Supplementary Material
http://www.jimmunol.org/content/suppl/2011/01/07/jimmunol.1000057.DC1

Subscription
Information about subscribing to The Journal of Immunology is online at:
http://jimmunol.org/subscription

Permissions
Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts
Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts

Upregulated IL-7 Receptor α Expression on Colitogenic Memory CD4+ T Cells May Participate in the Development and Persistence of Chronic Colitis
Tamako Shinohara, Yasuhiro Nemoto, Takanori Kanai, Kaori Kameyama, Ryuichi Okamoto, Kiichiro Tsuchiya, Tetsuya Nakamura, Teruji Totsuka, Koichi Ikuta and Mamoru Watanabe

J Immunol published online 7 January 2011
http://www.jimmunol.org/content/early/2011/01/07/jimmunol.1000057
Upregulated IL-7 Receptor α Expression on Colitogenic Memory CD4+ T Cells May Participate in the Development and Persistence of Chronic Colitis

Tamako Shinohara,*1 Yasuhiro Nemoto,*1 Takanori Kanai,† Kaori Kameyama,* Ryuichi Okamoto,* Kiichiro Tsuchiya,* Tetsuya Nakamura,* Teruji Totsuka,* Koichi Ikuta,‡ and Mamoru Watanabe*

We have previously demonstrated that IL-7 is essential for the persistence of colitis as a survival factor of colitogenic IL-7Rα-expressing memory CD4+ T cells. Because IL-7Rα is broadly expressed on various immune cells, it is possible that the persistence of colitogenic CD4+ T cells is affected by other IL-7Rα-expressing non-T cells. To test this hypothesis, we conducted two adoptive transfer colitis experiments using IL-7Rα−/− CD4+CD25− donor cells and IL-7Rα−/− × RAG-2−/− recipient mice, respectively. First, IL-7Rα expression on colitic lamina propria (LP) CD4+ T cells was significantly higher than on normal LP CD4+ T cells, whereas expression on other colitic LP immune cells, (e.g., NK cells, macrophages, myeloid dendritic cells) was conversely lower than that of paired LP cells in normal mice, resulting in predominantly higher expression of IL-7Rα on colitogenic LP CD4+ cells, which allows them to exclusively use IL-7. Furthermore, RAG-2−/− mice transferred with IL-7Rα−/− CD4+CD25− T cells did not develop colitis, although LP CD4+ T cells from mice transferred with IL-7Rα−/− × RAG-2−/− recipient mice were differentiated to CD4+CD44highCD62L− effector-memory T cells. Finally, IL-7Rα−/− × RAG-2−/− mice transferred with CD4+CD25− T cells developed colitis similar to RAG-2−/− mice transferred with CD4+CD25+ T cells. These results suggest that IL-7Rα expression on colitogenic CD4+ T cells, but not on other cells, is essential for the development of chronic colitis. Therefore, therapeutic approaches targeting the IL-7/IL-7R signaling pathway in colitogenic CD4+ T cells may be feasible for the treatment of inflammatory bowel diseases. *The Journal of Immunology, 2011, 186: 000–000.

Inflammatory bowel disease (IBD) is characterized by idiopathic chronic intestinal inflammation, which commonly takes a persistent course with lifelong recurrence (1–4). According to current understanding, IBD is caused by inappropriate responses of the activated immune system to intestinal commensal bacteria in patients with a genetically susceptible background. Above all, effector CD4+ T cells including Th1, Th2, and Th17 are highlighted in the pathogenesis of IBD, because some groups have reported the association between genes involved in the Th1/Th17/IL-23 pathway and IBD (5, 6). Alternatively, we have investigated the possibility that long-lived memory CD4+ T cells are the main cause of the persistence of IBD and have proved the importance of IL-7 for the maintenance system of memory CD4+ T cells in chronic colitis (7).

IL-7 is a stromal cell-derived cytokine that is secreted by fetal liver cells, stromal cells in the bone marrow, and the thymus and other epithelial cells, including intestinal goblet cells (8, 9). Recently, IL-7 has emerged as a critical key cytokine involved in controlling the survival of peripheral resting CD4+ T cells, including naive and memory cells, but not effector cells, and their homeostatic turnover proliferation (8–15). The effect of IL-7 on CD4+ T cells is controlled by the expression of the specific receptors for IL-7, the state of differentiation of the T cells, the available concentration of IL-7, and whether there is concomitant TCR signaling (16, 17).

In contrast to the role of IL-7 in naive and memory CD4+ T cells in the resting state, the pathologic role of IL-7 in chronic immune-mediated diseases, such as autoimmune diseases and IBD, remains largely unclear. We have previously demonstrated that 1) IL-7 is constitutively produced by intestinal epithelial cells, especially by goblet cells (18); 2) IL-7 transgenic mice developed chronic colitis that mimicked histopathologic characteristics of human IBD (19); 3) colonic lamina propria (LP) CD4+IL-7Rαhigh T cells in RAG-2−/− mice in which colitis was induced by adoptive transfer of CD4+CD45RBhigh T cells have characteristics of colitogenic memory T cells (20); 4) the selective elimination of CD4+IL-7Rαhigh T cells by administrating toxin-conjugated anti-IL-7Rα mAb completely ameliorated ongoing colitis in TCR-α−/− mice (21); and 5) IL-7 is essential for the persistence of colitis by showing that IL-7−/− × RAG-1−/− mice transferred with colitogenic LP CD4+ T cells did not develop colitis (22).
We hypothesize that the dysregulated IL-7/IL-7Rα pathway is critically involved in the pathogenesis of animal models of chronic colitis and human IBD, although IL-7 seems to be strictly regulated at a constant level as a homeostatic cytokine to maintain the number of CD4+ memory T cells in the body.

IL-7R consists of the α-chain (CD127) and the cytokine receptor γ-chain (IL-2Rγ; CD132), which is shared by the common γ-chain family cytokines (IL-2, IL-4, IL-9, IL-15, and IL-21) (14, 15). Because IL-7Rα is broadly expressed on CD4+ T and NK cells, macrophages, dendritic cells, fibroblasts, and epithelial cells (14, 15), the persistency of colitogenic memory CD4+ T cells may be affected by those cells in the form of “IL-7 competition”. To assess this possibility, we attempted to clarify the link between the expression of IL-7Rα on various cells in the whole body in normal and colitic conditions and the pathogenesis of chronic colitis.

In this study, we prove that IL-7Rα expression on CD4+ T cells, but not on other cells (NK cells, granulocytes, macrophages, and dendritic cells), is essential for the development of colitis by use of an adoptive transfer colitis model using IL-7Rα−/− donor cells and IL-7Rα−/− × RAG-2−/− recipient mice.

Materials and Methods

Animals

Female C57BL/6 mice were purchased from JapanCLEA (Tokyo, Japan). C57BL/6-background RAG-2−/− mice were obtained from Taconic Farms (Hudson, NY). C57BL/6-background IL-7Rα−/− mice have been described previously (23). IL-7Rα−/− mice were intercrossed with RAG-2−/− mice to generate IL-7Rα−/− × RAG-2−/− mice in the Animal Care Facility of Tokyo Medical and Dental University. Mice were maintained under specific pathogen-free conditions in the Animal Care Facility of Tokyo Medical and Dental University. Female donors and recipients were used at 6-12 wk of age. All experiments were approved by the regional animal study committees and were performed according to institutional guidelines and home office regulations.

Purification of T cell subsets

CD4+ T cells were isolated from spleen cells of IL-7Rα−/− or C57BL/6 mice using the anti-CD4 (L3T4)-MACS system (Miltenyi Biotec, Auburn, CA) according to the manufacturer’s instructions. Enriched CD4+ T cells (96–97% pure, as estimated by FACS Calibur [Becton Dickinson, Sunnyvale, CA]) were then labeled with PE-conjugated anti-CD4 (RM4-5; BD Pharmingen), anti–CD8α (53-6.7; BD Pharmingen), FITC-conjugated anti-CD5 (7D4; BD Pharmingen), and biotin–anti–CD69 mAb (BD Pharmingen). To measure cytokine production, 1 × 105 cells were cultured in 96-well plates (Costar, Cambridge, MA) precoated with 15 mg/ml hamster anti-mouse CD3ε mAb (145-2C11; BD Pharmingen) and 2 µg/ml hamster anti-mouse CD28 mAb (37.51; BD Pharmingen) in 20% FBS overnight at 4°C (24). Culture supernatants were removed after 48 h and assayed for cytokine production. Cytokine concentrations were determined by specific ELISA per the manufacturer’s recommendation (R&D Systems, Minneapolis, MN).

Flow cytometry

To detect the surface expression of various molecules, isolated splenocytes or LP mononuclear cells were preincubated with FcγR-blocking mAb (CD16/32; 2.4G2; BD Pharmingen) for 20 min followed by incubation with specific FITC-, PE-, PECy5-, or biotin-labeled Abs for 20 min on ice. The following mAbs, other than biotin-conjugated anti-mouse IL-7Rα (7A134; Immunological Laboratories (Takeeji, Japan), were obtained from BD Pharmingen: anti-CD4 mAb (RM4-5), anti-CD25 mAb (7D4), anti-CD45RB mAb (16A), anti-CD62L (Mel-14), anti-CD44 mAb (IM7), anti-CD69 mAb (HI.2F3), and anti-Bcl-2 mAb (3F11). Biotinylated Abs were detected with PE-streptavidin. Standard two- or three-color flow cytometric analyses were obtained using the FACS Calibur with CellQuest software. Background fluorescence was assessed by staining with control irrelevant isotype-matched mAbs. To analyze the TCR Vβ family repertoire, splenic cells were double-stained with PE-conjugated anti-CD4 mAb (RM4-5) and the following FITC-conjugated mAbs: VB2; KJ25; VB3; KT4; VB4; MR9-4; VB5; RR1-7; VB6; TR310; VB7; MR5-2; VB8.1/2; B21.14; VB8.3; MR10-2; VB9; B21.5; VB10; RR1-18; VB11; MR1-1; VB12; INI1; VB13; 14.2; VB14; and KJ23. VB17. All Abs were purchased from BD Pharmingen.

For intracellular staining of cytokines, CD4+ T cells were cultured for 12 h with ionomycin (500 ng/ml), PMA (50 ng/ml), and BD GolgiPlug (1 µl/ml BD Pharmingen). After the stimulation, cells were collected and their surface molecules were stained. Cells were fixed using Cytofix/Cytoperm Kit (BD Pharmingen) and stained with PE-conjugated anti–IFN-γ mAb (XMG1.2; BD Pharmingen) for 20 min (26). Statistical analysis

We examined the normality of each group. If either group was not normally distributed, we assessed the difference between two groups using the Mann-Whitney U test. If both groups were normally distributed, we assessed the variance of population within each group using F test. With homoscedasticity of both populations, we assessed the difference between two groups using the Student t test. Without homoscedasticity, we assessed the difference using Welch’s t test. We used the program Statcel for all statistical analysis. Differences were considered to be statistically significant when p < 0.05.

Results

IL-7Rα is expressed on various immune cells in WT and colitic mice

To first assess the role of the IL-7/IL-7R signaling pathway in the development of chronic colitis, we analyzed the expression of
IL-7Rα on various immune compartments in colonic LP of normal C57BL/6 mice (normal mice) and colitic C57BL/6-RAG-2−/− mice previously transferred with WT CD4+CD25− T cells (colitic mice). First, both normal and colitic LP CD3+CD4+ T cells highly expressed IL-7Rα, but the mean fluorescence intensity (MFI) of IL-7Rα expression on LP CD3+CD4+ T cells from colitic mice was significantly higher than in normal mice (Fig. 1A, 1B). Conversely, the MFI of IL-7Rα expression on colonic LP CD3−NK1.1+ NK cells, CD11b+Gr1low macrophages, CD11b+Gr1high granulocytes, CD11b+CD11c+ myeloid dendritic cells were significantly downregulated compared with those from normal mice (Fig. 1A, 1B). In addition, there were no differences in the expression of IL-7Rα on CD3−NKp46+ NK22-like cells (27–29) and CD11b−CD11c− lymphoid dendritic cells (Fig. 1A, 1B). These changes of IL-7Rα expression in LP cells of colitic mice resulted in the highest expression of IL-7Rα on CD3+CD4+ T cells as compared with that on other compartments (Fig. 1B), suggesting preferential use of IL-7 by CD3+CD4+ T cells in colitic conditions.

Naive CD4+ T cells are retained in substantial numbers in spleens of IL-7Rα+ mice

Given the evidence that various immune compartments constitutively express IL-7Rα, we next attempted to assess the role of IL-7Rα expression in the development of chronic colitis induced by adoptive transfer of CD4+CD25− T cells obtained from age-matched WT or IL-7Rα−/− mice into RAG-2−/− mice. It was particularly interesting that the expression level of IL-7Rα on colitic LP CD3+CD4+ T cells was significantly higher than that of other compartments in colitic conditions (Fig. 1). Because it is also known that IL-7/IL-7R signaling is critically involved in T cell development in thymus and the periphery (9, 10), we first assessed phenotypic characteristics of splenic CD4+ T cells in age-matched WT and IL-7Rα−/− mice before starting a series of adoptive transfer experiments. Consistent with previous reports (23, 30), the absolute cell number of CD3+CD4+ T cells recovered from spleen (SP) of IL-7Rα−/− mice was significantly lower than that of WT mice (data not shown). Although the ratio of naive (CD44low/CD62L+) versus memory (CD44high/CD62L−) T cells in SP of IL-7Rα−/− mice was markedly decreased compared with that of WT mice, a substantial number of naive CD4+ T cells were retained in SP of IL-7Rα−/− mice (Fig. 2A). In addition, we confirmed that SP CD4+ T cells of IL-7Rα−/− mice did not express IL-7Rα, and no differences in the expression of CD69, Foxp3, and CD25 were found between two groups (Fig. 2A). Of note, Bcl-2 expression in SP CD4+ T cells of IL-7Rα−/− mice was significantly lower than that of WT mice (p < 0.05; Fig. 2A), which seemed to be consistent with previous reports that IL-7 is essential for survival of CD4+ T cells (24). It was also possible that CD4+CD25− donor T cells in SPs of IL-7Rα−/− mice retain restricted clonality of CD4+ T cells because of the dysregulated differentiation of CD4+ T cells in the thymus as compared with that in WT mice. To test this possibility, we compared TCR Vβ repertoires of SP CD4+CD25− T cells from age-matched IL-7Rα−/− and WT mice. Flow cytometric analysis of these SP CD4+ cells using a panel of 15 anti-Vβ mAbs showed that the major Vβ population was Vβ8.1/8.2 in both groups, and the only significant difference in Vβ repertoires between the groups was Vβ8.3 (Fig. 2B).

RAG-2−/− mice transferred with IL-7Rα−/− CD4+CD25− T cells did not develop mild colitis

To then assess the role of the IL-7R signaling pathway in the development of chronic colitis, we used a chronic colitis model induced by adoptive transfer of SP CD4+CD25− T cells from IL-7Rα−/− or control WT mice into RAG-2−/− recipients (Fig. 3A).

**FIGURE 1.** IL-7Rα expression on various immune compartments obtained from colonic LP of normal and colitic mice. A, Dot plot analysis shows the IL-7α expression on each fraction of immune cells from colonic LP of normal and colitic mice. Numerical values on the dot plots and histograms express the mean percentage of each fraction. B, The bar graphs show the MFI of IL-7Rα on each immune compartment obtained from colonic LP of normal and colitic mice. The graph data are the mean ± SEM. *p < 0.05. N.S., not significant.

**FIGURE 2.** Phenotypic characterization of splenic CD4+ T cells obtained from age-matched WT and IL-7Rα−/− mice. A, FACS analysis shows the expression of CD44/CD62L, IL-7Rα, Foxp3, and Bcl-2 on/in splenic CD4+ T cells. The dotted line in the Bcl-2 histogram shows the baseline of isotype control. B, Flow cytometric analysis of Vβ families on the surface of splenic CD4+ T cells. To analyze the TCR Vβ family repertoire, splenic cells were double-stained with PE-conjugated anti-CD4 mAb (RM4-5) and a panel of 15 FITC-conjugated Vβ mAbs. The percentage value of each Vβ is the frequency pooled from three independent experiments (n = 6). The data are the mean ± SEM. *p < 0.05. N.S., not significant.
As a negative control, RAG-2\(^{-/-}\) mice were transferred with a mixture of SP CD4\(^{+}\)CD25\(^{-}\) T cells and CD4\(^{+}\)CD25\(^{+}\) Tregs obtained from WT mice. As depicted in Fig. 3B, RAG-2\(^{-/-}\) mice transferred with WT CD4\(^{+}\)CD25\(^{+}\) T cells manifested progressive weight loss from 4 wk after transfer (Fig. 3B). In contrast, RAG-2\(^{-/-}\) mice transferred with IL-7Ra\(^{-/-}\) CD4\(^{+}\)CD25\(^{+}\) T cells as well as RAG-2\(^{-/-}\) mice transferred with a mixture of CD4\(^{+}\) CD25\(^{+}\) T cells and CD4\(^{+}\)CD25\(^{+}\) Tregs appeared healthy and showed a gradual increase of body weight (Fig. 3B). To check the possibility that mice transferred with IL-7Ra\(^{-/-}\) CD4\(^{+}\)CD25\(^{+}\) T cells develop colitis with delayed kinetics, we observed all groups of mice until 11 wk after transfer. Eleven weeks after transfer, RAG-2\(^{-/-}\) mice transferred with WT CD4\(^{+}\)CD25\(^{+}\) T cells, but not those transferred with IL-7Ra\(^{-/-}\) CD4\(^{+}\)CD25\(^{+}\) T cells or WT CD4\(^{+}\)CD25\(^{+}\) T cells and CD4\(^{+}\)CD25\(^{+}\) Tregs, had enlarged colons with greatly thickened walls (Fig. 3C). The same mice also showed the enlargement of SP and mesenteric lymph nodes (Fig. 3C). The assessment of colitis by clinical scores showed a clear difference between RAG-2\(^{-/-}\) mice transferred with WT CD4\(^{+}\)CD25\(^{+}\) T cells and the other two groups (Fig. 3D).

Histologic examination showed prominent epithelial hyperplasia with glandular elongation and massive infiltration of mononuclear cells in LP of RAG-2\(^{-/-}\) mice transferred with WT CD4\(^{+}\)CD25\(^{+}\) T cells (Fig. 3E, middle panels). In contrast, these inflammatory changes were mostly abrogated, and only a few mononuclear cells were observed in the LP of the colon from RAG-2\(^{-/-}\) mice transferred with IL-7Ra\(^{-/-}\) CD4\(^{+}\)CD25\(^{+}\) T cells (Fig. 3E, left panels) or with a mixture of SP CD4\(^{+}\)CD25\(^{+}\) T cells and CD4\(^{+}\)CD25\(^{+}\) Tregs (Fig. 3E, right panels). This difference was also confirmed by the histologic scores of multiple colon sections: 5.35 ± 0.40 in RAG-2\(^{-/-}\) mice transferred with WT CD4\(^{+}\)CD25\(^{+}\) T cells versus 1.65 ± 0.57 in RAG-2\(^{-/-}\) mice transferred with IL-7Ra\(^{-/-}\) CD4\(^{+}\)CD25\(^{+}\) T cells and 2.00 ± 0.74 in RAG-2\(^{-/-}\) mice transferred with a mixture of SP CD4\(^{+}\)CD25\(^{+}\) T cells and CD4\(^{+}\)CD25\(^{+}\) Tregs (\(p < 0.001;\) Fig. 3F). Further quantitative evaluation of CD4\(^{+}\) T cell infiltration was made by calculating the absolute cell number of LP CD3\(^{+}\)CD4\(^{+}\) T cells recovered from the resected bowels. Significantly fewer CD4\(^{+}\) T cells were recovered from the colonic tissue of RAG-2\(^{-/-}\) mice transferred with IL-7Ra\(^{-/-}\) CD4\(^{+}\)CD25\(^{+}\) T cells or a mixture of SP CD4\(^{+}\)CD25\(^{+}\) T cells and CD4\(^{+}\)CD25\(^{+}\) Tregs as compared with colitic RAG-2\(^{-/-}\) mice transferred with WT CD4\(^{+}\)CD25\(^{+}\) T cells and CD4\(^{+}\)CD25\(^{+}\) Tcells or a mixture of SP CD4\(^{+}\)CD25\(^{+}\) T cells and CD4\(^{+}\)CD25\(^{+}\) Tregs produced significantly lower amounts of IFN-\(\gamma\) and TNF-\(\alpha\) than did colitic RAG-2\(^{-/-}\) mice transferred with WT CD4\(^{+}\)CD25\(^{+}\) T cells upon in vitro stimulation (Fig. 3H).

Histologic examination of the colon from RAG-2\(^{-/-}\) mice transferred with IL-7Ra\(^{-/-}\) CD4\(^{+}\)CD25\(^{+}\) T cells (left), RAG-2\(^{-/-}\) mice transferred with WT CD4\(^{+}\)CD25\(^{+}\) T cells (middle), and RAG-1\(^{-/-}\) mice transferred with WT CD4\(^{+}\)CD25\(^{+}\) T cells (right) at 11 wk after the transfer. Original magnification = ×40 (upper) and ×100 (lower). F. Histologic scoring at 11 wk after transfer. Data are indicated as the mean ± SEM of seven mice in each group. *\(p < 0.05.\) G. LP CD3\(^{+}\)CD4\(^{+}\) T cells were isolated at 11 wk after transfer, and the number was determined by flow cytometry. Data are indicated as the mean ± SEM of seven mice in each group. *\(p < 0.05.\) H. Cytokine production by LP CD4\(^{+}\) T cells. LP CD4\(^{+}\) T cells were isolated at 11 wk after transfer and stimulated with anti-CD3 and anti-CD28 mAbs for 48 h. IFN-\(\gamma\) and TNF-\(\alpha\) concentrations in culture supernatants were measured by ELISA. Data are indicated as the mean ± SD of seven mice in each group. *\(p < 0.05.\)
Importantly, further flow cytometric analysis revealed that almost all the SP and LP CD3+CD4+ T cells isolated from all three groups of mice at 11 wk after transfer were CD44highCD62L−/−CD69− effect-memory T (TEM) cells (Supplemental Fig. 1), indicating that the transferred CD4+ CD25− T cells could differentiate to activated TEM cells regardless of the expression of IL-7Rα or the presence or absence of Tregs. These results suggest that the lack of IL-7Rα prevented the development of colitis primarily by inhibiting the expansion or survival of cologenic CD4+ TEM cells in the colon in accordance with the lower expression of Bcl-2 (Fig. 2A). We found that SP and LP CD4+ T cells isolated from all groups of mice at 11 wk after transfer did not express IL-15Rβ, which is a critical receptor for IL-15 signaling, and thymic stromal lymphopoietin (TSLP) receptor, which is critical for TSLP signaling via TSLPR/IL-7Rα complex receptors (Supplemental Fig. 1), indicating that IL-15 and TSLP may not be involved in this colitis model.

To further assess whether IL-7Rα−/− CD4+ T cells are unable to produce inflammatory cytokines intrinsically or as the result of a secondary effect from disorder of cell proliferation or maintenance, we performed the following experiments. First, we assessed ex vivo cytokine production of IL-7Rα−/− or WT SP CD4+ T cells under Th1 polarizing conditions (Supplemental Fig. 2A). As shown in Supplemental Fig. 2B, IL-7Rα−/− SP CD4+ T cells expressed lower levels of IFN-γ than did WT SP CD4+ T cells under the Th1 polarizing ex vivo conditions. This finding was confirmed by the statistical analysis (Supplemental Fig. 2C). Next, we examined the ability of the IL-7Rα−/− CD4+ T cells to produce inflammatory cytokines under the same inflammatory conditions as the WT CD4+ T cells. For this purpose, the same number (3 × 10^5 cells per mouse) of Ly5.2+ IL-7Rα−/− SP CD4+ CD25− T cells and Ly5.1+ WT SP CD4+ CD25− T cells were cotransferred to RAG-2−/− recipients (Fig. 4A). The percentage of Ly5.2+−derived IL-7Rα−/− T cells in peripheral blood was gradually decreased after transfer, while that of Ly5.1+−derived WT T cells in peripheral blood was conversely increased, and the difference was significant 2 wk after transfer (Fig. 4B). Six weeks after transfer, all mice developed colitis (data not shown). Although the recovered cell number of Ly5.2+ SP or LP CD4+ T cells derived from IL-7Rα−/− donors at 6 wk after transfer was

![Figure 4](http://www.jimmunol.org/Downloadedfrom/132x157to452x500)

**FIGURE 4.** IL-7Rα−/− CD4+CD25− T cells cotransferred with WT CD4+CD25− T cells to RAG-2−/− mice could produce IFN-γ and IL-17, but could not survive. A, To discern why IL-7Rα−/− CD4+CD25− T cells could not induce colitis, we cotransferred the same number (3 × 10^5) of Ly5.2+ IL-7Rα−/− CD4+CD25− T cells and Ly5.1+ WT CD4+CD25− T cells to Ly5.2+−RAG-2−/− mice, and we compared the cell number and ability to produce Th1/Th17 cytokines between transferred IL-7Rα−/− and WT cells. PBMCs were collected 1, 2, 3, and 4 wk after the transfer. All mice were sacrificed and analyzed 6 wk after the transfer. B, Percentage of Ly5.1+ or Ly5.2+ cells in peripheral blood CD3+CD4+ cells at each time point were determined by flow cytometry. C, Percentage of Ly5.1+ or Ly5.2+ cells in SP and LP CD3+CD4+ cells 6 wk after the transfer. D, IFN-γ and IL-17 expression in recovered LP CD4+ T cells from IL-7Rα−/− or WT donor mice. LP CD4+ T cells were collected from RAG-2−/− recipients 6 wk after the transfer; they were cultured with ionomycin, PMA, and GolgiPlug for 12 h as mentioned in Materials and Methods. IFN-γ and IL-17 expression of them were determined by flow cytometry using intracellular staining methods. CD3+CD4+Ly5.1+ cells were considered as CD4+ T cells from WT donor mice, while CD3+CD4+Ly5.1− cells were considered as CD4+ T cells from IL-7Rα−/− donor mice. Numerical values on the histograms express the mean percentage of each fraction. E, Percentage of IFN-γ+ cells and IL-17+ cells in LP CD3+CD4+ T cells from IL-7Rα−/− or WT donor mice. Data are indicated as the mean ± SEM of five mice in each group. *p < 0.05.
were transferred with SP CD3⁺CD4⁺CD62L⁻CD44⁻ naive T cells obtained from age-matched WT or IL-7Rα⁻/⁻ mice (Fig. 5A). As a negative control, RAG-2⁻/⁻ mice were transferred with SP WT naive T cells and CD4⁻CD25⁺ Tregs (Fig. 5A). As expected, neither mice transferred with IL-7Rα⁻/⁻ naive T cells nor mice transferred with naive T cells and Tregs developed colitis as assessed by gross appearance of the colon (Fig. 5B), clinical (Fig. 5C) and histologic scorings (Fig. 5D, 5E), and the absolute cell number of LP CD3⁺CD4⁺ T cells (Fig. 5F) in sharp contrast to the diseased mice transferred with WT naïve T cells, confirming that IL-7Rα expression on CD4⁺ T cells is essential for the development of colitis, regardless of the different ratio of naive and memory cells in SP of IL-7Rα⁻/⁻ mice and WT mice. We further performed an apoptosis assay using annexin V/PI staining in this

![Diagram](https://example.com/diagram.png)

**FIGURE 5.** RAG-2⁻/⁻ transferred with IL-7Rα⁻/⁻ CD3⁺CD4⁺CD62L⁺CD44⁺ T cells did not develop chronic colitis. A, RAG-2⁻/⁻ mice were transferred with splenic CD3⁺CD4⁺CD62L⁺CD44⁺ T cells obtained from age-matched WT or IL-7Rα⁻/⁻ mice (3 × 10⁵ cells per mouse). As a negative control, RAG-2⁻/⁻ mice were transferred with splenic WT CD3⁺CD4⁺CD62L⁺CD44⁺ T cells (3 × 10⁵ cells per mouse) and CD4⁻CD25⁺ Tregs (1 × 10⁵ cells per mouse). B, Gross appearance of the colon, SP, and mesenteric lymph nodes from RAG-2⁻/⁻ mice transferred with IL-7Rα⁻/⁻ CD3⁺CD4⁺CD62L⁺CD44⁺ (top), RAG-2⁻/⁻ mice transferred with WT CD3⁺CD4⁺CD62L⁺CD44⁺ T cells (middle), and RAG-1⁻/⁻ transferred with WT CD3⁺CD4⁺CD62L⁺CD44⁺ T cells and CD4⁻CD25⁺ Tregs (bottom). C, Clinical scores were determined at 8 wk after the transfer as described in Materials and Methods. Data are indicated as the mean ± SEM of each group. *p < 0.05. D, Histologic examination of the colon from RAG-2⁻/⁻ mice transferred with IL-7Rα⁻/⁻ CD3⁺CD4⁺CD62L⁺CD44⁺ T cells (left), RAG-2⁻/⁻ mice transferred with WT CD3⁺CD4⁺CD62L⁺CD44⁺ T cells (middle), and RAG-1⁻/⁻ transferred with WT CD3⁺CD4⁺CD62L⁺CD44⁺ T cells and CD4⁻CD25⁺ Tregs (right) at 8 wk after the transfer. Original magnification ×40 (upper) and ×100 (lower). E, Histologic scoring at 8 wk after transfer. Data are indicated as the mean ± SEM of each group. *p < 0.05. F, LP CD3⁺CD4⁺ T cells were isolated at 8 wk after transfer, and the number was determined by flow cytometry. Data are indicated as the mean ± SEM of each group. *p < 0.05. G, The expression of propidium iodide (PI) and annexin V in SP CD4⁺ T cells from RAG-2⁻/⁻ mice transferred with IL-7Rα⁻/⁻ CD3⁺CD4⁺CD62L⁺CD44⁺ T cells, RAG-2⁻/⁻ mice transferred with WT CD3⁺CD4⁺CD62L⁺CD44⁺ T cells, and RAG-2⁻/⁻ transferred with WT CD3⁺CD4⁺CD62L⁺CD44⁺ T cells and CD4⁻CD25⁺ Tregs at 8 wk after the transfer. H, The percentage of early apoptotic cells (annexin V⁺PI⁻) and late apoptotic cells (annexin V⁺PI⁺). I, Intracellular staining of cytokines (IL-17/IFN-γ) in the colonic LP CD4⁺ T cells. Numerical values on the dot plots and histograms express the mean percentage of each fraction.
setting. IL-7Rα−/− SP CD4+ T cells underwent apoptosis more frequently than WT SP CD4+ T cells (Fig. 5G, 5H), which supports the hypothesis that expression of IL-7Rα on CD4+ T cells is important for their survival. Furthermore, the expression of IL-17 and IFN-γ in IL-7Rα−/− LP CD4+ T cells was markedly decreased compared with that in WT LP CD4+ T cells (Fig. 5f).

IL-7Rα−/− × RAG-2−/− mice transferred with CD4+CD25+ T cells developed colitis

To further assess the role of IL-7/IL-7R signaling in the development of chronic colitis, we next focused on IL-7Rα expression on non-T cells, such as APCs and NK cells that reside in RAG-2−/− recipients, because it is possible that IL-7 is competitively used by various IL-7Rα-expressing immune compartments, and the competition may affect the development of chronic colitis. To test this hypothesis, WT CD4+CD25− T cells were transferred into RAG-2−/− or IL-7Rα−/− × RAG-2−/− mice (Fig. 6A). As a negative control, a mixture of WT CD4+CD25− T cells and CD4+CD25+ Tregs was transferred into RAG-2−/− mice (Fig. 6A). When CD4+CD25+ T cells were transferred into the control RAG-2−/− mice, the recipients, as expected, rapidly developed severe wasting disease associated with clinical signs of severe colitis, in particular, weight loss, persistent diarrhea and occasionally bloody stool and anal prolapses, in sharp contrast to healthy RAG-2−/− mice transferred with a mixture of CD4+ CD25− T cells and CD4+CD25+ Tregs (Fig. 6B). When CD4+ CD25− T cells were transferred into the IL-7Rα−/− × RAG-2−/− mice, the recipients also developed severe wasting chronic colitis (Fig. 6B). These RAG-2−/− and IL-7Rα−/− × RAG-2−/− mice transferred with CD4+CD25+ T cells, but not RAG-2−/− mice transferred with a mixture of CD4+CD25+ T cells and CD4+CD25− Tregs, had enlarged colons with significantly thickened walls accompanied with enlarged SPs and mesenteric lymph nodes 8 wk after transfer (Fig. 6C). Consistent with this finding, clinical scores of RAG-2−/− and IL-7Rα−/− × RAG-2−/− mice transferred with CD4+CD25+ T cells were significantly higher than those of RAG-2−/− mice transferred with a mixture of CD4+ CD25− T cells and CD4+CD25+ Tregs (Fig. 6D). No significant difference in clinical scores was found between RAG-2−/− and IL-7Rα−/− × RAG-2−/− mice transferred with CD4+CD25+ T cells, although the score of IL-7Rα−/− × RAG-2−/− mice tended to be higher than that of RAG-2−/− mice transferred with CD4+CD25− T cells (Fig. 6D).

Histologic examination showed that tissue sections from RAG-2−/− and IL-7Rα−/− × RAG-2−/− mice transferred with CD4+ CD25− T cells were characterized by inflammatory infiltrate, epithelial hyperplasia, crypt cell damage, and goblet cell depletion, in contrast to RAG-2−/− mice transferred with a mixture of CD4+CD25+ T cells and CD4+CD25− Tregs, which showed no features of colitis (Fig. 6E). This difference was also confirmed by histologic scoring of multiple colon sections (Fig. 6F). Consistent with the histologic assessment, the numbers of LP CD4+ T cells recovered from RAG-2−/− and IL-7Rα−/− × RAG-2−/− mice transferred with CD4+CD25+ T cells were similar to each other but significantly higher than that from noncolitic RAG-2−/− mice transferred with a mixture of CD4+CD25+ T cells and CD4+CD25− Tregs (Fig. 6G). Cytokine production by LP CD4+ T cells is depicted in Fig. 6H. LP CD4+ T cells from RAG-2−/− and IL-7Rα−/− × RAG-2−/− mice transferred with CD4+CD25+ T cells produced significantly higher levels of IFN-γ and TNF-α than did those from the control mice transferred with a mixture of CD4+ CD25− T cells and CD4+CD25+ Tregs (Fig. 6H).

Flow cytometric analysis revealed that the LP CD4+ T cells isolated from all groups of mice at 8 wk after transfer were CD44hiCD62LhiCD69+ TEm cells (Supplemental Fig. 3A), indicating that the transferred CD4+CD25− T cells could differentiate to activated TEm cells regardless of the presence or absence of IL-7Rα on non-T cells in the RAG-2−/− recipient mice. Intracellular analysis further showed that almost the same fraction of LP CD4+ T cells from both RAG-2−/− and IL-7Rα−/− × RAG-2−/− mice transferred with CD4+CD25+ T cells had differentiated to IFN-γ-producing Th1 or IL-17–producing Th17 (Supplemental Fig. 3B). In contrast, the expression of IFN-γ in LP CD4+ T cells from RAG-2−/− mice transferred with a mixture of CD4+CD25+ and CD4+CD25− T cells was markedly reduced as compared with the groups with colitis (Supplemental Fig. 3B).

To further clarify whether the lower number of CD4+CD25− T cells in the transfer experiment makes this difference significant, RAG-2−/− mice and IL-7Rα−/− × RAG-2−/− mice were transferred with 3 × 10^5 or 1 × 10^5 WT SP CD4+CD25− T cells as a negative control, RAG-2−/− mice were transferred with splenic WT CD4+CD25− T cells (3 × 10^5 cells per mouse) and CD4+CD25− Tregs (3 × 10^5 cells per mouse; Supplemental Fig. 4A). However, no differences were found in clinical and histologic colitis scores or the absolute number of LP CD3+CD4+ T cells between IL-7Rα−/− and WT transferred groups, irrespective of lower or higher number of donor T cells (Supplemental Fig. 4B–E).

Although we also checked the expression of MHC class II on CD11b+CD11c+ classical dendritic cells and CD11b+CD11c+ myeloid dendritic cells in this experiment (Supplemental Fig. 4F), no differences were detected between any groups. Diminished expression of MHC class II on dendritic cells in RAG-2−/− mice, which is caused by elevated level of IL-7 with lymphopenia, may recover after transferred CD4+ T cells consume IL-7.

Discussion

This study has demonstrated that the high expression of IL-7Rα on colitic CD4+ T cells, but not on non-T cells, is essential for the development and persistence of colitis. This finding is supported by the findings that 1) the MFI of IL-7Rα expression of LP CD4+ T cells is significantly higher than that of other non-CD4+ T cells in colitic conditions, 2) the MFI of IL-7Rα expression of colitic LP CD4+ T cells is significantly higher than that of normal LP CD4+ T cells, 3) RAG-2−/− mice transferred with IL-7Rα−/− × CD4+CD25+ T cells do not develop colitis, and 4) IL-7Rα−/− × RAG-2−/− mice transferred with WT CD4+CD25+ T cells develop colitis similar to that in transferred IL-7Rα−/− × RAG-2−/− mice. Collectively, IL-7Rα expression on colitic CD4+ T, but not on other cells, is essential for the development and persistence of chronic colitis.

It was originally reported that IL-7Rα is highly expressed on lymphocytes such as T cells (16). Consistent with this report, we have previously reported that the IL-7/IL-7R signaling pathway is critical for the maintenance of IL-7Rαhi colitogenic CD4+ memory T cells (18, 20). Furthermore, we showed that treatment with neutralizing anti–IL-7Rα mAb ameliorated ongoing chronic colitis (18). More recently, several reports have proved the importance of the IL-7/IL-7R signal in nonlymphocytes. Guimond et al. (31) have reported that IL-7Rα is expressed on some types of dendritic cells, and that in the lymphopenic environment the IL-7/IL-7R signal of dendritic cells leads to depression of its MHC class II molecule, which results in the suppression of the proliferation of CD4+ T cells. Other recent reports that IL-7Rα is broadly expressed on NK cells, dendritic cells, and macrophages in normal conditions (16, 17), suggesting the need for us to further investigate the importance of the IL-7/IL-7R signaling pathway in non-T cells for the development and persistence of chronic colitis. Although IL-7Rα expression on
some LP populations, such as NK cells, granulocytes, macrophages, and CD11b+CD11c+ myeloid dendritic cells, in colitic mice was significantly downregulated compared with that in normal mice, the expression level of IL-7Rα on colitic CD4+ T cells was conversely high, with the result that colitogenic memory CD4+ T cells sustain the highest expression of IL-7Rα in inflammatory conditions.

IL-7Rα−/− mice are originally lymphopenic, because of the loss of IL-7/IL-7R signaling pathway in lymphocytes, which is a critical factor for their development in the thymus and their maintenance in the periphery. Comparison of the surface phenotypes of SP CD4+ T cells in IL-7Rα−/− and WT mice by flow cytometric analysis revealed no significant differences in the expression of CD69, CD25, and Foxp3 (Fig. 2A). Manifestation of an antiapoptosis molecule Bcl-2 of CD4+ T cells from IL-7Rα−/− mice was lower than that of CD4+ T cells from WT mice, which corresponds to the previous reports that the IL-7/IL-7R signal maintains T cells, upregulating the antiapoptosis molecule. Nevertheless, we detected a substantial number of CD44lowCD62L+ naive CD4+ T cells resident in the SPs of IL-7Rα−/− mice, although their relative number in IL-7Rα−/− mice was significantly lower than that in WT mice. Because of the scarcity of naive CD4+ T cells in IL-7Rα−/− mice, it was possible that the failure of some part of naive T cells to develop might occur in the thymus, which would lead to the loss of some TCR repertoires needed for the onset of colitis. Thus, we compared the TCR Vβ repertoire of SP CD4+ T cells in IL-7Rα−/− mice to those in WT mice. However, except in the ratio of Vβ8.3, no evidence was found of skewed development in TCR Vβ repertoires between age-matched IL-7Rα−/− and WT mice.

As expected, RAG2−/− mice transferred with SP IL-7Rα−/−CD4+CD25− T cells did not develop colitis, in sharp contrast to colitic CD4+CD25− T cells differentiated to CD44highCD62L− TEM cells as well as those from colitic RAG2−/− mice transferred with WT CD4+CD25− T cells. This result suggests that IL-7Rα−/− mice causes the disorder of cell proliferation or maintenance rather than the impaired development of memory CD4+ T cells, in accordance with the downmodulated Bcl-2 expression of IL-7Rα−/− CD4+ T cells. As shown in Fig. 3H, production of Th1 cytokines from recovered LP CD4+ T cells of the IL-7Rα−/−CD25− group was significantly lower than that of the WT CD25+ group. However, IL-7Rα−/− CD4+ T cells could express Th1 and Th17 cytokines to an extent similar to that in WT CD4+ T cells in the colitic condition (Fig. 4). Therefore, we conclude that disorder of IL-7Rα−/− CD4+ T cells to proliferate and survive is the main mechanism underlying their inability to induce colitis, whereas their reduced inflammatory cytokine production is a secondary effect. Furthermore, we also analyzed other common γ-receptor-associated receptor IL-15Rβ to determine whether it was upregulated to compensate for the lack of IL-7Rα. However, no dif-

**FIGURE 6.** IL-7Rα−/− × RAG2−/− transferred with WT CD4+CD25− T cells developed chronic colitis. A. RAG2−/− mice and IL-7Rα−/− × RAG2−/− mice were transferred with splenic WT CD4+CD25− T cells (3 × 10^6 cells per mouse). As a negative control, RAG2−/− mice were transferred with splenic WT CD4+CD25− T cells (3 × 10^6 cells per mouse) and CD4+CD25− Tregs (1 × 10^6 cells per mouse). B. Disease activity index during 8 wk after transfer. *p < 0.05. C. Gross appearance of the colon, SP, and mesenteric lymph nodes from IL-7Rα−/− × RAG2−/− mice transferred with CD4+CD25− T cells (top), RAG2−/− mice transferred with CD4+CD25− T cells (middle), and RAG2−/− mice transferred with CD4+CD25− T cells and CD4+CD25− Tregs (right). Original magnification ×40 (upper) and ×100 (lower). F. Histologic scoring 8 wk after transfer.
ference was found in the expression of IL-15Rβ on SP or LP CD4^+ T cells from each group. These results suggest that IL-7Rα expression on colitogenic CD4^+ T cells is essential for the development and persistence of colitis.

Next, we used IL-7Rα−/− × RAG2−/− mice to access the importance of the IL-7/IL-7Rα signaling pathway in non-T cells. At the start of this project, we hypothesized that IL-7Rα−/− × RAG2−/− mice transferred with CD4^+CD25^− T cells would develop more severe colitis than the control transferred RAG2−/− recipient mice by considering two points. First, we thought that the availability of IL-7 for colitogenic CD4^+ T cells might increase in IL-7Rα−/− × RAG2−/− mice as a result of the loss of IL-7 consumption by IL-7Rα−lacking non-T cells. Actually, IL-7 concentration in serum from IL-7Rα−/− mice is reported to be higher than that from WT mice (31). Thus, it was possible that the persistence of colitogenic memory CD4^+ T cells is affected by those cells in the form of IL-7 competition. Second, we had to consider the presence of newly identified RORγ^T^+ IL-22-producing NK cells (so called NK-22 cells) (27–29, 32, 33) for the development of chronic colitis, because it has been shown that these NK-22 cells constitutively express IL-7Rα. Importantly, it has been reported recently that IL-22 is protective in murine DSS-induced colitis model using IL-22−/− × RAG2−/− mice (33), leading to speculation that these NK-22 cells reside in intestinal LP of RAG-2−/− mice and may be regulated by the IL-7/IL-7Rα signaling pathway. Unexpectedly, we could not detect any significant differences regarding the severity of colitis between RAG-2−/− and IL-7Rα−/− × RAG2−/− recipient mice. This finding was also confirmed by the experiment using a smaller number of CD4^+CD25^− T cells as donor cells. Instead, we found that the expression of IL-7Rα on colitic LP CD4^+ T cells was significantly higher than that on normal LP CD4^+ T cells (Fig. 1), suggesting a mechanism for exclusive use of IL-7 by highly IL-7Rα-expressing colitogenic CD4^+ T cells.

Previously, we showed that IL-7Rα expression on LP CD4^+ T cells in CD4^+CD45RB^hi^ T cell-transferred RAG-2−/− mice is downmodulated at the early effector phase of colitogenic CD4^+ T cell differentiation (1–2 wk after transfer) and is again upregulated at the memory phase when colitis is established (1–2 wk after transfer) (22). Thus, it is possible that the competition for IL-7 between colitogenic CD4^+ T cells and other non-T cells occurs during such an early phase of colitis development. Otherwise, IL-7 competition between T cells versus non-T cells may occur at more acute immune responses, such as acute bacterial infections, which is mainly regulated by IL-7Rα–downmodulating effector T cells (10).

Finally, it is important to discuss the therapeutic strategies for the treatment of IBD. Because IL-7 is the most important cytokine for the maintenance of homeostasis of all the resting memory CD4^+ T cells, it seems to be unsafe to adopt the blockade of IL-7/IL-7Rα signaling pathway for the treatment of IBD. As shown in this study, however, it should be emphasized that the highest expression of IL-7Rα is found in colitogenic memory LP CD4^+ T cells as compared with non-CD4^+ T cell compartments and normal CD4^+ T cells. In such a situation, it is possible that a neutralizing or depleting anti-IL-7Rα mAb would preferentially target colitogenic memory CD4^+ T cells with the highest expression of IL-7Rα. Consistent with this notion, a recent report has shown that targeted depletion of pathogenic Th1 and Th17 cells, which express high levels of lymphotixin-α, inhibits autoimmune diseases (34). In addition, it may be necessary to develop a molecular targeting therapy against the IL-7Rα molecule that is more specific for the target organ, rather than a systemic therapy, using effective drug delivery to inflamed mucosa of IBD.

Collectively, we have shown that IL-7Rα expression on CD4^+ T cells is essential for the development of colitis in this model. This finding suggests that IL-7Rα on colitogenic memory LP CD4^+ T cells is one of the important targets in IL-7/IL-7R signaling blocking therapy.

Disclosures

The authors have no financial conflicts of interest.

References


SUPPLEMENTAL INFORMATION

Supplemental Figure Legends

Figure S1

CD3⁺CD4⁺ T cells isolated from RAG-2⁻/⁻ transferred with IL-7Rα⁻/⁻ CD4⁺CD25⁻ T cells differentiated into CD44^{high}CD62L⁻ effector-memory T cells. FACS analysis shows the expression of CD44/CD62L, CD69, IL-7Rα, IL-15Rβ, and TSLPR on SP and LP CD4⁺ T cells from RAG-2⁻/⁻ mice transferred with IL-7Rα⁻/⁻ CD4⁺CD25⁻ T cells, RAG-2⁻/⁻ mice transferred with WT CD4⁺CD25⁻ T cells, and RAG-2⁻/⁻ transferred with WT CD4⁺CD25⁻ T cells and CD4⁺CD25⁺ T_R cells at 11 wk after the transfer. Numerical values on the dot plots and histograms express the mean percentage of each fraction.

Figure S2

Th-1 polarized IL-7Rα⁻/⁻ CD4⁺ T cells did not produce IFN-γ in vitro. (A) SP CD4⁺ T cells were obtained from IL-7Rα⁻/⁻ mice or WT mice. They were cultured with anti-CD3 antibody and recombinant IL-12 and anti-IL-4 antibody for three days, then they were collected, washed and cultured with ionomycine, PMA and GolgiPlug for 12 hours as mentioned in Materials and Methods. Finally, IFN-γ expression of them were determined by flow cytometry using intra-cellular staining methods. (B) IFN-γ expression in collected CD4⁺ T cells in each group. Numerical values on the histograms express the mean percentage of each fraction. (C) Percentage of IFN-γ⁺ cells in collected CD4⁺ T cells in each group. Data are indicated as the mean ± SEM of five
mice in each group. *, P<0.05.

Figure S3

CD3⁺CD4⁺ T cells isolated from IL-7Rα⁻/⁻ x RAG-2⁻/⁻ mice transferred with CD4⁺CD25⁻ T cells differentiated into CD44⁺CD62L⁻ effector-memory T cells. (A) FACS analysis shows the expression of CD44/CD62L, and CD69 on SP and LP CD4⁺ T cells from IL-7Rα⁻/⁻ x RAG-2⁻/⁻ mice and RAG-2⁻/⁻ mice transferred with CD4⁺CD25⁻ T cells, and RAG-2⁻/⁻ mice transferred with WT CD4⁺CD25⁻ T cells and CD4⁺CD25⁺ T_R cells at 8 wks after transfer. (B) Intracellular staining of cytokines (IL-17/IFN-γ) in the colonic LP CD4⁺ T cells. Numerical values on the dot plots and histograms express the mean percentage of each fraction.

Figure S4

Titration of WT CD4⁺CD25⁻ T cells transferred to IL-7Rα⁻/⁻ X RAG-2⁻/⁻ mice or RAG-2⁻/⁻ mice. (A) Further to know whether there are any difference in disease severity between IL-7Rα⁻/⁻ x RAG-2⁻/⁻ mice transferred with CD4⁺CD25⁻ T cells and RAG-2⁻/⁻ mice transferred with CD4⁺CD25⁻ T cells or not, we performed titration of numbers of transferred CD4⁺CD25⁻ T cells. RAG-2⁻/⁻ mice and IL-7Rα⁻/⁻ x RAG-2⁻/⁻ mice were transferred with 3x10⁵ or 1x10⁵ splenic WT CD4⁺CD25⁻ T cells. As a negative control, RAG-2⁻/⁻ mice were transferred with splenic WT CD4⁺CD25⁻ T cells (3x10⁵ cells/mouse) and CD4⁺CD25⁺ T_R cells (3x10⁵ cells/mouse). (B) Clinical scores were determined at 10 wk after the transfer as described in Materials and Methods. Data are indicated as the mean ± SEM of five mice in each group. *, P<0.05. (C) Histological
examination of the colon from the mice in each group. Original magnification x100. (D) Histological scoring after 10 wk after transfer. Data are indicated as the mean ± SEM of five mice in each group. *, P<0.05. (E) LP CD3⁺CD4⁺ T cells were isolated at 10 wk after transfer, and the number was determined by flow cytometry. Data are indicated as the mean ± SEM of five mice in each group. *, P<0.05. (F) Expression of MHC-classII on LP CD11b⁺CD11c⁺ and CD11b⁻CD11c⁺ dendritic cells. Numerical values on the dot plots and histograms express the mean percentage of each fraction.