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IL-7 Is Essential for the Development and the Persistence of Chronic Colitis

Teruji Totsuka, Takanori Kanai, Yasuhiro Nemoto, Shin Makita, Ryuichi Okamoto, Kiichiro Tsuchiya, and Mamoru Watanabe

Although IL-7 has recently emerged as a key cytokine involved in controlling the homeostatic turnover and the survival of peripheral resting memory CD4+ T cells, its potential to be sustained pathogenic CD4+ T cells in chronic immune diseases, such as inflammatory bowel diseases, remains unclear. In this study, we demonstrate that IL-7 is essential for the development and the persistence of chronic colitis induced by adoptive transfer of normal CD4+CD45RB<sub>high</sub> T cells or colitogenic lamina propia (LP) CD4+ memory T cells into immunodeficient IL-7<sup+/−</sup> × RAG-1<sup−/−</sup> and IL-7<sup−/−</sup> × RAG-1<sup−/−</sup> mice. Although IL-7<sup+/−</sup> × RAG-1<sup−/−</sup> recipients transferred with CD4+CD45RB<sub>high</sub> splenocytes developed massive inflammation of the large intestinal mucosa concurrent with massive expansion of Th1 cells, IL-7<sup−/−</sup> × RAG-1<sup−/−</sup> recipients did not. Furthermore, IL-7<sup−/−</sup> × RAG-1<sup−/−</sup>, but not IL-7<sup+/−</sup> × RAG-1<sup−/−</sup>, mice transferred with LP CD4<sup+</sup>CD44<sub>high</sub>CD62L<sub>−</sub>IL-7R<sub>α</sub> effector-memory T cells (TEM) isolated from colitic CD4<sup+</sup>CD45RB<sub>high</sub>-transferred mice did not develop colitis. Although rapid proliferation of transferred colitogenic LP CD4<sup+</sup> TEM cells was observed in the in IL-7<sup−/−</sup> × RAG-1<sup−/−</sup>-mice to a similar extent of those in IL-7<sup+/−</sup> × RAG-1<sup−/−</sup> mice, Bcl-2 expression was significantly down-modulated in the transferred CD4<sup+</sup> TEM cells in IL-7<sup−/−</sup> × RAG-1<sup−/−</sup> mice compared with those in IL-7<sup+/−</sup> × RAG-1<sup−/−</sup> mice. Taken together, IL-7 is essential for the development and the persistence of chronic colitis as a critical survival factor for colitogenic CD4<sup+</sup> TEM cells, suggesting that therapeutic approaches targeting IL-7/IL-7R signaling pathway may be feasible in the treatment of inflammatory bowel diseases. The Journal of Immunology, 2007, 178: 4737–4748.

Inflammatory bowel diseases (IBD) is caused by excessive and tissue-damaging chronic inflammatory responses in the gut wall and commonly take persistent, disabling courses. In some patients, disease progresses steadily, while in others, relapses alter with remissions. According to present understanding, disease is caused and controlled by pathogenic effector and memory CD4<sup+</sup> T cells, which are accumulated in their target tissues and thus determine activity and clinical character. However, the nature of pathogenic memory CD4<sup+</sup> T cells over time and the correlation between effector and memory CD4<sup+</sup> T cells in chronic colitis in the presence of commensal bacteria remains largely unknown.

IL-7 is a stromal cell-derived cytokine that is secreted by fetal liver cells, stromal cells in the bone marrow and thymus, and other epithelial cells. Recently, IL-7 has emerged as a key cytokine involved in controlling the survival of peripheral resting CD4<sup+</sup> T cells, including naive and memory cells and their homeostatic turnover. The effect of IL-7 on T cells is controlled by the expression of the specific receptor for IL-7, the state of differentiation of the T cell, the available concentration of the cytokine, and whether there is concomitant TCR signaling. IL-7R consists of the α-chain (CD127) and the common cytokine receptor γ-chain, which is shared by the common γ-chain family cytokines (IL-2, IL-4, IL-9, IL-15, and IL-21). In contrast to the role of IL-7 in naive and memory CD4<sup+</sup> T cells in the resting state, the pathological role of IL-7 in chronic immune-mediated diseases, such as autoimmune diseases and IBD, remains largely unclear.

In this study, we attempt to clarify the link between the colitogenic CD4<sup+</sup> T cells and IL-7 more extensively in terms of pathogenesis of chronic colitis using adoptive transfer system. The adoptive transfer of CD4<sup+</sup>CD45RB<sub>high</sub> T cells into syngeneic immunodeficient mice, such as SCID mice and RAG-1 or RAG-2-deficient mice, induces human IBD-like diseases. The key factors for the development of colitis are an expanding CD4<sup+</sup> T cell subset in lymphopenic condition, an intact gut flora of the host, and various cytokines.

lymphoid organ aberrations and the lack of lymphocytes (3), we, in this study, used IL-7\(\times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \time...
5–10 days. At the indicated time points, isolated splenic cells were stained with allophycocyanin-labeled anti-CD4 mAb (L3T4), PerCP-labeled anti-CD3 mAb (2C11), and PE-labeled Annexin V (MBL), and the intensity of CFSE and annexin V was determined after gating on CD3+ CD4+ T cells. To assess the role of commensal bacteria in cell division, a group of mice were treated with a set of four antibiotics, i.e., ampicillin (1 g/L; Sigma-Aldrich), vancomycin (500 mg/L; Abbott Laboratories), neomycin sulfate (1 g/L; Pharmacia), and metronidazole (1 g/L; Sidmack) in drinking water 2 wk before beginning the adoptive transfer and during the course of experiment based on a variation of the commensal depletion protocol of Fagarasan et al. (18). At the indicated time points, isolated spleen cells were stained with APC-labeled anti-CD4 mAb and PerCP-labeled anti-CD3 mAb, and the intensity of CFSE was determined after gating on CD3+ CD4+ T cells.

Statistical analysis
The results were expressed as the mean ± SD. Groups of data were compared by Mann-Whitney U test. Differences were considered to be statistically significant when p < 0.05.
RESULTS

Lack of colitis in IL-7−/− × RAG-1−/− mice transferred with CD4+CD45RBhigh T cells

We used a chronic colitis model induced by an adoptive transfer of splenic CD4+CD45RBhigh T cells from normal C57BL/6 mice into IL-7−/− × RAG-1−/− and IL-7+/+ × RAG-1−/− littermate recipients to assess a requirement of IL-7 for the development of chronic colitis (Fig. 1A). Consistent with previous reports (14), the control IL-7+/+ × RAG-1−/− mice manifested progressive weight loss from 3 wk after transfer (data not shown) and clinical symptoms of colitis as shown as clinical scores estimated by diarrhea with increased mucus in the stool, anorectal prolapse, hunched posture, and weight loss by 8 wk after transfer (Fig. 1B). In contrast, the IL-7−/− × RAG-1−/− mice transferred with
CD4⁺CD45RB<sup>high</sup> T cells showed no clinical signs of colitis and weight loss throughout the entire observation periods (Fig. 1B). At 8 wk after transfer, the colon from the transferred IL-7<sup>7/7</sup> × RAG-1<sup>−/−</sup> mice, but not that from the transferred IL-7<sup>−/−</sup> × RAG-1<sup>−/−</sup> mice, was enlarged and had a greatly thickened wall (Fig. 1C). In addition, the enlargement of the spleen and mesenteric lymph nodes was also present in the control IL-7<sup>7/7</sup> × RAG-1<sup>−/−</sup> mice transferred with CD4⁺CD45RB<sup>high</sup> T cells as compared with the IL-7<sup>−/−</sup> × RAG-1<sup>−/−</sup> mice transferred with CD4⁺CD45RB<sup>high</sup> T cells (Fig. 1C). Totally, the assessment of colitis by clinical scores showed a clear difference between the transferred IL-7<sup>7/7</sup> × RAG-1<sup>−/−</sup> mice and the transferred IL-7<sup>−/−</sup> × RAG-1<sup>−/−</sup> mice (Fig. 1B). Histological examination showed prominent epithelial hyperplasia with glandular elongation with a massive infiltration of mononuclear cells in the LP of the colon from the transferred IL-7<sup>7/7</sup> × RAG-1<sup>−/−</sup> mice (Fig. 1D). In contrast, the glandular elongation was mostly abrogated, and only a few mononuclear cells were observed in the LP of the colon from the transferred IL-7<sup>−/−</sup> × RAG-1<sup>−/−</sup> mice (Fig. 1D). This difference was also confirmed by histological scoring of multiple colon sections, which was 0.16 ± 0.04 in the transferred IL-7<sup>7/7</sup> × RAG-1<sup>−/−</sup> mice vs 5.16 ± 0.19 in the transferred IL-7<sup>−/−</sup> × RAG-1<sup>−/−</sup> mice (p < 0.01) (Fig. 1E). We confirmed that the IL-7<sup>−/−</sup> × RAG-1<sup>−/−</sup> mice transferred with CD4⁺CD45RB<sup>high</sup> T cells did not develop intestinal inflammation cells until 20 wk of observation after transfer (data not shown).

A further quantitative evaluation of CD4<sup>+</sup> T cell infiltration was made by isolating LP and splenic CD4<sup>+</sup> T cells. Only a few CD4<sup>+</sup> T cells were recovered from the colonic tissue of the transferred IL-7<sup>−/−</sup> × RAG-1<sup>−/−</sup> mice as compared with the transferred IL-7<sup>7/7</sup> × RAG-1<sup>−/−</sup> mice (Fig. 1F). The number of CD4<sup>+</sup> cells recovered from the colon of the transferred IL-7<sup>7/7</sup> × RAG-1<sup>−/−</sup> mice (150.5 ± 3.23 × 10<sup>5</sup>) far exceeded the number of originally injected cells (3.0 ± 10<sup>5</sup>), indicating an extensive T cell proliferation and survival in the inflamed colon, which was mostly abrogated in the transferred IL-7<sup>−/−</sup> × RAG-1<sup>−/−</sup> mice (0.60 ± 0.65 × 10<sup>5</sup>) (Fig. 1F). Furthermore, the number of CD4<sup>+</sup> splenocytes from the transferred IL-7<sup>−/−</sup> × RAG-1<sup>−/−</sup> mice was also significantly increased as comparable to that from the transferred IL-7<sup>7/7</sup> × RAG-1<sup>−/−</sup> recipients (Fig. 1G). We also examined the cytokine production by LP CD4<sup>+</sup> T cells from the transferred IL-7<sup>7/7</sup> × RAG-1<sup>−/−</sup> mice and the transferred IL-7<sup>−/−</sup> × RAG-1<sup>−/−</sup> mice. As shown in Fig. 1H, LP CD4<sup>+</sup> T cells from the transferred IL-7<sup>−/−</sup> × RAG-1<sup>−/−</sup> mice produced significantly less IFN-γ, IL-2, and TNF-α as compared with those from the transferred IL-7<sup>7/7</sup> × RAG-1<sup>−/−</sup> mice upon in vitro stimulation. Importantly, flow cytometry analysis revealed that the LP CD4<sup>+</sup> T cells isolated from both IL-7<sup>7/7</sup> × RAG-1<sup>−/−</sup> and IL-7<sup>−/−</sup> × RAG-1<sup>−/−</sup> recipients 8 wk after an adoptive transfer of CD4<sup>+</sup>CD45RB<sup>high</sup> T cells were CD4<sup>+</sup>CD62L<sup>−/−</sup>CD69<sup>−/−</sup>CD45RB<sup>−/−</sup>IL-7Ra<sup>−/−</sup> (Fig. 1I), indicating that the transferred CD4<sup>+</sup>CD45RB<sup>high</sup> T cells could differentiate to activated T<sub>EM</sub> cells even in the absence of IL-7. These results suggest that the lack of IL-7 prevented the development of colitis primarily by inhibiting the expansion and/or survival of colitigenic CD4<sup>+</sup> T<sub>EM</sub> cells in the colon and secondarily by inhibiting the development of the Th1 T<sub>EM</sub> cells.

**Kinetics of development of colitis and IL-7Ra expression**

When the immune system is first challenged by exposure to Ags, naive CD4<sup>+</sup> T cells become activated and undergo many rounds of expansion as they differentiate into effector and memory CD4<sup>+</sup> T cells. Because it is believed that IL-7 is not involved in the initial expansion of the effector cells but its role in maintaining pool size may influence either the onset or maintenance of colitis, we assessed the kinetics of the development of colitis and IL-7Ra expression to determine whether IL-7 is crucial for the onset of colitis in our model (Fig. 2A). At 1 wk after an adoptive transfer of CD4<sup>+</sup>CD45RB<sup>high</sup> T cells into IL-7<sup>7/7</sup> × RAG-1<sup>−/−</sup> and IL-7<sup>−/−</sup> × RAG-1<sup>−/−</sup> mice, both mice did not develop colitis (Fig. 2B), and the average histological scores in both mice were 0 (Fig. 2C). At 2 wk after the transfer, however, the transferred IL-7<sup>7/7</sup> × RAG-1<sup>−/−</sup> recipients develop mild colitis, characterized with the infiltration of a small number of mononuclear cells in the LP, but the transferred IL-7<sup>−/−</sup> × RAG-1<sup>−/−</sup> recipients did not (Fig. 2B). The average histology scores revealed that the transferred IL-7<sup>7/7</sup> × RAG-1<sup>−/−</sup> recipients had significantly higher colitis scores as compared with the transferred IL-7<sup>−/−</sup> × RAG-1<sup>−/−</sup> recipients (Fig. 2C). Four weeks after the transfer, the difference between two groups was more apparent, as the transferred IL-7<sup>7/7</sup> × RAG-1<sup>−/−</sup> mice developed more severe colitis, but the transferred IL-7<sup>−/−</sup> × RAG-1<sup>−/−</sup> mice did not (Fig. 2B and C). To examine the effects of IL-7 on the expansion of CD4<sup>+</sup> T cells in the recipients, we compared the infiltration of CD4<sup>+</sup> T cells by determining the number of CD4<sup>+</sup> T cells in the spleen and the LP by FACs analysis. In accordance with the histological scores, the average number of LP and splenic CD4<sup>+</sup> T cells recovered from the transferred IL-7<sup>7/7</sup> × RAG-1<sup>−/−</sup> recipients were significantly increased from 2 wk after the transfer as compared with those from the transferred IL-7<sup>−/−</sup> × RAG-1<sup>−/−</sup> recipients (Fig. 2D).

To obtain a more comprehensive understanding of the development of effector and memory CD4<sup>+</sup> T cells over time in this setting, we carefully examined the cell surface expression of IL-7Ra on LP CD4<sup>+</sup> T cells obtained from the recipients at the indicated time points. As shown in Fig. 2E, the splenic CD4<sup>+</sup> T cells (donor CD4<sup>+</sup>CD45RB<sup>high</sup> T cells) before the transfer (0 wk) and the LP CD4<sup>+</sup> T cells after 1 wk of transfer highly expressed IL-7Ra, but approximately half of CD4<sup>+</sup> T cells from both the transferred IL-7<sup>7/7</sup> × RAG-1<sup>−/−</sup> and IL-7<sup>−/−</sup> × RAG-1<sup>−/−</sup> recipients had down-regulated IL-7Ra at 2 wk after transfer. However, a large portion of LP CD4<sup>+</sup> T cells from both the transferred mice at 4 wk after the transfer had regained higher expression of IL-7Ra to >80% per total CD4<sup>+</sup> T cells (Fig. 2E). These data indicate that IL-7Ra expression was regulated during T cell differentiation from...
CD4+CD45RBhigh IL-7Ra high naive T cells (0 wk before the transfer), CD4+IL-7Ra low effector T cells (1 and 2 wk after the transfer), to CD4+IL-7Rahigh memory T cells (4 wk after the transfer), suggesting that naive and memory CD4+ T cells may be more responsive to IL-7-mediated signaling.

**TCR Vβ repertoires in IL-7−/− × RAG-1−/− and IL-7−/− × RAG-1−/− mice transferred with CD4+CD45RBhigh T cells**

Analysis of the TCR repertoire of the immunodeficient SCID/Rag-1/−/− recipients in the CD4+CD45RBhigh T cell transfer model may provide an opportunity to characterize the unique T cell population present in the diseased individuals in a manner not possible in clinical studies on human patients. Furthermore, it was also possible that the adoptive transfer of CD4+CD45RBhigh T cells to IL-7−/− × RAG-1−/− recipients gains more skewed and restricted clonality of CD4+ T cells as compared with that in IL-7−/− × RAG-1−/− recipients. To assess this possibility, we next made a comparison between TCR Vβ repertoires of splenic CD4+ T cells from colitic CD4+CD45RBhigh T cell-transferred and IL-7−/− × RAG-1−/− and IL-7−/− × RAG-1−/− and normal age-matched wild-type mice. Flow cytometric analysis of these splenic CD4+ T cells using a panel of 15 anti-Vβ mAbs showed that most major
Vβ population was Vβ8.1/8.2 in all three groups, i.e., age-matched C57BL/6J and IL-7−/− × RAG-1−/− and IL-7+/+ × RAG-1−/− mice transferred with CD4+CD45RB+ T cells. Although only a Vβ4 ratio in colitic IL-7+/+ × RAG-1−/− recipients was significantly decreased as compared with that in normal C57BL/6J mice, there were no significant differences of other Vβ repertoires between these two groups (Fig. 3). In noncolitic IL-7−/− × RAG-1−/− recipients, however, the repertoires of Vβ3 and Vβ10b were significantly decreased as compared with those in normal C57BL/6J mice and colitic IL-7+/+ × RAG-1−/− recipients (Fig. 3), indicating that the lack of IL-7 suppressed the expansion of certain type of Vβ populations as compared with other repertoires.

Lack of colitis in IL-7−/− × RAG-1−/− mice transferred with colitogenic LP CD4+ T<sub>EM</sub> cells

To next assess the role of IL-7 in the persistent colitis without the impact of naive T cell priming, activation, and differentiation, we first isolated LP CD4+ T<sub>EM</sub> cells as colitogenic CD4+ T<sub>EM</sub> cells from CD4+CD45RB+ T cell-transferred colitic RAG-2−/− mice at 4–6 wk after transfer because flow cytometry analysis revealed that the colitic LP CD4+ T cells were CD44<sup>hi</sup> CD62L− IL-7Rα<sup>hi</sup> T<sub>EM</sub> cells (Fig. 1I), and we previously demonstrated that the adoptive transfer of these cells into new IL-7-competent RAG-2−/− mice induces chronic colitis (17). We then transferred these LP CD4+ T<sub>EM</sub> cells into IL-7−/− × RAG-1−/− mice (IL-7−/− × RAG-1−/−) and the IL-7−/− × RAG-1<sup>−/−</sup> mice (IL-7−/− × RAG-1<sup>+/+</sup>) to focus on the persistence of colitogenic CD4<sup>+</sup> T<sub>EM</sub> cells (Fig. 4A). IL-7−/− × RAG-1<sup>−/−</sup> recipients developed a severe colitis 4 wk after the transfer, characterized by significant weight loss, diarrhea, and higher total clinical scores (Fig. 4B) and thickening of the colonic wall with inflammation (Fig. 4C). Average histological scores characterized by transmural inflammation with high numbers of lymphocytes in the LP and submucosa, and prominent epithelial hyperplasia with loss of goblet...
cells (Fig. 4D) were 4.83 ± 0.98 in IL-7−/−→IL-7+/+ mice (Fig. 4E). In contrast, IL-7+/+→IL-7−/− recipients appeared healthy and did not exhibit any signs of colitis (Fig. 4B), with gradual increase of body weight and no apparent thickening of the colonic wall (Fig. 4C). No evident pathological changes were observed in the colon (Fig. 4D). This histological difference was also confirmed by histological scoring, which was 0 in IL-7−/−→IL-7−/− recipient mice (p < 0.005 as compared with IL-7+/+→IL-7+/+ mice) (Fig. 4E). The average recovered numbers of LP and splenic CD4+ T cells from colitic IL-7+/−→IL-7+/+ recipients were 173.7 ± 11.2 × 10^5 cells/colon (Fig. 4F) and 51.5 ± 11.3 × 10^5 cells/spleen (Fig. 4G), respectively, whereas those from IL-7+/−→IL-7−/− mice were 0.64 ± 0.7 × 10^5 cells/colon (Fig. 4F) (p < 0.01) and 0.54 ± 0.29 × 10^5 cells/spleen (Fig. 4G) (p < 0.05), respectively. As shown in Fig. 4H, LP CD4+ T cells from IL-7+/−→IL-7+/+ recipients produced significantly less IFN-γ, IL-2, and TNF-α as compared with those from IL-7+/−→IL-7+/+ mice upon in vitro stimulation (Fig. 4H). Furthermore, flow cytometry analysis showed that the LP CD4+ T cells isolated from both IL-7+/−→IL-7+/+ and IL-7+/−→IL-7−/− recipients were sustained by the phenotype of CD44<sup>high</sup>CD62L<sup>−</sup>IL-7Rα<sup>high</sup>TEM cells (Fig. 4I).

**IL-7 is essential for the survival of colitogenic CD4+ TEM cells**

To further examine the effect of IL-7 on the proliferation and the survival of the colitogenic LP CD4+ TEM cells in vivo, we used the CFSE dilution method since dilution of CFSE provided a division history of the cells, allowing us to examine cells undergoing proliferation. First, the LP CD4+ TEM cells obtained from CD4+ CD45RB<sup>high</sup>-transferred colitic mice were labeled with CFSE and adoptively transferred into IL-7+/−×RAG-1−/− or IL-7−/−×RAG-1−/− mice. Although the relatively delayed division of expanded CD4+ T cells in IL-7+/−×RAG-1−/− mice at the indicated time points, the LP CD4+ T cells had markedly divided until 10 days after transfer both in the IL-7−/−×RAG-1−/− mice and the IL-7+/−×RAG-1−/− recipients, indicating that it appears IL-7 is not essential for colitogenic CD4+ TEM cells to undergo lymphopenia-driven rapid proliferation (19). Interestingly, consistent with the markedly and significantly decreased cell numbers of splenic and LP CD4+ T cells in CD4+ CD45RB<sup>high</sup> TEM cells (Fig. 1, F and G), or colitogenic LP CD4+ T cell (Fig. 4, F and G)-transferred IL-7−/−×RAG-1−/− mice as compared with those in the paired transferred IL-7+/−×RAG-1−/− mice, CFSE− cells that have divided more than eight times (19) in IL-7−/−×RAG-1−/− mice were stained with annexin V with higher percentages at day 7 after transfer as compared with those in IL-7+/−×RAG-1−/− mice (Fig. 5A), indicating that rapidly dividing cells in IL-7+/−×RAG-1−/− mice could not survive to maintain their cell number. To further address the survival checkpoint, we next assessed whether regulation of Bcl-2 requires IL-7 at 7 days after the transfer since induction of the anti-apoptotic protein, Bcl-2, is a hallmark of responses to IL-7 (20). As expected, the splenic CD4+ T cells in the transferred IL-7+/−×RAG-1−/− mice expressed significantly less level of Bcl-2 as compared with those in the transferred IL-7+/−×RAG-1−/− mice (Fig. 5, B and C).

**Commensal bacteria-partially dependent rapid proliferation and IL-7-dependent slow proliferation**

Because we found that IL-7 is essential for the development and persistence of colitis, and it has been previously demonstrated that the presence of commensal bacteria are needed to develop and sustain chronic colitis in various models of colitis (1), we finally addressed this point in the adoptive transfer setting using IL-7−/−×RAG-1−/− and IL-7+/−×RAG-1−/− recipients treated with or without antibiotics treatment by CFSE dilution assay at 7–21 days after transfer. In this experiment, we used normal splenic CD4+ CD25− T cells, including CD4+ CD45RB<sup>high</sup> naive T cells and CD4+ CD45RB<sup>low</sup> TEM cells, but not CD4+ CD25+ regulatory T cells, rather than colitogenic LP CD4+ T cells as donor cells (Fig. 5) to assess two types of cell division, rapid (spontaneous, endogenous) proliferation and slow (homeostatic) proliferation (19, 21, 22). As shown in Fig. 6, slow proliferation in IL-7+/−×RAG-1−/− recipients at days 7–14 after transfer was observed as two to three peaks of dividing cells regardless of the antibiotic treatment, whereas none of slowly dividing cells was observed in IL-7−/−×RAG-1−/− recipients, indicating that slow proliferation is dependent on the presence of IL-7. In contrast, rapid proliferation in IL-7−/−×RAG-1−/− and IL-7+/−×RAG-1−/− recipients treated with antibiotics at days 7 and 14 after transfer was partially but not completely impaired as compared with that in both IL-7+/−×RAG-1−/− and IL-7+/−×RAG-1−/− recipients without antibiotics treatment (Fig. 6), indicating that rapid proliferation in these recipients is driven not only by the presence of commensal bacterial Ags, but also presumably by other environmental Ags, such as food and bedding, and/or self-Ags. Furthermore, although rapid-proliferating cells in IL-7−/−×RAG-1−/− recipients should
have divided over eight times (19) as is in a similar manner with IL-7<sup>+</sup>/RAG-1<sup>-/-</sup> recipients, rapid-dividing area of IL-7<sup>-/-</sup>/RAG-1<sup>-/-</sup> recipients was markedly decreased as compared with that of IL-7<sup>-/-</sup>/RAG-1<sup>-/-</sup> recipients, indicating that rapid-proliferating cells in IL-7<sup>-/-</sup>/RAG-1<sup>-/-</sup> recipients were subjected to undergo apoptosis or the rate of rapid-proliferating cells in IL-7<sup>-/-</sup>/RAG-1<sup>-/-</sup> recipients were significantly faster as undetectable as for cells divided over eight times (19) by this CSFE method.

**Sustained CD4<sup>+</sup> T cells in IL-7<sup>-/-</sup>/RAG-1<sup>-/-</sup> recipients do not have a potential to induce colitis when transferred to IL-7<sup>+</sup>/RAG-1<sup>-/-</sup> recipients**

Finally, we address a question whether sustained CD4<sup>+</sup>CD44<sup>high</sup>CD62L<sup>-</sup> effector-memory type of T cells in IL-7<sup>-/-</sup>/RAG-1<sup>-/-</sup> mice transferred with CD4<sup>+</sup>CD45RB<sup>high</sup> T cells (Fig. 1) have a potential to induce colitis if they were transferred to new IL-7-competent IL-7<sup>+</sup>/RAG-1<sup>-/-</sup> mice. Because it was very important to assess a possibility that a small but substantial number of CD4<sup>+</sup> T cells would be maintained by other factors, such as commensal bacterial Ag-driven TCR signaling and IL-15, as suggested by others (16, 23–25) in CD4<sup>+</sup>CD45RB<sup>high</sup> T cell-transferred IL-7<sup>-/-</sup>/RAG-1<sup>-/-</sup> mice, but not enough to expand to induce colitis due to the absence of IL-7, we isolated splenic CD4<sup>+</sup> T cells from colitic CD4<sup>+</sup>CD45RB<sup>high</sup> T cell-transferred colitic IL-7<sup>-/-</sup>/RAG-1<sup>-/-</sup> mice and noncolitc IL-7<sup>-/-</sup>/RAG-1<sup>-/-</sup> mice at 8 wk after transfer (Fig. 7A). We next retransferred these splenic CD4<sup>+</sup> T cells into new IL-7<sup>+</sup>/RAG-1<sup>-/-</sup> mice (Fig. 7A). Expectedly and similarly with the result from the adoptive transfer of colitic LP CD4<sup>+</sup> T cells (Fig. 4), IL-7<sup>+</sup>/RAG-1<sup>-/-</sup>
recipients transferred with splenic CD4+ T cells from colitic CD4+ CD45RBhigh T cell-transferred IL-7+/+ × RAG-1−/− (IL-7+/+ → IL-7+/+) mice developed a severe colitis until 4–6 wk after the transfer, characterized by significant weight loss, diarrhea, and higher total clinical scores (Fig. 7B) and thickening of the colonic wall with inflammation (Fig. 7, C and D). In contrast, IL-7−/− × RAG-1−/− recipient (IL-7−/− → IL-7+/+) mice appeared healthy and did not exhibit any signs of colitis until 9 wk after transfer (Fig. 7B), and no apparent thickening of the colonic wall (Fig. 7C). No evident pathological changes were observed in the colon (Fig. 7D). Average histological scores characterized by severe inflammation and epithelial hyperplasia (Fig. 7D) were 4.90 ± 0.87 in those IL-7−/− → IL-7+/+ mice in contrast to 0.42 ± 1.13 in those IL-7−/− → IL-7+/+ mice (p < 0.001) (Fig. 7E). The average recovered numbers of LP and splenic CD4+ T cells from colitic IL-7+/+ → IL-7+/+ mice were 190.8 ± 77.3 × 105 cells/colon (Fig. 7F) and 31.3 ± 18.3 × 105 cells/spleen (Fig. 7G), respectively, whereas those from noncolitic IL-7−/− → IL-7+/+ mice were 29.7 ± 39.9 × 105 cells/colon (Fig. 7F) (p < 0.005) and 6.23 ± 13.68 × 105 cells/spleen (Fig. 7G) (p < 0.05), respectively. As shown in Fig. 7H, LP splenic CD4+ T cells from noncolitic IL-7−/− → IL-7+/+ mice produced significantly less IFN-γ, IL-2, and TNF-α as compared with those from colitic IL-7+/+ → IL-7+/+ mice (Fig. 7H). Furthermore, flow cytometry analysis showed that the LP CD4+ T cells isolated from both colitic IL-7+/+ → IL-7+/+ recipients and noncolitic IL-7−/− → IL-7+/+ mice were sustained the phenotype of CD44highCD62L− TEM cells (Fig. 7H).

Discussion

A central pursuit in the field of chronic immune-mediated diseases, such as IBD, has been to identify the specific factors that are responsible for the persistence of the diseases. In this study, we demonstrated that IL-7 is essential for the development and the persistence of chronic colitis by a series of adoptive transfer of normal CD4+CD45RBhigh T cells and colitogenic LP CD4+ CD44highCD62L− TEM cells into IL-7+/+ × RAG-2−/− and IL-7−/− × RAG-2−/− recipients. Although rapidly proliferative responses of donor colitogenic LP CD4+ TEM cells was observed in IL-7−/− × RAG-2−/− recipients to a similar extent of those in recipient IL-7+/+ × RAG-2−/− mice after transfer, expression of Bcl-2 was significantly down-modulated in LP CD4+ T cells, and the number of recovered LP CD4+ T cells was markedly decreased in the IL-7−/− × RAG-2−/− recipients as compared with IL-7+/+ × RAG-2−/− recipients. These results suggest that IL-7 is critical for the persistence of chronic colitis as a survival factor for colitogenic CD4+ TEM cells rather than proliferative factor to sustain the intestinal inflammation.

We have previously shown a potential role for IL-7/IL-7R-mediated immune responses in intestinal inflammation. First, IL-7 transgenic mice developed chronic colitis that mimicked histopathological characteristics of human IBD (12). As chronic colitis developed, IL-7 transgenic mice showed significant infiltration of IL-7RhighCD4+ T cells in the colonic LP. Second, we clarified that mucosal IL-7RhighCD4+ T cells in colitic TCRα-deficient mice are the pathogenic T cells that can induce chronic colitis by the adoptive transfer of these cells into syngeneic immunodeficient RAG-2−/− mice, and the selective elimination of IL-7RhighCD4+ T cells by administrating toxin-conjugated anti-IL-7Rα mAb completely ameliorated colitis (13). Third, in vitro stimulation by IL-7 enhanced the significant proliferative responses and the survival of colitic LP CD4+, but not normal LP CD4+, T cells (26). These previous results suggest that IL-7 might be a crucial factor for the development of chronic colitis and prompted us to investigate to prove it directly using the adoptive transfer system in the completely IL-7-deficient condition. Because adult IL-7−/− mice are highly lymphopenic in the peripheral blood and lymphoid organs due to the defective lymphopoiesis (27), it was impossible to compare wild-type mice and littermate IL-7−/− mice in terms of disease susceptibility. To overcome this issue, we generated littermate IL-7−/− × RAG-1−/− and IL-7−/− × RAG-1−/− mice and used as recipients for the adoptive transfer of CD4+CD45RBhigh T cells or the colitogenic LP CD4+ CD44highCD62L− TEM cells into these mice. Importantly, because IL-7 is not detected in lymphocytes, the present adoptive transfer system could provide a clue whether IL-7 is essential for the development and the persistence of chronic colitis.

In this study, we found that IL-7−/− × RAG-1−/− transferred with CD4+CD45RBhigh T cells never developed chronic colitis 8 wk after the transfer (Fig. 1) and even 20 wk after the transfer (data not shown). The results showed that IL-7 is essentially needed to develop colitis in terms of disease susceptibility in this model, but it was still unclear whether IL-7 is critical for the initiation of T cell activation or the persistence of colitogenic CD4+ TEM cells. To clarify this issue in detail, we next conducted another adoptive transfer experiment using colitogenic LP CD4+ CD44highCD62L− TEM cells into IL-7+/+ × RAG-1−/− mice and IL-7−/− × RAG-1−/− mice without the impact of T cell priming, activation, and differentiation of naive CD4+ T cells. Again, we found that IL-7−/− × RAG-1−/− transferred with the colitogenic LP CD4+ TEM cells never developed chronic colitis after transfer (Fig. 2) in contrast to the transferred IL-7+/+ × RAG-1−/− mice that developed severe colitis. The results showed that IL-7 is especially essential for the persistence of colitogenic CD4+ TEM cells.

Of note, however, de Latour et al. (28) very recently reported that IL-7−/− × RAG-1−/− mice transferred with CD4+CD45RBhigh T cells developed a wasting disease and colitis at 6 wk after transfer that was performed by very similar protocol of ours albeit to less inflammatory severity as compared with those in IL-7+/+ × RAG-1−/− recipients. However, the discrepancy between their result and ours was not surprising because they used recipients that were colonized by Helicobacter hepaticus, a bacteria known to be associated with colitis in immunodeficient mice, such as Rag-deficient and SCID mice, indicating that their result might be due to the activated innate immune responses induced by mucosal Helicobacter hepaticus infection, resulting in increasing production of signal 3 cytokines, such as IL-12, type I IFNs (IFN-α/β), and type II IFN (IFN-γ), to promote expansion and survival of colitogenic effector and memory T cells (29). Because we demonstrated that the small but substantial number of memory-type of mucosal CD4+ T cells were resided even in CD4+CD45RBhigh T cell-transferred IL-7−/− × RAG-1−/−, it is likely that H. hepaticus-induced activation of innate immunity in their setting might have accelerated the development of Helicobacter-mediated T cell expansive colitis or just T cell-independent innate immune-mediated colitis by increasing activated macrophages and granulocytes. Although we performed the specific PCR for Helicobacter species, including H. hepaticus using stool samples from mice in our facility, all data were all negative for Helicobacter species (data not shown). Further studies will be needed this issue.

Somewhat at odds, however, we found that rapid proliferation of donor CD4+ TEM cells was observed after transfer of CFSE-labeled colitogenic LP CD4+ TEM cells into IL-7−/− × RAG-1−/− mice as well as into IL-7+/+ × RAG-1−/− mice (Fig. 4), although the total number of recovered CD4+ T cells from IL-7−/− × RAG-1−/− mice was markedly decreased as compared with that from IL-7+/+ × RAG-1−/− mice (Fig. 3). Consistent with these results, we found that Bcl-2 expression was significantly decreased...
and conversely the ratio of annexin V+ cells in rapidly proliferating CFSE CD4+ cells was significantly increased in CD4+ T cells from the transferred IL-7−/−/RAG-1−/− mice as compared with those from the transferred IL-7+/−/RAG-1−/− mice. These results suggest that IL-7 is not required for rapid proliferation of colitogenic LP CD4+ TEM cells in the lymphopenic condition but is critical for the survival of colitogenic CD4+ TEM cells, followed by the essential contribution for the persistent colitis. Furthermore, the kinetics study to assess an early effector phase showed no colonic inflammation at 1 and 2 wk after transfer of CD4+ CD45RBhigh T cells into the IL-7−/− × RAG-1−/− recipients and thus suggests that chronic persistent colitis is not induced by only the expansion of effector cells, but what may be needed is the continuous conversion to TEM and the equilibrium between effector cells and TEM cells to maintain the diseases. Another possibility, which we do not favor, is that IL-7 might be essential for the maintenance of the colitogenic LP effector CD4+ T cells to sustain the disease.

It should be discussed this colitis model induced by the adoptive transfer into lymphopenic mice from the standpoint of homeostatic regulation of T lymphocytes. Conditions present in congenital mutant mice have been exploited for many years as an animal model for chronic wasting IBD, which occur several weeks after adoptive transfer of syngeneic naive CD4+ CD45RBhigh T cells to the condition, which lacks regulatory T cells (14, 15). Chronic colitis results from secretion of large amounts of inflammatory cytokines, especially IFN-γ and TNF-α, by infiltrated LP CD4+ cells that are chronically activated presumably by the bacterial Ags in the colon (30). The essential role of enteric bacteria is affirmed by the fact that intestinal inflammation cannot be induced if the mutant mice are reared under germfree conditions (15), indicating that enteric bacterial Ags might be responsible for the expansion of colitogenic CD4+ T cells. Apart from this model, it is now well accepted that the transfer of naive CD4+ T cells into a lymphocyte-deficient environment initiates proliferative responses (5, 21, 22). Careful analysis reveals that some of the transferred cells proliferate rapidly and undergo robust differentiation to memory cells, a process designated “rapid proliferation” responding to external Ags, including enteric bacteria, and other cells proliferate relatively slowly, designated “slow proliferation” responding to self-Ags (21, 22). Min et al. (19) recently demonstrated that rapid proliferation of T cells is IL-7 independent, whereas slow proliferation is IL-7 dependent. Although the mechanism of our colitis model induced by the adoptive transfer of CD4+CD45RBhigh T cells would fit with enteric bacteria-inducing rapid proliferation model because rapid proliferation of donor LP CD4+ TEM cells was observed into IL-7−/− × RAG-1−/− mice (Fig. 4), we found that IL-7 is critically required for the development and the persistence of colitis in mice transferred with CD4+CD45RBhigh T cells or the colitogenic CD4+ TEM cells. The discrepancy may be due to the difference of IL-7 dependency between the rapid proliferation, which is IL-7 independent, and the following survival step of CD4+ TEM cells, which is IL-7 dependent. In other words, IL-7 may be critically needed to survive the colitogenic CD4+ TEM cells after their rapid proliferation in the lymphopenic condition. However, it should be also noted that rapid proliferation in antibiotics-treated mice, regardless of IL-7−/− × RAG-1−/− and IL-7+/− × RAG-1−/− mice, could be not fully abolished (Fig. 7). This indicates that other environmental Ags, such as food and bedding and self-Ags themselves, might be involved in rapid proliferation in the lymphopenic condition.

Such characteristics of our colitis model raise another important question whether the colitogenic CD44+CD44highCD62L− T cells can be defined as TEM cells rather than effector T cells in the presence of Ags, in this case, intestinal bacteria. In general, immunological memory has evolved to warrant rapid and efficient elimination of microbial agents that repeatedly enter the organism. As a rule, immunological memory builds up, following successful elimination from the organism. In contrast, persistence of Ag, like in chronic infectious diseases, often leads to the exhaustion of the immune response (31). In immune responses in mice with chronic colitis, the target commensal bacteria are never eliminated but persist throughout life. Thus, would the colitogenic CD4+ T cells in CD4+CD45RBhigh-transferred colitis model build up memory against Ags? If so, do colitogenic memory CD4+ T cells play a role in the course of chronic disease? First, we found that the colitogenic CD4+ T cells highly expressed both CD44 and IL-7Ra. It is generally thought that highly expressed IL-7Ra is one of accepted memory, but not effector, T cell markers, and also IL-7Ra is down-regulated via TCR stimulation in the presence of Ags. Second, memory, but not effector, CD4+ T cells are critically controlled by the survival by IL-7 (3, 4). Consistent with this, we also found that the colitogenic LP CD4+ T cells were markedly decreased in IL-7−/− × RAG-1−/− mice transferred with the colitogenic CD4+ T cells as compared with the transferred IL-7+/− × RAG-1−/− mice. Collectively, these data indicate that the colitogenic CD4+ T cells are TEM cells rather than effector CD4+ T cells. In fact, Zaph and colleagues (32) recently demonstrated that Leishmania-specific central memory T cells develop in the presence of parasites. Although it could be argued that IBD, including murine model of chronic colitis, are due to chronic infection (persistence) of intestinal bacteria, it is likely that host and intestinal bacteria must establish some form of long-term relationship in which the immunological rules may be somewhat different from those of a brief encounter. Thus, a hallmark of T cell-mediated immune reaction to persistently expressed commensal bacterial Ags in chronic colitis would be the continual generation of Ag-specific effector and memory T cells. Because effector T cells are short-lived cells, there must exist cellular mechanisms by which effector and memory T cells specific for persistent bacterial Ags are maintained in the colitic mice.

Taken together, IL-7 is essential for the development and the persistence of chronic colitis as a critical survival factor for colitogenic CD4+ TEM cells rather than proliferative factor to sustain the intestinal inflammation, suggesting that therapeutic approaches targeting IL-7/IL-7R signal pathway may be feasible in the treatment of IBD.

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Disclosures

The authors have no financial conflict of interest.

References


