IL-7 exacerbates chronic colitis with expansion of memory IL-7R<sup>high</sup> CD4<sup>+</sup> mucosal T cells in mice

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We have also shown a potential role for IL-7/IL-7R-mediated immune responses in intestinal inflammation (30, 31). First, IL-7 transgenic (Tg) mice developed chronic colitis that mimicked histopathological characteristics of human ulcerative colitis (UC). As chronic colitis developed, IL-7 Tg mice showed decreased expression of IL-7 protein in epithelial cells and significant infiltration of CD4<sup>+</sup>IL-7<sup>+</sup> T cells in the lamina propria. Second, we clarified that mucosal CD4<sup>+</sup> T cells expressing high levels of IL-7R (IL-7R<sup>high</sup>) are the pathogenic T cells that induce chronic colitis and are thus potential targets for treatment of murine chronic colitis (32). We demonstrated that transfer of IL-7R<sup>high</sup> CD4<sup>+</sup> LPLs isolated from colitic mice in immunodeficient mice induces chronic severe colitis. Importantly, the selective elimination of IL-7R<sup>high</sup> LPLs by administering small amounts of toxin-conjugated anti-IL-7R antibody completely ameliorated ongoing colitis (32). Third, dysregulation of the IL-7/IL-7R system is also observed in colonic mucosa of patients with UC at the active stage (unpublished data).

The mechanism of expansion and survival of IL-7R<sup>high</sup> CD4<sup>+</sup> LPLs in intestinal mucosa is, however, poorly understood. IL-7 is believed to act synergistically with IL-15 to regulate the potential for survival and homeostatic proliferation of memory CD8<sup>+</sup> T cells but not memory CD4<sup>+</sup> T cells (5, 24, 27), but controversy remains about whether IL-7 itself has a role in supporting the turnover of memory CD4<sup>+</sup> T cells. Recent reports indicated that memory CD4<sup>+</sup> T cells expressed high levels of IL-7R and responded to IL-7 by prolonged survival in vitro (10, 13, 25). This result indicated that IL-7 has previously unrecognized roles in the regulation of the survival and maintenance in at least a certain subset of memory CD4<sup>+</sup> T cells. Especially, it remains unknown whether IL-7 is involved in the dysregulated homeostasis of memory-type pathogenic T cells in autoimmune disorders. Because we have shown that CD4<sup>+</sup> LPLs in colitic mice express high levels of IL-7R, we sought to address whether IL-7 contributes to the expansion of those cells under pathogenic autoimmune conditions.

Here we provide evidence that, among LPLs generally exhibiting memory cell phenotypes, only those in mice with...
chronic colitis express high levels of IL-7R. Stimulation with exogenous recombinant IL-7 (rIL-7) alone in vitro induced significant proliferation of IL-7R$$^{hi}$$ memory CD4$$^{+}$$ LPLs isolated from colitic mice but had no effect on that of memory CD4$$^{+}$$ LPLs from wild-type (WT) mice. Other epithelial cell-derived cytokines, including IL-15 and thymic stromal lymphopoietin (TSLP), did not induce the proliferation of IL-7R$$^{hi}$$ CD4$$^{+}$$ LPLs. Of note, in vivo administration of rIL-7 caused colitis more quickly and in greater severity in mice transferred with CD4$$^{+}$$ LPLs from colitic mice. Moreover, inhibition of the IL-7/IL-7R pathway by administration of anti-IL-7R monoclonal antibody (MAb) significantly ameliorated colitis. These data indicate that IL-7 plays a crucial role in expansion of IL-7R$$^{hi}$$ mucosal T cells in memory CD4$$^{+}$$ cells and development of chronic colitis. They also suggest that therapeutic approaches targeting this pathway may be feasible in the treatment of human inflammatory bowel disease.

METHODS

Mice. Female C57BL/6 mice were purchased from Japan Clea (Tokyo, Japan). Female TCR-$$\alpha$$-deficient (TCR-$$\alpha$$-$$^{-/-}$$) mice with a background of C57BL/6 were purchased from Jackson Laboratory (Bar Harbor, ME). Female C57BL/6 recombinase-activating gene (RAG)-2-deficient (RAG-2$$^{-/-}$$) mice were kindly provided by Central Laboratories for Experimental Animals (Kawasaki, Japan). In some experiments, WT littermates were used as controls. Mice used for the transfer colitis experiments were aged 8–12 wk. All mice were maintained in the Animal Care Facility of Tokyo Medical and Dental University. The review board of the University approved the experimental animal studies.

Purification of T cell subsets. Spleens and mesenteric lymph nodes (MLNs) were mechanically dissociated, and the red blood cells were lysed by treatment with 0.84% NH4Cl. Cells were filtered through a 100-$$\mu$$m-pore-size nylon mesh and suspended in RPMI (Sigma-Aldrich, St. Louis, MO) containing 10% FCS. Splenic CD4$$^{+}$$ T cells were isolated from the cell suspension using the anti-CD4 MACS beads system (Miltenyi Biotec, Auburn, CA) according to the manufacturer’s instructions. For isolation of colonic LPLs, entire colons were opened longitudinally, washed with PBS, and cut into small (~3 mm) pieces. The dissected specimens were incubated twice with Ca$$^{2+}$$- and Mg$$^{2+}$$-free Hank’s balanced salt solution containing 1 mM dithiothreitol (GIBCO-BRL, Gaithersburg, MD) for 30 min at 37°C with gentle stirring. The residual tissue fragments were washed and incubated with collagenase A (Roche, Mannheim, Germany) for 2 h at 37°C. The supernatants were collected and washed, and the lymphocyte fraction was isolated on discontinuous Percoll gradients of 75 and 40%. Enriched CD4$$^{+}$$ T cells were obtained by positive selection using the anti-CD4 MACS magnetic separation system as described above. The resultant cells were shown to contain >96% CD4$$^{+}$$ cells by analysis using the FACS Calibur (Becton-Dickinson, Sunnyvale, CA).

Induction of colitis. In this study, the well-characterized CD4$$^{+}$$-CD45RB$$^{hi}$$-induced RAG-2$$^{-/-}$$ colitis (23) and spontaneous TCR-$$\alpha$$-$$^{-/-}$$ colitis (15) models were used. The LP CD4$$^{+}$$ T cells isolated from colitic TCR-$$\alpha$$-$$^{-/-}$$ (20-wk-old) mice were transferred into RAG-2$$^{-/-}$$ mice as previously described (32). All animals were weighing, and diarrhea was evaluated as an indicator of colitis.

Flow cytometry. The profiles of spleen, MLN and LP T cells were analyzed by flow cytometry. Cells were preincubated with a F(ab’)$_2$-blocking MAb (CD16/16; 2.4G2; BD PharMingen, San Diego, CA) for 20 min and then incubated with FITC-, phycoerythrin (PE)-, or biotin-labeled specific antibodies for 30 min on ice. Antibodies used were anti-CD4 MAb (RM4–5; BD PharMingen), anti-TCR-$$\beta$$ MAb (H57–597; BD PharMingen), anti-CD25 MAb (HT-2; BD PharMingen), anti-CD44 MAb (IM7; BD PharMingen), and anti-CD62L MAb (H57–597; BD PharMingen). To measure cytokine production, isolated CD4$$^{+}$$ T cells were seeded on 96-well round microtiter plates and cultured in 10% FCS-containing RPMI, supplemented with 2-mercaptoethanol (GIBCO), penicillin-streptomycin (GIBCO), l-glutamine (GIBCO), and HEPES (GIBCO). After 72 h of incubation, cells were pulsed for 12 h with [$$^{3}$$H]thymidine (1 $$\mu$$Ci/well), and the [% of annexin V-negative] by 10.220.33.2 on July 7, 2017 http://ajpgi.physiology.org/ Downloaded from

Proliferation assay. Splenic (2 × 10$$^{5}$$ cells/well), MLN (1 × 10$$^{5}$$ cells/well) and LP (1 × 10$$^{5}$$ cells/well) CD4$$^{+}$$ T cells were seeded on 96-well round microtiter plates and cultured in 10% FCS-containing RPMI, supplemented with 2-mercaptoethanol (GIBCO), penicillin streptomycin (GIBCO), l-glutamine (GIBCO), and HEPES (GIBCO). After 72 h of incubation, cells were pulsed for 12 h with [$$^{3}$$H]thymidine (1 $$\mu$$Ci/well), and the [% of annexin V-negative] by 10.220.33.2 on July 7, 2017 http://ajpgi.physiology.org/ Downloaded from

Detection of apoptosis. Isolated colonic CD4$$^{+}$$ LPLs resuspended in RPMI 1640 medium supplemented with 10% FCS and 1% penicillin-streptomycin were cultured in 96-well round microtiter plates at 1 × 10$$^{5}$$ cells/well in the absence or presence of rmIL-7 (50 ng/ml). After 10 days of culture, cells were washed with PBS and resuspended in a mixture of 100 µl diluted binding buffer and 5 µl of annexin V-FITC (BD PharMingen) solution, and then 2 µl of 7-aminoactinomycin D were added. After 15 min of incubation at room temperature in the dark, flow cytometry analysis was performed using a FACS Caliber (Becton-Dickinson). A portion of cells was stained with PE-anti-CD4 MAb in parallel, and the percentage of surviving CD4$$^{+}$$ cells was determined by evaluating annexin V-negative cells. To analyze Bcl-2 expression in CD4$$^{+}$$ LPLs, these cells were cultured in medium supplemented with rmIL-7 (50 ng/ml). After 16 h of incubation, cells were fixed and permeabilized with BD Cytofix/Cytopherm solution before intracellular cytokine staining with FITC-conjugated anti-Bcl-2 MAb (3F11; BD PharMingen).

Cytokine assay. To measure cytokine production, isolated CD4$$^{+}$$ LPLs were cultured in medium supplemented with 1 µg/ml soluble anti-CD28 MAb (37.51; BD PharMingen) in 96-well plates precoated with 10 µg/ml anti-CD3 MAb (145–2C11; BD PharMingen) in PBS. Culture supernatants were collected, and the cytokine concentrations of IFN-$$\gamma$$, TNF-$$\alpha$$, IL-2, IL-4, and IL-5 were determined by flow cytometry with a cytokine bead array set (BD PharMingen) according to the manufacturer’s instructions.

Administration of IL-7 in colitic memory IL-7R$$^{hi}$$ CD4$$^{+}$$ LPL-exposed RAG-2$$^{-/-}$$ mice. CD4$$^{+}$$ LT cells were isolated from colitic TCR-$$\alpha$$-$$^{-/-}$$ mice (20 wk of age) by use of anti-CD4 MACS beads. The purified CD4$$^{+}$$ LPLs (1.5 × 10$$^{5}$$) were intraperitoneally transferred to RAG-2$$^{-/-}$$ mice. Either rmIL-7 (5 µg/day ip on days 0–7) or PBS alone was administered to these mice intraperitoneally, and the effects of IL-7 on the development of colitis were evaluated.

Administration of anti-IL-7 MAb for diseased TCR-$$\alpha$$-$$^{-/-}$$ mice. TCR-$$\alpha$$-$$^{-/-}$$ mice (8-wk-old) were treated with rat anti-murine IL-7R MAb (A7R34) by intraperitoneal injection of 1 mg dose one time per week for 8 wk. Control mice were treated with the same amounts of purified rat IgG (Sigma-Aldrich). All mice were killed on the day after the last treatment, and colitic lesions were evaluated.

Clinical and histological analysis. Mice were weighed and monitored for appearance and signs of soft stool or diarrhea three times per week. After death, clinical scores were assessed as the sum of the following four parameters: hunching and wasting, 0 or 1; colon thickening, 0–3 (0, no colon thickening; 1, mild thickening; 2, moderate thickening; 3, extensive thickening); and stool consistency, 0–3 (0, normal beaded stool; 1, soft stool; 2, diarrhea; 3, gross bloody stool; see Ref. 9). For histological scoring, the area most affected was

G746 IL-7 EXACERBATES MURINE CHRONIC COLITIS

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selected and graded by the number and severity of lesions. The mean degree of inflammation in the colon was calculated using a modification of a previously described scoring system (2).

Statistical analysis. The results are expressed as means ± SD. For statistical analysis, we used the program Statview for Macintosh and MS Office (Excel) and analyzed the data by Student’s t-test.

**RESULTS**

**IL-7R<sup>high</sup> expression in memory CD4<sup>+</sup> LPLs from colitic mice.** To determine whether IL-7 directly promotes the expansion of CD4<sup>+</sup> T cells in colonic mucosa and the development of chronic colitis, we first investigated the levels of IL-7R expression on CD4<sup>+</sup> LPLs in nondiseased (6-wk-old) or diseased TCR-α<sup>-/-</sup> (18-wk-old) mice, diseased C57BL/6 RAG-2<sup>-/-</sup> mice reconstituted with CD4<sup>+</sup>CD45RB<sup>high</sup> cells for 6 wk, and WT mice (8 wk old). Flow cytometric analysis revealed that the degree of IL-7R expression on CD4<sup>+</sup> LPLs from colitic TCR-α<sup>-/-</sup> mice was significantly higher than that in nondiseased TCR-α<sup>-/-</sup> or WT mice (*P* < 0.01; Fig. 1A). The mean fluorescence intensities (MFI) of IL-7R expression in the flow cytometric histogram were 490 ± 34 in CD4<sup>+</sup> LPLs from diseased TCR-α<sup>-/-</sup> mice, 243 ± 27 from nondiseased TCR-α<sup>-/-</sup> mice, and 173 ± 37 from WT mice. The degree of IL-7R expression on CD4<sup>+</sup> LPLs from colitic RAG-2<sup>-/-</sup> mice transferred with CD4<sup>+</sup>CD45RB<sup>high</sup> cells was also high (MFI: 266 ± 74), but not comparable to the level in CD4<sup>+</sup> LPLs from diseased TCR-α<sup>-/-</sup> mice. In contrast, the expression levels of IL-7R on CD4<sup>+</sup> T cells from spleen and MLNs showed no significant differences between the groups (data not shown).

We next assessed CD44, CD62L, and CD45RB expression on CD4<sup>+</sup> LPLs from colitic mice and WT mice. All CD4<sup>+</sup> LPLs from colitic TCR-α<sup>-/-</sup> mice, colitic RAG-2<sup>-/-</sup> mice transferred with CD4<sup>+</sup>CD45RB<sup>high</sup> cells and WT mice showed phenotypes of memory cells with CD44<sup>high</sup>, CD62L<sup>low</sup>, and CD45RB<sup>high</sup> (Fig. 1B). These results indicate that CD4<sup>+</sup> LPLs from all mice show phenotypes of memory cells, but only LPLs in colonic mucosa from colitic mice, especially colitic TCR-α<sup>-/-</sup> mice, express high levels of IL-7R.

**Stimulation with rIL-7 alone in vitro induced expansion and survival of IL-7R<sup>high</sup> memory CD4<sup>+</sup> LPLs isolated from colitic mice.** To clarify the mechanism by which CD4<sup>+</sup>IL-7R<sup>high</sup> LPLs infiltrated and expanded in the LP, we performed in vitro cell proliferation studies. To determine the effect of IL-7 on CD4<sup>+</sup> LPLs from diseased or nondiseased TCR-α<sup>-/-</sup> mice, diseased CD4<sup>+</sup>CD45RB<sup>high</sup> T cell-transferred mice and WT mice were stimulated in vitro with exogenously added rIL-7. Surprisingly, stimulation with rIL-7 alone induced significant increase in DNA synthesis of IL-7R<sup>high</sup> memory CD4<sup>+</sup> LPLs from both types of colitis mice in a concentration-dependent manner (Fig. 2). In sharp contrast, rIL-7 alone did not enhance the proliferation of CD4<sup>+</sup> LPLs from WT mice. Interestingly, CD4<sup>+</sup> LPLs from diseased TCR-α<sup>-/-</sup> mice showed higher proliferative responses to rIL-7 than with those from diseased CD4<sup>+</sup>CD45RB<sup>high</sup>-transferred mice, reflecting the higher levels of IL-7R expression, as shown in Fig. 1A. Consistent with the lower expression of IL-7R on LPLs from nondiseased TCR-α<sup>-/-</sup> mice (Fig. 1A) than that from the paired diseased TCR-α<sup>-/-</sup> mice, the proliferative responses of LPLs by rIL-7 from nondiseased mice were significantly lower than those from the paired diseased mice (Fig. 2). Furthermore, rIL-7 alone induced a marginal effect on the proliferation of spleen CD4<sup>+</sup> cells and MLN CD4<sup>+</sup> cells from diseased and nondiseased TCR-α<sup>-/-</sup> mice or diseased CD4<sup>+</sup>CD45RB<sup>high</sup>-transferred mice and WT mice.
Stimulation with recombinant (r) IL-7 alone in vitro induced expansion of colitic IL-7R<sup>high</sup> memory CD4<sup>+</sup> LPLs. Stimulation with rIL-7 alone in vitro induced significant increase in DNA synthesis of IL-7R<sup>high</sup> memory CD4<sup>+</sup> LPLs from diseased TCR<sup>+</sup> mice (n = 9), but not from nondiseased TCR<sup>+</sup> mice (n = 4) or WT mice (n = 16), in a concentration-dependent manner (*P < 0.01, **P < 0.001). CD4<sup>+</sup> LPLs from colitic CD4<sup>+</sup> CD45RB<sup>high</sup>-transferred mice showed smaller proliferative responses to rIL-7 than those from colitic TCR<sup>+</sup> mice, reflecting relatively high levels of IL-7R expression (see Fig. 1A). rIL-7 alone induced a marginal effect on the proliferation of spleen CD4<sup>+</sup> cells and mesenteric lymph node (MLN) cells from diseased or nondiseased TCR<sup>+</sup> mice, colitic RAG-2<sup>−/−</sup> mice transferred with CD4<sup>+</sup> CD45RB<sup>high</sup> cells, and WT mice.

Effects of different epithelial cell-derived cytokines on the proliferation of the CD4<sup>+</sup> LPLs. The responses of CD4<sup>+</sup> LPLs to rIL-7, rIL-15, and recombinant thymic stromal lymphopoietin (rTSLP) were compared. rIL-7 alone significantly increased DNA synthesis in CD4<sup>+</sup> LPLs from colitic TCR<sup>−/−</sup> mice or colitic CD45RB<sup>high</sup>-trf mice. In contrast, neither rIL-15 alone nor rTSLP alone could induce the proliferation of CD4<sup>+</sup> LPLs from colitic mice or WT mice (n = 5/group).
To further examine whether IL-7 is specifically involved in the proliferative responses of colitic IL-7R<sup>hi</sup>CD4<sup>+</sup> LPLs, we assessed the effect of other cytokines secreted by colonic epithelial cells on the proliferation of the CD4<sup>+</sup> LPLs. One such factor is IL-15, since previous studies revealed that IL-15 is secreted from intestinal epithelial cells (6) and aberrant production of IL-15 results in chronic colitis (14). Another factor that might induce proliferation of IL-7R<sup>hi</sup>CD4<sup>+</sup> LPLs is TSLP. This cytokine shares the IL-7R<sub>x</sub> chain with IL-7 (3, 20, 22), and its expression is increased in colitic mucosa from TCR<sub>+</sub>/H9251/H11002<sub>+</sub> mice. TSLP is secreted from intestinal epithelial cells (6) and aberrant production of TSLP has been shown to exacerbate murine chronic colitis (7). We next tested whether IL-7 promotes the survival of IL-7R<sup>hi</sup> memory CD4<sup>+</sup> LPLs isolated from colitic CD4<sup>+</sup>CD45RB<sup>hi</sup>-transferred mice. As shown in Fig. 4A, the number of IL-7R<sup>hi</sup>CD4<sup>+</sup> LPLs isolated from colitic mice significantly increased in culture with rIL-7 alone compared with that in culture in the absence of rIL-7. Counts of cultured cells with rIL-7 reached 10-fold of those cultured in the absence of rIL-7. We further examined the apoptosis of those cells in the presence or the absence of rIL-7. After 10 days of culture, >85% of IL-7R<sup>hi</sup>CD4<sup>+</sup> LPLs cultured in the presence of rIL-7 were annexin V negative, whereas almost all cells cultured in the absence of rIL-7 were annexin V positive (Fig. 4B). To further analyze Bcl-2 expression in CD4<sup>+</sup> LPLs cultured with rIL-7, isolated CD4<sup>+</sup> LPLs and splenic CD4<sup>+</sup> cells from colitic CD45RB<sup>hi</sup>-transferred mice were cultured in medium supplemented with rIL-7 (50 ng/ml). Flow cytometric analysis revealed that the degree of Bcl-2 expression in isolated CD4<sup>+</sup> LPLs or splenic CD4<sup>+</sup> cells cultured with rIL-7 was upregulated (Fig. 4C). All these data raise the possibility that IL-7 promotes both in vitro proliferative expansion and survival of IL-7R<sup>hi</sup>CD4<sup>+</sup> memory LPLs that infiltrate the colonic mucosa with chronic colitis.

Because Park and colleagues (21) have recently demonstrated that IL-7 itself suppressed IL-7R<sub>x</sub> expression as a novel mechanism for maximizing IL-7-dependent T cell survival, we next evaluated the IL-7R expression on cultured IL-7R<sup>hi</sup>CD4<sup>+</sup> LPLs cells with rIL-7. Interestingly, CD4<sup>+</sup> LPLs from colitic TCR<sub>+</sub>/H9251/H11002<sub>+</sub> mice, which initially expressed a high level of IL-7R, were significantly downmodulated after the

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Fig. 4. IL-7 promotes survival in vitro of colitic IL-7R<sup>hi</sup> memory CD4<sup>+</sup> LPLs. A: no. of IL-7R<sup>hi</sup>CD4<sup>+</sup> LPLs isolated from colitic CD45RB<sup>hi</sup>-transferred mice increased significantly (*P < 0.0001, **P < 0.001, and †P < 0.01) in culture with rIL-7 alone (50 ng/ml), reaching 10-fold the count in culture without rIL-7 (n = 5). B: IL-7 suppresses apoptosis of colitic IL-7R<sup>hi</sup>CD4<sup>+</sup> LPLs. To determine whether IL-7 increased the survival of memory-phenotype IL-7R<sup>hi</sup>CD4<sup>+</sup> LPLs, we examined apoptosis of colitic IL-7R<sup>hi</sup>CD4<sup>+</sup> LPLs in the presence or the absence of rIL-7 (50 ng/ml). After 10 days of culture, >85% of IL-7R<sup>hi</sup>CD4<sup>+</sup> LPLs treated with rIL-7 were annexin V negative, whereas almost all cells cultured in the absence of rIL-7 were annexin V positive (*P < 0.0001, **P < 0.001, and †P < 0.01). C: IL-7 upregulated Bcl-2 in colitic IL-7R<sup>hi</sup>CD4<sup>+</sup> LPLs. To analyze Bcl-2 expression in CD4<sup>+</sup> LPLs simulated with rIL-7, isolated CD4<sup>+</sup> LPLs and spleen CD4<sup>+</sup> cells from colitic CD45RB<sup>hi</sup>-transferred mice were cultured with rIL-7 (50 ng/ml). Flow cytometric analysis revealed that the degree of Bcl-2 expression was substantially increased after stimulation with rIL-7 in CD4<sup>+</sup> LPLs from colitic mice. D: IL-7 suppresses expression on cultured IL-7R<sup>hi</sup>CD4<sup>+</sup> LPLs with rIL-7. CD4<sup>+</sup> LPLs from colitic TCR<sub>+</sub>/H9251/H11002<sub>+</sub> mice, which initially expressed a high level of IL-7R, were significantly (*P < 0.001 and **P < 0.01) downmodulated after the culture with rIL-7 compared with those cultured without rIL-7.
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IL-7 EXACERBATES MURINE CHRONIC COLITIS

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culture with rIL-7 compared with those cultured without rIL-7 (Fig. 4D).

In vivo administration of IL-7 exacerbates colitis in RAG-2−/− mice transferred with IL-7R<sup>hi</sup>CD4<sup>+</sup> TCR-α−/− LPLs. To assess whether the enhancing effect of IL-7 on the expansion of IL-7R<sup>hi</sup>CD4<sup>+</sup> LPLs in vitro is likewise observed in vivo, we tested whether in vivo rIL-7 administration might influence the colonic inflammation-induced RAG-2−/− mice transferred with IL-7R<sup>hi</sup>CD4<sup>+</sup> LPLs isolated from colitic TCR-α−/− mice (Fig. 5A). Our previous studies showed that recipient mice transferred with 1.5 × 10<sup>5</sup> IL-7R<sup>hi</sup>CD4<sup>+</sup> LPLs from colitic TCR-α−/− mice developed colitis within 6 wk after the transfer. rIL-7 was intraperitoneally injected in those recipient mice daily on days 0–7 after the cell transfer. Intriguingly, IL-7–treated recipient mice, but not PBS–treated mice, rapidly showed severe weight loss and wasting disease at 2–3 wk after the transfer, and we killed all recipient mice at 3 wk after the transfer.

At 3 wk after the transfer, gross inspection of the colon in recipient mice treated with rIL-7 revealed increased inflammatory activity compared with the PBS–injected control group (Fig. 5B). Moreover, the clinical score of mice treated with rIL-7 was significantly (P < 0.01) higher than that of PBS–treated controls (Fig. 5C). Histological analysis of colonic mucosa showed development of severe colitis in recipient mice treated with rIL-7 (Fig. 5D). In contrast, mice treated with PBS alone developed only mild colitis at 3 wk after the transfer. As expected, the histological score assessing the severity of inflammation was significantly (P < 0.05) increased in mice treated with rIL-7 compared with PBS–injected mice (Fig. 5E). Total cell numbers of isolated colonic LPLs were significantly (P < 0.05) increased in mice treated with rIL-7 administration (Fig. 5F). These results indicate that IL-7–treated mice have an escalating effect on the onset of colitis in RAG-2−/− mice transferred with IL-7R<sup>hi</sup>CD4<sup>+</sup> memory T cells.

Cytokine production of isolated CD4<sup>+</sup> LPLs in RAG-2−/− mice after stimulation with anti-CD3 MAbs and anti-CD28 MAbs were quite different. Isolated CD4<sup>+</sup> LPLs from mice treated with rIL-7 produced significantly higher amounts of IFN-γ (P < 0.001) and TNF-α (P < 0.001), but not IL-2, IL-4, and IL-5 (P < 0.05), compared with those from PBS–treated mice (Fig. 5G).

Interestingly, flow cytometric analysis revealed that the degree of IL-7R expression in isolated CD4<sup>+</sup> LPLs of recipient mice treated with rIL-7 or PBS was significantly decreased compared with that in freshly isolated CD4<sup>+</sup> LPLs from colitic TCR-α−/− mice but still higher than that on normal CD4<sup>+</sup> LPLs (Fig. 5H). In CD4<sup>+</sup> LPLs of recipient mice treated with rIL-7, TCR-β<sup>dim</sup> cells were significantly increased and IL-7R expression on CD4<sup>+</sup> LPLs was higher than that in spleen CD4<sup>+</sup> cells (Fig. 5I). In contrast, the expression of IL-7R on LPLs from WT mice was much lower than that in mice treated with rIL-7. These infiltrated CD4<sup>+</sup> IL-7R<sup>hi</sup> LPLs from rIL-7–treated recipient mice with severe colitis mainly consisted of memory cells with CD44<sup>high</sup>, CD62L<sup>low</sup>, and CD45RB<sup>low</sup> (Fig. 5J). Collectively, these data indicate that in vivo administration of rIL-7 worsens chronic colitis, probably through the expansion of memory CD4<sup>+</sup> LPLs in RAG-2−/− mice transferred with LPLs from colitic mice.

**Blockade of the IL-7/IL-7R pathway ameliorated colitis with decrease of memory CD4<sup>+</sup> LPLs.** To prove the role of IL-7 in the exacerbation of chronic colitis with expansion of IL-7R<sup>hi</sup> LPLs, we finally attempted to control chronic colitis by inhibiting the IL-7/IL-7R pathway using a blocking antibody–based strategy. We found that 50 μg/ml of anti-IL-7R MAb used in this assay efficiently inhibited in vitro proliferation of CD4<sup>+</sup> LPLs from chronically inflamed mucosa of TCR-α−/− mice (data not shown). In this setting, we used established TCR-α−/− mice rather than the above–mentioned RAG-2−/− mice reconstituted with CD4<sup>+</sup> IL-7R<sup>hi</sup> memory T cells, with the scope of clinical application to human inflammatory bowel disease. Because TCR-α−/− mice were shown to develop colitis at 12–16 wk of age in our breeding laboratory, we started the treatment at 8 wk of age. We diagnosed these mice by observing weight loss, ruffle appearance, signs of soft stool and diarrhea. We treated chronic colitis in TCR-α−/− mice by intraperitoneal injection of 1 mg anti-IL-7R MAb weekly for 8 wk (Fig. 6A). Blockade of the IL-7/IL-7R pathway by anti-IL-7R MAb significantly ameliorated the development of chronic colitis in TCR-α−/− mice. In clear contrast, TCR-α−/− mice treated in parallel with an isotype–matched control antibody developed severe colitis, as shown by diarrhea, bloody stool, and weight loss. Gross inspection and histological analysis of the colon in anti-IL-7R MAb–treated TCR-α−/− mice revealed marked reduction in the inflammatory activity (Fig. 6B). This difference was confirmed by histological scoring of multiple colon sections, which gave scores of 1.33 ± 0.21 in anti-IL-7R MAb–treated mice vs. 2.67 ± 0.21 in control rat IgG–treated mice (P < 0.01; Fig. 6C). In addition, the number of CD4<sup>+</sup> LPLs was significantly decreased in mice treated with anti-IL-7R MAb (Fig. 6D). These results indicate that blockade of the IL-7/IL-7R pathway leads to inhibition of chronic colitis with decreased memory CD4<sup>+</sup> LPLs.

**DISCUSSION**

The most important findings of the present study were that exogenously added IL-7 directly promoted the expansion and...
The number of CD4\(^{+}\)/IL-10\(^{+}\)MAb-treated TCR-\(\alpha^{+}\)/IL-10\(^{+}\)transferred LPLs reached 100 times the colonic mucosa of recipient mice, and their selective elimination by small amounts of toxin-conjugated anti-IL-7R\(\alpha\) antibody completely ameliorated ongoing colitis (32). The reason for the substantial proliferative response of the IL-7R\(^{+}\)/CD4\(^{+}\)LPLs remains unclear, because IL-7 production in the epithelial cells was conversely decreased according to goblet depletion (unpublished observations). Recent studies have indicated that IL-7 is implicated in the survival and homeostatic proliferation of naive CD4\(^{+}\) T cells (12, 24, 27, 28). It was also demonstrated that IL-7 could have previously unrecognized roles in the survival and generation of memory CD4\(^{+}\) cells (10, 13, 25). For instance, low doses of IL-7 serve as a survival factor for effector memory CD4\(^{+}\) cells, and high doses of IL-7 induce proliferation of memory cells (13). IL-7 directly regulates both survival and expansion of memory CD4\(^{+}\) cells (10, 13, 25).

Kondrack et al. (10) demonstrated that memory CD4\(^{+}\) cells expressing comparable levels of IL-7R to naive T cells show sustained cellular survival in response to IL-7 in vivo. Thus, IL-7 would be a good candidate for proliferation and survival of colitogenic IL-7R\(^{+}\)/CD4\(^{+}\) memory LPLs. In the current study, we demonstrated that in vitro stimulation with IL-7, but not IL-15 and TSLP, induced a proliferative response in IL-7R\(^{+}\)/CD4\(^{+}\) LPLs from colitic TCR-\(\alpha^{+}\)/IL-15\(^{+}\) mice or colitic CD4\(^{+}\)CD45RB\(^{+}\)/T cell-transferred mice. In sharp contrast, memory CD4\(^{+}\) LPLs from nondiseased TCR-\(\alpha^{+}\)/IL-15\(^{+}\) mice or WT mice did not respond to IL-7. Interestingly, CD4\(^{+}\) LPLs from colitic TCR-\(\alpha^{+}\)/IL-15\(^{+}\) mice showed more potent ability to proliferate than those from colitic CD4\(^{+}\)CD45RB\(^{+}\)/transferred mice, suggesting that the proliferation response on IL-7 stimulation depends on the expression levels of IL-7R. However, the possibility cannot be excluded that reactivities to IL-7 are dependent on the Th2-dominant (TCR-\(\alpha^{+}\)/IL-15\(^{+}\)) or Th1-dominant (CD4\(^{+}\)CD45RB\(^{+}\)/transferred) mouse strain. Upon continuous in vitro stimulation with IL-7, colitic CD4\(^{+}\) LPLs survived and expanded for 10 days in this study (Fig. 4D), and actually survived for several weeks in the following study (unpublished observation). These data indicate that IL-7 promotes both expansion and survival in vitro of IL-7R\(^{+}\)/memory CD4\(^{+}\) LPLs that infiltrated the colonic mucosa with chronic colitis.

In the present study, we also demonstrated that in vivo administration of IL-7 worsened chronic colitis with expansion of IL-7R\(^{+}\)/memory CD4\(^{+}\) LPLs in RAG-2\(^{-/-}\) mice transferred with those LPLs from colitic TCR-\(\alpha^{+}\)/IL-15\(^{+}\) mice. Moreover, blockade of IL-7R by an antibody-based strategy in vivo led to inhibition of colitis with decreased memory CD4\(^{+}\) LPLs. Therefore, we conclude that IL-7 itself plays crucial roles in the expansion of IL-7R\(^{+}\)/memory CD4\(^{+}\) mucosal T cells and exacerbation of chronic colitis, although it is also possible that yet-unknown cytokines are involved in this mechanism.

We have recently demonstrated that intestinal epithelial cells also express IL-7R and that bone marrow-derived epithelial cells can differentiate into the epithelium of the gastrointestinal tract in humans (18, 19). Epithelial cells of male donor origin were distributed throughout the entire gastrointestinal tract of female bone marrow transplant recipients. Donor-derived epithelial cells remarkably repopulated the gastrointestinal tract during epithelial regeneration. Therefore, we suggest that the IL-7-IL-7R pathway may have previously unrecognized roles in the mucosal immune system.
Surprisingly, however, we found that IL-7R expression on IL-7-stimulated IL-7R$^{high}$CD4$^{+}$ LPLs was significantly downmodulated compared with that on freshly isolated IL-7R$^{high}$CD4$^{+}$ LPLs in vitro. Similarly, Park and colleagues (21) very recently demonstrated a novel regulatory mechanism that specifically suppressed IL-7R$\alpha$ expression on normal murine T cells in response to IL-7. Furthermore, we have previously demonstrated that IL-7 expression in colonic epithelial cells is decreased in colonic mucosa with murine chronic colitis, such as IL-7 Tg mice and TCR-\(\alpha\)/\(\beta\)-/ mice (30). Therefore, we assessed the effect of other cytokines, such as IL-15 and TSLP, secreted by colonic epithelial cells on the expansion of IL-7R$^{high}$ memory CD4$^{+}$ LPLs in chronic colitic lesions. Our results revealed that neither IL-15 nor TSLP alone induced the proliferation of IL-7R$^{high}$ memory CD4$^{+}$ LPLs from colitic mice or IL-7R$^{low}$ memory CD4$^{+}$ LPLs from WT mice. Collectively, these results indicate that IL-7 from a source outside the intestine or yet-unknown cytokines may be responsible for the proliferation of IL-7R$^{high}$CD4$^{+}$ LPLs on colitic mucosa. Consistent with this hypothesis, a recent report has shown that serum concentration of IL-7 is strongly upregulated, and IL-7 is produced by dendritic-like cells within peripheral lymphoid tissues in human immunodeficiency virus-infected patients (17). Furthermore, it is also possible that colitogenic IL-7 highly sensitive IL-7R$^{high}$ CD4$^{+}$ LPLs can sufficiently respond to decreased IL-7 in the inflamed mucosa, although the mechanism of the sustained IL-7R$^{high}$ expression on pathogenic CD4$^{+}$ LPLs remains largely unclear. Further study will be needed to address this issue.

We have some evidence that the IL-7-IL-7R pathway in the colonic mucosa is disturbed in human UC (unpublished observation). Human inflammatory bowel disease is thought to result from inappropriate activation of the mucosal immune system driven by luminal antigens. The activation of important cell populations is eventually accomplished by the production of a wide variety of nonspecific mediators of inflammation, including many other inflammatory and proinflammatory cytokines, chemokines, and growth factors. We suggest that IL-7R$^{high}$ memory CD4$^{+}$ LPLs are one such important cell population. Therefore, this study provides a basis for practical application of therapy targeting the IL-7-IL-7R pathway for the treatment of chronic intestinal inflammation in human inflammatory bowel disease.

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