 1640 medium (Sigma-Aldrich) containing 10% FCS (BD Biosciences) plus penicillin and streptomycin (both from Meiji Seika). Erythrocytes were eliminated with 0.155 M NaN3, and 0.5% Triton X-100, pH 7.5), the cells were stained with PE-Cy5–TCRβ mAb (H57-597; BD Biosciences) and allophycocyanin–IFN-γ mAb (XM1G1; ebioscience). Fluorescence signals from the cells were acquired by FACSCalibur and analyzed with CellQuest software (both from BD Biosciences). Data were collected with logarithmic amplification.

ELISA. Serum KC and SAA levels were determined by the mouse KC ELISA kit (R&D Systems) and the mouse SAA ELISA kit (Invitrogen), respectively. Assays were performed according to the manufacturers’ instructions.

Immunohistochemical staining. Colon sections collected from mice were treated with optimum cutting temperature compound (Sakura Finetechnical Co.) and immediately frozen in liquid nitrogen. 4-μm sections of the colon tissues, fixed with acetone at 4°C for 10 min followed by blocking for 30 min, were incubated with anti–mouse CD8α mAb (53-6.7; BD Biosciences) on 96-well flat-bottom plates for 6 h and treated with Brefeldin A for the final 2 h. The stimulated cells were stained with PE-Cy5–TCRβ mAb (H57-597; BD Biosciences) and fixed with 4% paraformaldehyde phosphate buffer solution (Wako). After treatment with permeabilizing solution (50 mmol/liter NaCl, 5 mmol/liter EDTA, 0.02% NaN3, and 0.5% Triton X-100, pH 7.5), the cells were stained with PE–IL-17 mAb (TC11-18H10.1; BD Biosciences) and allophycocyanin–IFN-γ mAb (XM1G1; ebioscience). Flow cytometric analysis. For analysis of cell-surface molecules, the cell samples indicated in the figures were stained with fluorescent dye-conjugated mAbs against the selected markers on ice. Cell proliferation was evaluated by monitoring the fluorescence intensity of the cells prestained with CSFE (Invitrogen), according to the manufacturer’s instructions. For detection of cyttoplasmic cytokines, the indicated cells were stimulated with anti–CD3ε mAb (145-2C11; BD Biosciences) on 96-well flat-bottom plates for 6 h and treated with Brefeldin A for the final 2 h. The stimulated cells were stained with PE-Cy5–TCRβ mAb (H57-597; BD Biosciences) and fixed with 4% paraformaldehyde phosphate buffer solution (Wako). After treatment with permeabilizing solution (50 mmol/liter NaCl, 5 mmol/liter EDTA, 0.02% NaN3, and 0.5% Triton X-100, pH 7.5), the cells were stained with PE–IL-17 mAb (TC11-18H10.1; BD Biosciences) and allophycocyanin–IFN-γ mAb (XM1G1; ebioscience). Fluorescence signals from the cells were acquired by FACSCalibur and analyzed with CellQuest software (both from BD Biosciences). Data were collected with logarithmic amplification.

Antibiotics treatment. Drinking water containing 1 g/liter ampicillin sodium (Meiji Seika), 1 g/liter neomycin sulfate (Nacalai Tesque), 500 mg/liter vancomycin (Nacalai Tesque), and 1 g/liter metronidazole (Nacalai Tesque) was provided for RAG2-/- mice at 4 wk before cell transfer. 5 × 108 naive CD8+ T cells stained with CSFE were intravenously injected into the antibiotic-treated mice, and the proliferation was determined by flow cytometry.

Real-time PCR. Total RNA was extracted from LNs or colon tissues of the mice indicated in the figures using the Isogen RNA extraction kit (Nippongene), according to the manufacturer’s instructions. cDNA was prepared from the total RNA with RT (Invitrogen), oligo dT, and dNTP mixture (Promega). The indicated gene cDNAs were specifically amplified using a thermal cycler system (ABI PRISM 7700 Sequence; Applied Biosystems) and using the corresponding primer pairs for mouse IL-7, IL-15, IL-6, TGF-β, IL-1β, TNF-α, IFN-γ, IL-17A, and β-actin. The sequences used were as follows: IL-7, sense 5'-GAGGTGGGTGTTAGTCTGATGACT-3', antisense 5'-GGGTCCTGTGCTGACCTC-3', (probe) 5'-TTTGAGGTCAACAACCCACAGG-3'; TNF-α, sense 5'-GGATCACCTCACCACCAAGGC-3', antisense 5'-CAAGCTCAAGGAGGTCCAGAAGAA-3', and (probe) 5'-CCACCTTGGACACATGGCTC-3'; IFN-γ, sense 5'-GAGATGCCATCATCGTGTGCTCATACA-3', and (probe) 5'-AGAAATCCCGCTGACGACACTT-3'; IL-15, sense 5'-ATGCTGATACCCGCTCTTCCT-3', and (probe) 5'-CCACCTCTGGACACATGGCTC-3'; IFN-γ, sense 5'-AGGTCCTTGGACACATGGCTC-3', and (probe) 5'-CCACCTCTGGACACATGGCTC-3'.

Flow cytometric analysis. For analysis of cell-surface molecules, the cell samples indicated in the figures were stained with fluorescent dye-conjugated mAbs against the selected markers on ice. Cell proliferation was evaluated by monitoring the fluorescence intensity of the cells prestained with CSFE (Invitrogen), according to the manufacturer’s instructions. For detection of cyttoplasmic cytokines, the indicated cells were stimulated with anti–CD3ε mAb (145-2C11; BD Biosciences) on 96-well flat-bottom plates for 6 h and treated with Brefeldin A for the final 2 h. The stimulated cells were stained with PE-Cy5–TCRβ mAb (H57-597; BD Biosciences) and fixed with 4% paraformaldehyde phosphate buffer solution (Wako). After treatment with permeabilizing solution (50 mmol/liter NaCl, 5 mmol/liter EDTA, 0.02% NaN3, and 0.5% Triton X-100, pH 7.5), the cells were stained with PE–IL-17 mAb (TC11-18H10.1; BD Biosciences) and allophycocyanin–IFN-γ mAb (XM1G1; ebioscience). Fluorescence signals from the cells were acquired by FACSCalibur and analyzed with CellQuest software (both from BD Biosciences). Data were collected with logarithmic amplification.
(probe) 5′-TGTCCTGATGCGATGCTGTACCA-3′. Samples were normalized to the housekeeping gene β-actin according to the ΔΔCT method: ΔΔCT = ΔCTsample – ΔCNTarget. Percentages against the WT control sample were calculated for each sample.

Statistics. All experiments were repeated at least three times. Mean values and SDs were calculated for data from three independent experiments and are shown in the figures. Statistical significance was calculated using the Student’s t test. P < 0.05 was considered significant in the present experiments, as indicated with an asterisk.

Online supplemental material. Fig. S1 demonstrates that injection of anti-IL-6R mAb blocks proliferation of adoptively transferred naive CD8+ T cells in mLNs. Fig. S2 shows that TGF-β signaling is not required for SP of CD8+ T cells. Fig. S3 demonstrates that anti-IL-6R mAb treatment does not induce apoptosis of naive CD8+ T cells. Online supplemental material is available at http://www.jem.org/cgi/content/full/jem.20071133/DC1.

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REFERENCES

1. Min, B., H. Yamane, J. Hu-li, and W.E. Paul. 2005. Spontaneous and homeostatic proliferation of CD4 T cells are regulated by different mechanisms. J. Immunol. 174:6039–6044.

2. Hue, S., P. Ahern, S. Buonocore, M.C. Kullberg, D.J. Cua, and K.J. Maloy. 2006. Interleukin-23 drives innate and T cell-mediated intestinal inflammation. J. Exp. Med. 203:2473–2483.

3. King, C., A. Ilie, K. Koelsch, and N. Sarvetnick. 2004. Homeostatic expansion of T cells during immune insufficiency generates autoreactivity. Cell. 117:265–277.

4. Murphy, K.M., and S.L. Reiner. 2002. The lineage decisions of helper T cells. Nat. Rev. Immunol. 2:933–944.

5. Takaoka, A., Y. Tanaka, T. Tsuji, T. Jinushi, A. Hoshino, Y. Asakura, Y. Mita, K. Watanabe, S. Nakaake, Y. Togashi, et al. 2001. A critical role for IL-10 in the induction of IL-17 producing T cells to mediate autoimmune encephalomyelitis. J. Exp. Med. 203:1685–1691.

6. Nakae, S., S. Saio, R. Horai, K. Sudo, S. Mori, and Y. Iwakura. 2003. IL-17 production from activated T cells is required for spontaneous development of destructive arthritis in mice deficient in IL-1 receptor antagonist. Proc. Natl. Acad. Sci. USA. 100:5986–5990.

7. Yen, D., J. Cheung, H. Scheerens, F. Poulet, T. McClanahan, B. McKenzie, M.A. Kleinmschek, A. Owenyong, J. Matsson, W. Blumenschein, et al. 2006. IL-23 is essential for T cell-mediated colitis and promotes inflammation via IL-17 and IL-6. J. Clin. Invest. 116:1310–1316.

8. Kullberg, M.C., D. Jankovic, C.G. Feng, S. Hue, P.L. Gorelick, B.S. McKenzie, D.J. Cua, F. Powere, A.W. Cheever, K.J. Maloy, and A. Sher. 2006. IL-23 plays a key role in Helicobacter hepaticus-induced T cell-dependent colitis. J. Exp. Med. 203:2485–2494.

9. Bouma, G., C. Foucau, J.L. Fuss, and R.S. Blumberg. 2002. The immunology of mucosal models of inflammation. Annu. Rev. Immunol. 20:495–549.

10. Schutz, B., K. Chamoto, H. Kitamura, and T. Nishimura. 2007. Blocking of IL-6 antagonist 3 protects against DSS-induced acute colitis. Inflamm. Bowel Dis. 13:856–864.

11. Blumberg, H., H. Dinh, E.S. Trueblood, J. Prezinos, D. Kugler, N. Weng, S.T. Kanaly, J.E. Towne, C.B. Willis, M.K. Kuechle, et al. 2007. Opposing activities of two novel members of the IL-1 ligand family regulate skin inflammation. J. Exp. Med. 204:2603–2614.

12. Harrington, L.E., R.D. Hatton, P.R. Mangan, H. Turner, T.L. Murphy, K. Chamoto, H. Kitamura, and T. Nishimura. 2007. A crucial role for interleukin (IL)-1 in the induction of IL-17–producing T cells that mediate autoimmune encephalomyelitis. J. Exp. Med. 203:1685–1691.

13. Sutton, C., C. Brereton, B. Keogh, K.H. Mills, and E.C. Lavelle. 2006. A crucial role for interleukin (IL)-1 in the induction of IL-17–producing T cells that mediate autoimmune encephalomyelitis. J. Exp. Med. 203:1685–1691.