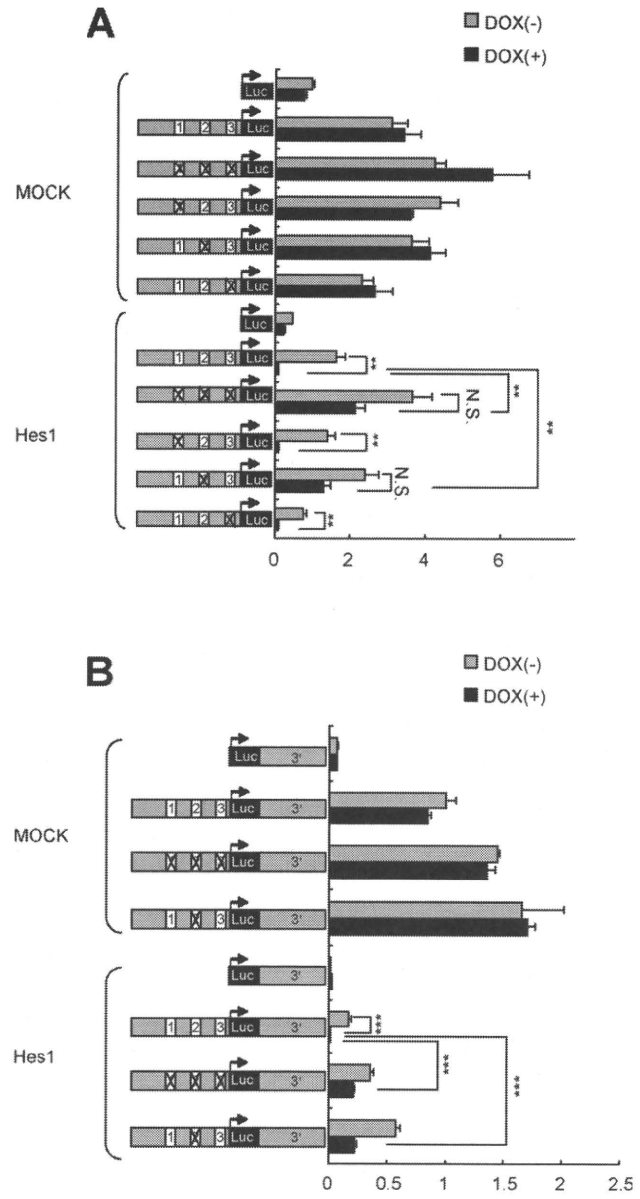


FIGURE 4. Hes1 regulates the transcriptional activity of Hath1 via 5′ promoter region. (A) 5′ Hath1 reporter plasmid containing the 1000-bp upstream region of Hath1 was transfected into LS174T Tet-on Hes1 cells and LS174T cells transfected with a mock plasmid. The induction of Hes1 by DOX significantly decreased the transcriptional activity on Hath1, whereas the transcriptional activity of the mock plasmid did not change. Three regions that matched the consensus sequence for binding Hes1, the Class C site, in the 1000-bp upstream region of Hath1 are indicated as square numbers. Reporter activity of a mutant with all regions of the Hes1 binding site deleted was not suppressed by Hes1 expression. A mutant construct in which only the second region of the Hes1 binding site was deleted was also unaffected by Hes1. (B) Hath1 reporter plasmid containing the 3′ enhancer region of Hath1 behind the luciferase sequence was inserted into 5′ Hath1 reporter plasmid. Hes1 also suppressed Hath1 transcriptional activity enhanced by 3′ enhancer region. The deletion mutants of the Hes1 binding site in the 5′ region of Hath1 were also unaffected by Hes1 expression (B). (**$P < 0.01$, ***$P < 0.001$, $n = 3$).

including the Hes1 binding sites but not 3′ region of the Hes1 binding sites (Fig. 5B), supporting the idea that Hes1 binds directly to the 5′region of Hath1 to suppress the transcriptional activity in IEC.

## Hes1 Does Not Completely Block the Transcriptional Activity of Hath1 Promoted by CDX2

To clarify the balance between the enhancer and the repressor in Hath1 transcriptional activity, we next assessed whether CDX2, which promotes *Atoh1* gene transcription in mice, is affected by Notch signaling on Hath1 transcription. Treatment with GSI showed slight induction of CDX2 in LS174T cells (Fig. 6A). Moreover, HES1 expression did not affect the expression of CDX2 (Fig. 6B), suggesting that the expression of CDX2 may be independent of Notch signaling. To assess the effect of CDX2 on Hath1 transcription regulated by HES1, a reporter assay of Hath1 was performed. Although CDX2 did not promote Hath1 transcription via the 5′ promoter region of Hath1 (Fig. 6C), CDX2 cotransfected with the reporter plasmid containing the 3′ enhancer region of Hath1 showed significant increase of transcriptional activity of Hath1 (Fig. 6D). Interestingly, the transcriptional activity of Hath1 promoted by CDX2 was not suppressed by Hes1 induction in LS174T tet-HES1 cells. These results suggest that Hes1 at the 5′ region of Hath1 could not completely abrogate the transcriptional activity of Hath1 promoted via the 3′ enhancer region by CDX2, and *Hath1* gene expression might be regulated by the balance between HES1 and CDX2.

## Hath1 Protein Expression Is Decreased in the Goblet Cell Depletion of UC

We finally assessed whether Hath1 is decreased in colon mucosa with goblet cell depletion in line with the former results in vitro. In normal colonic mucosa, Hath1 and CDX2 were expressed in almost all IECs. In contrast, Hes1 was expressed in IECs situated in the lower half of the villi (Fig. 7). In UC patients, both Hath1 and CDX2 disappeared, while Hes1-positive cells were extended at the top of the villi (Fig. 7), indicating that the suppression of Hath1 in goblet cell depletion might be caused by both the disappearance of CDX2 and the extension of Hes1-positive cells.

## DISCUSSION

This study reveals for the first time that Hes1 directly suppresses *Hath1* gene expression via the Notch signal, indicating that downregulation of Hath1 is associated with goblet cell depletion in human UC in combination with the disappearance of CDX2. Previous reports have suggested that Notch signaling suppressed the phenotypic gene expression of goblet cells by suppressing *Atoh1* gene expression,[5] although it remains unknown how Notch signaling suppresses

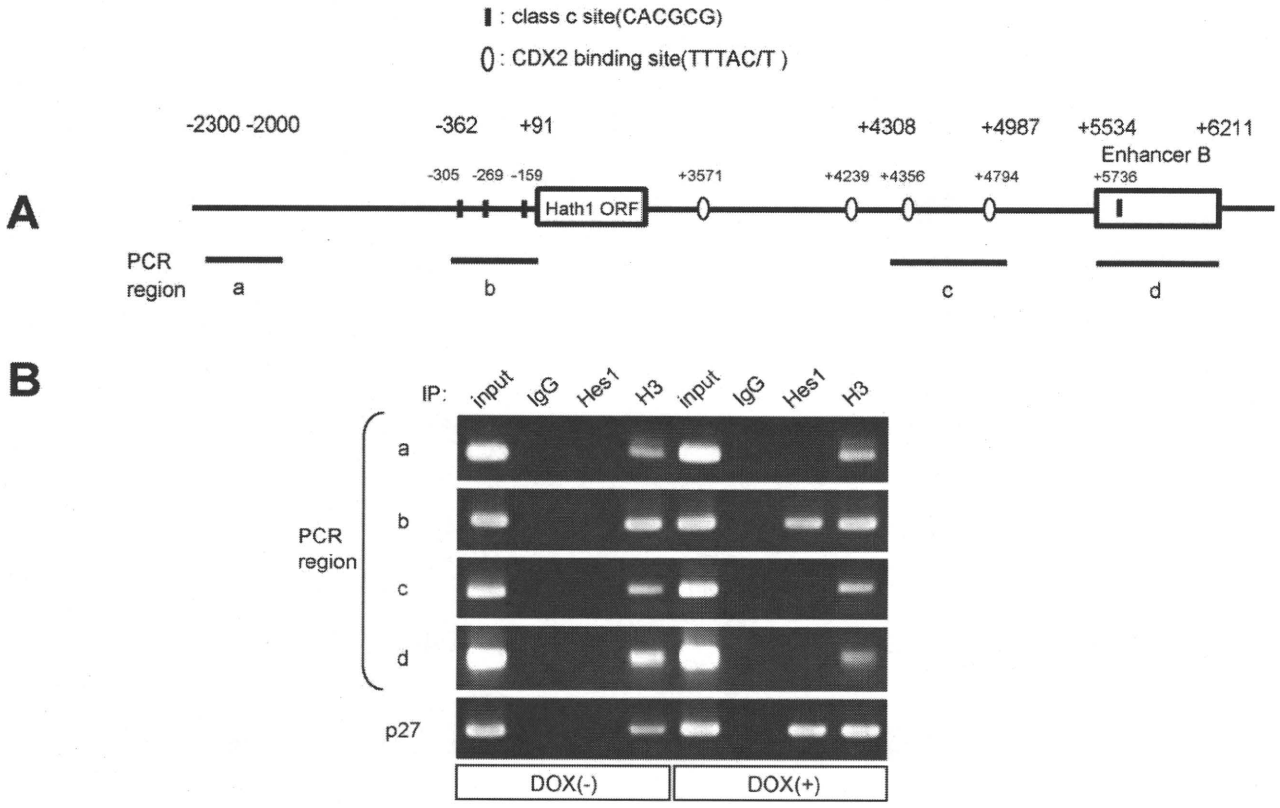


FIGURE 5. Hes1 binds to 5′ Hath1 promoter region. (A) Schematic presentation of Hath1 genome. (B) ChIP assay was performed using LS174T Tet-on Hes1 cells with or without DOX treatment for 24 hours. Each region indicated by a letter in (A) was amplified from the immunoprecipitant by each antibody. The amplification of p27 from the immunoprecipitant by Hes1 antibody was confirmed to be the known region of the Hes1 binding site. Only the 5′ region including the Hes1 binding sites of Hath1 (region b) was amplified from the immunoprecipitant by Hes1 antibody under the induction of Hes1 expression by DOX.

*Hath1* gene expression. We first found that Hes1, but not HeyL, was necessary and sufficient for the suppression of *Hath1* gene expression by Notch signaling in IEC. Canonical Notch signaling leads to transcriptional activation of Hes family and Hey family genes such as Hes1, Hes5, Hes7, Hey1, Hey2, and HeyL by binding NICD to RBP-Jk.[20] Hes and Hey family genes play important roles in the differentiation of various tissues,[21,22] but it has not been clarified how the function of each gene is assigned via Notch signaling. While we found that all Hes and Hey family genes were upregulated by NICD expression in intestinal cells, we also noticed that Hes1 and HeyL were exorbitantly expressed by NICD than other Hes and Hey family genes (data not shown), suggesting that the functional assignment of Notch signaling is regulated by the quantity of each Hes and Hey family gene expressed. HeyL has been identified as one of the target genes of Notch3 receptor, because HeyL is expressed in smooth muscle cells of the digestive tract and the vasculature following Notch3 expression in later stages of development.[23] In this study, we could not identify the function of HeyL in goblet cell differentiation; rather, its function is expected to be assessed in future study of the effect of Notch signaling on IEC.

On the other hand, we found that Hes1 is critical for the differentiation into goblet cells via Notch signaling, since the binding of HES1 to the Hath1 5′ promoter region silences *Hath1* gene expression. Although the 3′ region of Atoh1 has been characterized as the enhancer and repressor region to regulate *Hath1* gene expression by CDX2, Zic1, and Hic1, the function of the 5′ region of Atoh1 has not been clarified. This study revealed that the 5′ region of Hath1 is necessary not only for basic transcription but also for the regulation by HES1 via Notch signaling to presumably suppress the transcriptional activity of the basic transcription factors. It has been reported that Hes1 binds not only to the N-box sequence but also to class C sites to suppress the expression of genes such as $P27^{kip118}$ and achaete-scute homolog-1,[24] through which it plays a central role in cell proliferation and differentiation, respectively. In this study we identified a class C site at position −289 of the 5′ region of Hath1, playing a crucial role in the regulation of *Hath1* gene expression by the Notch signal. We therefore suspected that Hes1 might completely shut out the transcriptional activity via the

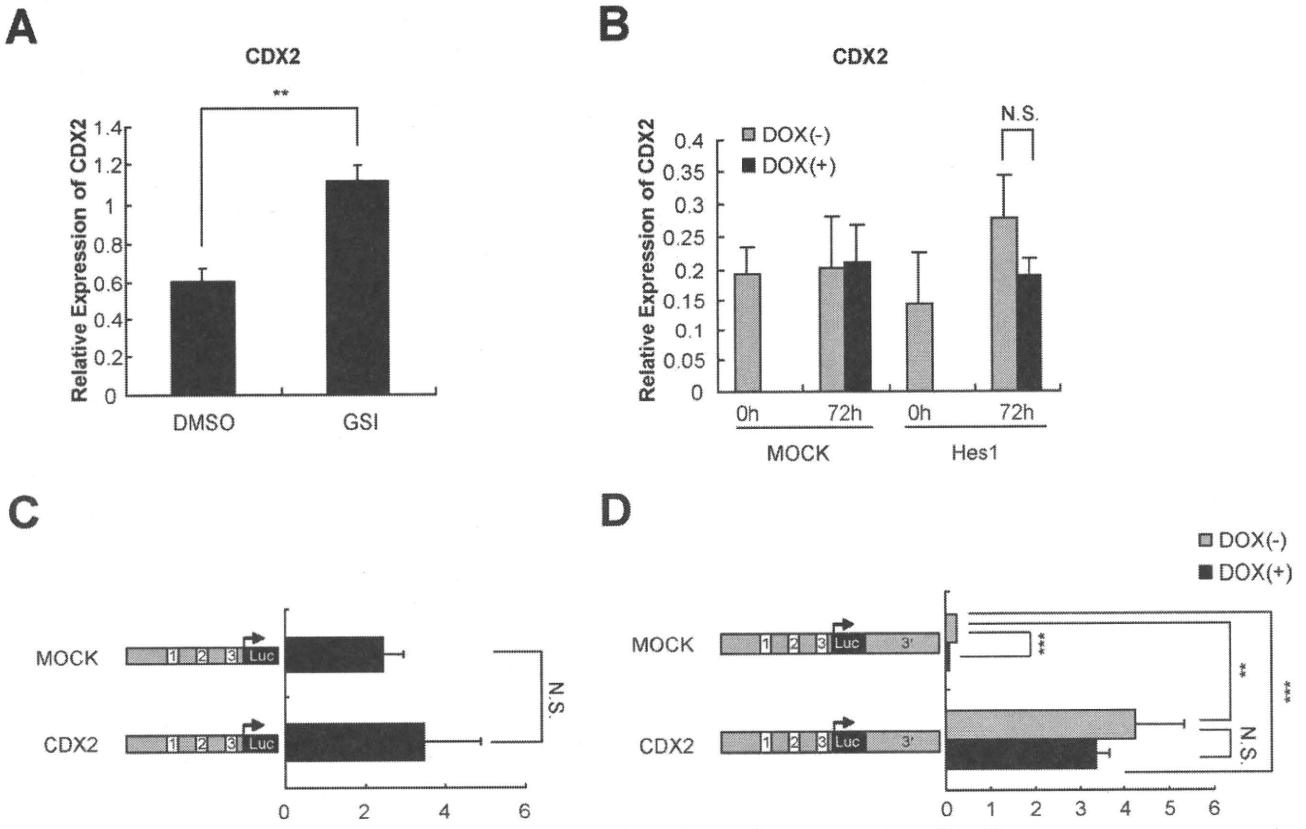


FIGURE 6. CDX2 enhances the transcriptional activity of Hath1 independently of Notch signaling. (A) *CDX2* gene expression was analyzed by treatment of LS174T cells with GSI for 72 hours. CDX2 was slightly upregulated by Notch signal inhibition. (B) *CDX2* gene expression was analyzed by the Hes1 expression induced by DOX in LS174T Tet-on Hes1 cells. *CDX2* gene expression was not affected by Hes1 expression. (C) Transcriptional activity of Hath1 via the 5′ region by CDX2 was assessed in LS174T cells for 72 hours after transfection of both the *CDX2* gene and 5′ Hath1 reporter plasmid. CDX2 did not affect the transcriptional activity via the 5′ promoter region of Hath1. (D) HES1 did not suppress the transcriptional activity via the 3′ region of Hath1 by forced expression of CDX2. The transcriptional activity of Hath1 was assessed for 72 hours after transfection of both the *CDX2* gene and 3′ Hath1 reporter plasmid with or without DOX in LS174T Tet-on HES1 cells. (**$P < 0.01$, ***$P < 0.001$, $n = 3$).

3′ enhancer region, but that forced expression of CDX2 could induce the transcriptional activity of Hath1 even with Hes1 expression. Moreover, the expression of CDX2 was not affected by Notch signaling, suggesting that CDX2 and HES1 independently regulate *Hath1* gene expression. Thus, regulation by Hes1 via Notch signaling is not sufficient to suppress the gene transcription of *Hath1*, indicating that the transcriptional activity of Hath1 is regulated by the balance between CDX2 and HES1 expression.

Importantly, the present study also indicated that Hath1 is essential to regulate goblet cell formation in UC. Although the expression of Hath1 in inflamed mucosa of UC has been reported,[25] the correlation between goblet cell content and Hath1 expression in UC has not been elucidated. We confirmed that Hath1 was expressed in inflamed mucosa with conserved goblet cell formation in UC (data not shown), since goblet cell content might correlate with Hath1 expression in UC. In Atoh1-deficient mice, secretory lineages of IEC including goblet cells are completely lost,[9,26] indicating that Hath1 might have the function of not only mucus production but also differentiation toward goblet cells in human intestine.

Moreover, this study suggested that goblet cell depletion in UC caused by the disappearance of Hath1 required not only HES1 expression but also CDX2 suppression of IEC. CDX2 has been reported to be downregulated in UC mucosa,[27] but it remains unknown how CDX2 expression is suppressed by colonic inflammation even though CDX2 is upregulated by inflammation in the esophagus and stomach.[28,29] One previous report indicated that CDX2 expression is suppressed by hypoxia inducible factor 1 (HIF1).[30] Another report found that HIF1 is overexpressed in UC mucosa,[31] suggesting that HIF1 might suppress CDX2 expression in UC. Whatever the case, the regulation of CDX2 expression of IEC should be assessed to clarify the mechanism of goblet cell depletion in UC.

In conclusion, we have revealed for the first time that Hes1 is sufficient to suppress *Hath1* gene transcription via the Notch signal, but insufficient to suppress *Hath1* gene transcription by CDX2. The cooperation between Hes1 and

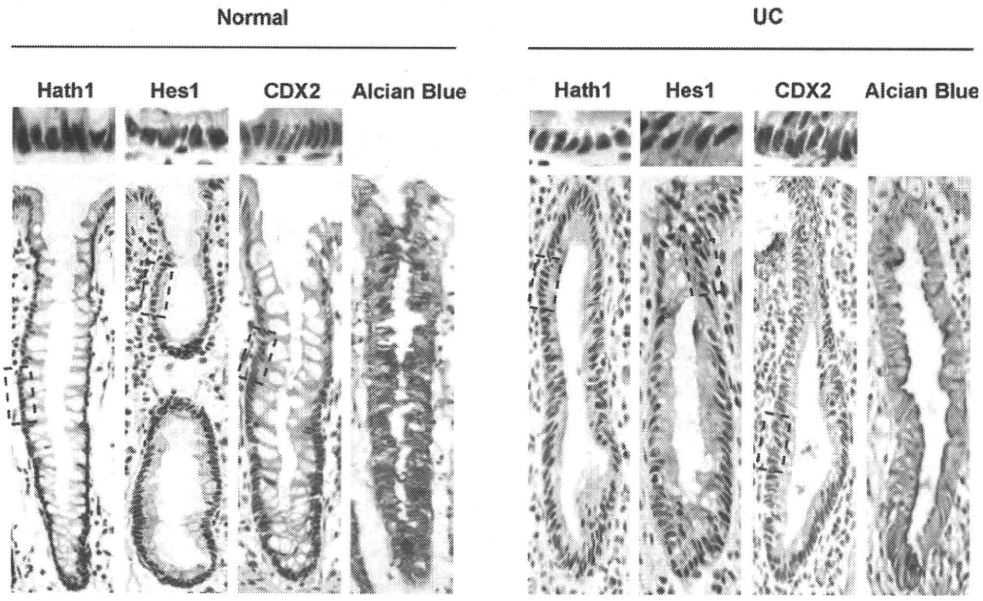


FIGURE 7. Immunohistochemistry of intestinal mucosa in UC. In normal colonic mucosa, Hath1 and CDX2 were expressed in most IEC. Hes1 was expressed in intestinal epithelial cells in the lower half of villi. In UC mucosa with goblet cell depletion, neither Hath1 nor CDX2 was expressed, whereas Hes1 was expressed up to the top of the villi. Upper column shows magnified view of the upper villus areas identified by dashed line in the lower column. Blue staining with Alcian blue represents goblet cells. The examination was performed by using the sections from three different individuals.

CDX2 is important to regulate *Hath1* gene expression, which is involved in goblet cell formation in UC. More detailed analysis of Hath1 expression at various stages of UC or other enteritis diseases associated with goblet cell depletion will lead us understand the regulation of Hath1 reduction under the inflammation state with various cytokines and inflammatory cells infiltration. Finally, elucidation of the mechanism of goblet cell depletion in UC will help us to develop novel therapies for strengthening the barrier function of colonic mucosa.

## REFERENCES


1. Booth C, Brady G, Potten CS. Crowd control in the crypt. *Nat Med.* 2002;8:1360–1361.
2. El-Assal ON, Besner GE. HB-EGF enhances restitution after intestinal ischemia/reperfusion via PI3K/Akt and MEK/ERK1/2 activation. *Gastroenterology.* 2005;129:609–625.
3. Haramis AP, Begthel H, van den Born M, et al. De novo crypt formation and juvenile polyposis on BMP inhibition in mouse intestine. *Science.* 2004;303:1684–1686.
4. Clevers H. Wnt/beta-catenin signaling in development and disease. *Cell.* 2006;127:469–480.
5. Fre S, Huyghe M, Mourikis P, et al. Notch signals control the fate of immature progenitor cells in the intestine. *Nature.* 2005;435:964–968.
6. Oshima S, Nakamura T, Namiki S, et al. Interferon regulatory factor 1 (IRF-1) and IRF-2 distinctively up-regulate gene expression and production of interleukin-7 in human intestinal epithelial cells. *Mol Cell Biol.* 2004;24:6298–6310.
7. Crosnier C, Stamataki D, Lewis J. Organizing cell renewal in the intestine: stem cells, signals and combinatorial control. *Nat Rev Genet.* 2006;7:349–359.
8. Okamoto R, Tsuchiya K, Nemoto Y, et al. Requirement of Notch activation during regeneration of the intestinal epithelia. *Am J Physiol Gastrointest Liver Physiol.* 2009;296:G23–35.
9. Yang Q, Bermingham N, Finegold M, et al. Requirement of Math1 for secretory cell lineage commitment in the mouse intestine. *Science.* 2001;294:2155–2158.
10. Tsuchiya K, Nakamura T, Okamoto R, et al. Reciprocal targeting of Hath1 and beta-catenin by Wnt glycogen synthase kinase 3beta in human colon cancer. *Gastroenterology.* 2007;132:208–220.
11. Helms A, Abney A, Ben-Arie N, et al. Autoregulation and multiple enhancers control Math1 expression in the developing nervous system. *Development.* 2000;127:1185–1196.
12. Ebert PJ, Timmer JR, Nakada Y, et al. Zic1 represses Math1 expression via interactions with the Math1 enhancer and modulation of Math1 autoregulation. *Development.* 2003;130:1949–1959.
13. Murata K, Hattori M, Hirai N, et al. Hes1 directly controls cell proliferation through the transcriptional repression of p27Kip1. *Mol Cell Biol.* 2005;25:4262–4271.
14. Katz JP, Perreault N, Goldstein BG, et al. The zinc-finger transcription factor Klf4 is required for terminal differentiation of goblet cells in the colon. *Development.* 2002;129:2619–2628.
15. Jensen J, Pedersen EE, Galante P, et al. Control of endodermal endocrine development by Hes-1. *Nat Genet.* 2000;24:36–44.
16. Zheng H, Pritchard D, Yang X, et al. KLF4 gene expression is inhibited by the notch signaling pathway that controls goblet cell differentiation in mouse gastrointestinal tract. *Am J Physiol Gastrointest Liver Physiol.* 2009;296:G490–498.
17. Briggs KJ, Corcoran-Schwartz IM, Zhang W, et al. Cooperation between the Hic1 and Ptch1 tumor suppressors in medulloblastoma. *Genes Dev.* 2008;22:770–785.
18. Murata K, Hattori M, Hirai N, et al. Hes1 directly controls cell proliferation through the transcriptional repression of p27Kip1. *Mol Cell Biol.* 2005;25:4262–4271.
19. Mutoh H, Sakamoto H, Hayakawa H, et al. The intestine-specific homeobox gene Cdx2 induces expression of the basic helix-loop-helix transcription factor Math1. *Differentiation.* 2006;74:313–321.
20. Katoh M. Notch signaling in gastrointestinal tract (review). *Int J Oncol.* 2007;30:247–251.
21. Kageyama R, Ohtsuka T, Kobayashi T. The Hes gene family: repressors and oscillators that orchestrate embryogenesis. *Development.* 2007; 134:1243–1251.

22. Monastirioti M, Giagtzoglou N, Koumbanakis K, et al. Drosophila Hey is a target of Notch in asymmetric divisions during embryonic and larval neurogenesis. *Development*. 2010;137:191–201.
23. Mukhopadhyay A, Jarrett J, Chlon T, et al. HeyL regulates the number of TrkC neurons in dorsal root ganglia. *Dev Biol*. 2009;334:142–151.
24. Chen H, Thiagalingam A, Chopra H, et al. Conservation of the Drosophila lateral inhibition pathway in human lung cancer: a hairy-related protein (HES-1) directly represses achaete-scute homolog-1 expression. *Proc Natl Acad Sci U S A*. 1997;94:5355–5360.
25. Gersemann M, Becker S, Kubler I, et al. Differences in goblet cell differentiation between Crohn's disease and ulcerative colitis. *Differentiation*. 2009;77:84–94.
26. Shroyer NF, Helmrath MA, Wang VYC, et al. Intestine-specific ablation of mouse atonal homolog 1 (Math1) reveals a role in cellular homeostasis. *Gastroenterology*. 2007;132:2478–2488.
27. Dahan S, Roda G, Pinn D, et al. Epithelial: lamina propria lymphocyte interactions promote epithelial cell differentiation. *Gastroenterology*. 2008;134:192–203.
28. Colleypriest B, Palmer R, Ward S, et al. Cdx genes, inflammation and the pathogenesis of Barrett's metaplasia. *Trends Mol Med*. 2009;15:313–322.
29. Eda A, Osawa H, Yanaka I, et al. Expression of homeobox gene CDX2 precedes that of CDX1 during the progression of intestinal metaplasia. *J Gastroenterol*. 2002;37:94–100.
30. Zheng J, Sun X, Wang W, et al. Hypoxia-inducible factor-1alpha modulates the down-regulation of the homeodomain protein CDX2 in colorectal cancer. *Oncol Rep*. 2010;24:97–104.
31. Giatromanolaki A, Sivridis E, Maltezos E, et al. Hypoxia inducible factor 1alpha and 2alpha overexpression in inflammatory bowel disease. *J Clin Pathol*. 2003;56:209–213.

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# IL-2 is positively involved in the development of colitogenic CD4$^+$ IL-7R$\alpha^{high}$ memory T cells in chronic colitis

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**IL-2 and IL-7 share a common γ-chain receptor and are critical for T-cell homeostasis. We aimed to clarify the reciprocal roles of IL-2 and IL-7 in the development and persistence of chronic colitis. We performed a series of adoptive transfers of IL-2$^{-/-}$ CD4$^+$CD45RB$^{high}$ T cells into RAG-2$^{-/-}$ mice and assessed the role of IL-2 in the induction of IL-7Rα on colitogenic CD4$^+$ T cells and the development of chronic colitis. RAG-2$^{-/-}$ mice transferred with WT but not with IL-2$^{-/-}$ CD4$^+$CD45RB$^{high}$ T cells developed Th1/Th17-mediated colitis. Consistently, re-expression of IL-7Rα was severely impaired on IL-2$^{-/-}$ but not on WT CD4$^+$ T cells from the transferred mice. To exclude a contribution of the preclinical autoimmunity of IL-2$^{-/-}$mice, WT Ly5.1$^+$ or IL-2$^{-/-}$ Ly5.2$^+$ CD4$^+$CD45RB$^{high}$ T cells from GFP mice previously transplanted with the same number of WT and IL-2$^{-/-}$ BM cells were transferred into RAG-2$^{-/-}$ mice. RAG-2$^{-/-}$ mice transferred with IL-2$^{-/-}$-derived CD4$^+$ CD45RB$^{high}$ T cells did not develop colitis, but their splenic CD4$^+$ T cells changed from effector-memory to central-memory type. These results show that IL-2 is critically involved in the establishment and maintenance of IL-7-dependent colitogenic memory CD4$^+$IL-7R$\alpha^{high}$ T cells.**

**Key words:** Animal models · CD4$^+$ T cell · Cytokines · Memory cell · Mucosal immunity

## Introduction

The inflammatory bowel diseases (IBD), Crohn's disease and ulcerative colitis, are caused by chronic inflammatory responses in the gut wall [1–3]. Although the aetiology of IBD is uncertain, there is much evidence suggesting that the pathogenesis of IBD involves dysregulated recognition of intestinal bacterial antigens, resulting in the generation of colitogenic CD4$^+$ effector and memory T cells [4–10]. However, how colitogenic CD4$^+$ T cells are generated and maintained in patients with IBD remains unknown.

During T-cell priming and maintenance of colitogenic CD4$^+$ effector and memory T cells, cytokines may provide critical signals. IL-2 is produced by activated T cells early after antigenic stimulation and is essential for proliferation of T cells in the effector phase, at least *in vitro* [11, 12]. More recently, it has been shown that exposure to IL-2 in the effector phase is required for successful long-term survival of CD4$^+$ T cells and their

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differentiation into memory T cells [13]. Interestingly, IL-2 does not seem to be needed for T-cell proliferation *in vivo* because IL-$2^{-/-}$ and IL-$2R\alpha^{-/-}$ mice develop autoimmune diseases, including chronic colitis, and extensive proliferation of T cells [14, 15]. Thus, the development of such diseases *in vivo* is explained entirely by the lack of $CD4^+CD25^+Foxp3^+$ Treg cells, which are dependent on IL-2 for their development and maintenance [16–18].

In contrast to IL-2, IL-7 is produced not by lymphocytes but by stromal cells in the BM and thymus and by epithelial cells [19–21]. This cytokine is important for supporting the survival of naïve and memory $CD4^+$ T cells, but not that of effector $CD4^+$ T cells [21–23]. Our previous studies on the pathogenesis of IBD demonstrated that: (i) IL-7 is constitutively produced by intestinal goblet epithelial cells [20], (ii) IL-7 transgenic mice developed chronic colitis [24], (iii) mucosal $CD4^+$IL-$7R\alpha^{high}$ T cells in $CD4^+CD45RB^{high}$ T-cell-transferred colitic mice are colitogenic [25, 26], and (iv) IL-7 is essential for the persistence of colitis because IL-$7^{-/-}$ × RAG-$1^{-/-}$ mice transferred with colitogenic $CD4^+$ T cells did not develop colitis [27].

IL-2 and IL-7 share a common γ-chain receptor and are critical cytokines for T-cell homeostasis [21, 22]. To clarify the reciprocal roles of IL-2 and IL-7 in the development and persistence of chronic colitis, we performed a series of adoptive transfers of WT or IL-$2^{-/-}$ $CD4^+CD45RB^{high}$ T cells into RAG-$2^{-/-}$ mice.

## Results

### IL-$2^{-/-}$ mice sustain substantial numbers of naïve $CD4^+$ T cells that have reduced levels of IL-7Rα

IL-$2^{-/-}$ mice develop spontaneous autoimmune syndrome from 4–6 wk of age and develop IBD at 10–12 wk of age [14]. We first analysed the phenotypic characteristics of $CD4^+$ T cells of young (3.5-wk-old) IL-$2^{-/-}$ and WT mice. The proportions of $CD4^+$ $CD44^{high}$ or $CD45RB^{low}$ memory T cells and $CD4^+CD44^{low}$ or $CD45RB^{high}$ naïve T cells in the spleen (SP) of IL-$2^{-/-}$ mice were reciprocally higher or lower, respectively, than those of the paired WT mice (Fig. 1A and B). However, it is noteworthy that naïve T cells were detected in the SP of young IL-$2^{-/-}$ mice at this stage, indicating that continuous generation of naïve T cells occurs in these mice. As IL-2 is essential for the development and maintenance of $CD4^+CD25^+Foxp3^+$ Treg [17], expression of CD25 and Foxp3 on/in SP $CD4^+$ T cells in IL-$2^{-/-}$ mice was markedly impaired compared with that in WT mice (Fig. 1A and B). In contrast, SP $CD4^+CD45RB^{high}$ populations from IL-$2^{-/-}$ and WT mice did not contain Treg (Fig. 1B). Interestingly, the positive frequency of IL-7Rα expression on SP $CD4^+$ T cells in IL-$2^{-/-}$ mice significantly reduced, which is in sharp contrast to that in WT mice (Fig. 1A and C). This suggests that SP $CD4^+$ T cells in IL-$2^{-/-}$ mice include a substantial number of effector T cells at a young age despite the absence of clinical manifestations or impaired naïve or memory T cells. Surprisingly, we found that the positive frequency of IL-7Rα expression on the SP $CD4^+$ $CD45RB^{high}$ naïve T cells of IL-$2^{-/-}$ mice was also significantly reduced compared with that of WT mice (Fig. 1B and D), suggesting that IL-2 is involved in the development and maintenance of $CD4^+$ naïve T cells (Fig. 1B). This was also the case with the SP $CD4^+CD45RB^{low}$ T cells of IL-$2^{-/-}$ mice, which may be explained by an increased number of effector T cells and/or impaired development of memory T cells.

### RAG-$2^{-/-}$ mice transferred with IL-$2^{-/-}$ $CD4^+$ CD45 $Rb^{high}$ T cells do not develop colitis

Since the positive frequency of IL-7Rα expression on IL-$2^{-/-}$ $CD4^+CD45RB^{high}$ T donor cells reduced (Fig. 1), the possibility remained that the impaired naïve $CD4^+$ T cells themselves were critically involved in the development of spontaneous colitis in IL-$2^{-/-}$ mice. To first assess the effect of IL-2 deficiency on the initial developmental process of colitis, $CD4^+CD45RB^{high}$ T cells from young WT or non-colitic IL-$2^{-/-}$ mice were injected intraperitoneally into RAG-$2^{-/-}$mice (Fig. 2A). As a negative control, RAG-$2^{-/-}$ mice were transferred with WT $CD4^+$ $CD45RB^{high}$ T cells and WT $CD4^+CD25^+$ Treg (Fig. 2A). Consistent with previous report [27], the RAG-$2^{-/-}$ mice transferred with WT $CD4^+CD45RB^{high}$ T cells manifested weight loss from 5 wk after transfer (Fig. 2B). Clinical symptoms of colitis as shown by clinical scores were apparent 7 wk after transfer (Fig. 2D). In contrast, RAG-$2^{-/-}$ mice transferred with WT $CD4^+CD45RB^{high}$ T cells and Treg showed no weight loss or clinical symptoms of colitis (Fig. 2B and D). However, RAG-$2^{-/-}$ mice transferred with IL-$2^{-/-}$ $CD4^+CD45RB^{high}$ T cells did not show wasting (Fig. 2B) or clinical symptoms of colitis throughout the observation period of 7 wk after transfer (Fig. 2D). Overall, the assessment of colitis according to clinical score showed a clear difference between RAG-$2^{-/-}$ mice transferred with IL-$2^{-/-}$ and those transferred with WT $CD4^+CD45RB^{high}$ T cells (Fig. 2D). At 7 wk after transfer, the colon of RAG-$2^{-/-}$ mice transferred with WT $CD4^+CD45RB^{high}$ T cells, but not with IL-$2^{-/-}$ $CD4^+$ $CD45RB^{high}$ T cells or a combination of WT $CD4^+CD45RB^{high}$ and Treg, was enlarged and had a greatly thickened wall (Fig. 2C). Enlargement of the SP was also present in RAG-$2^{-/-}$ mice transferred with WT $CD4^+CD45RB^{high}$ T cells but not in other groups (Fig. 2C).

Histological examination showed a massive infiltration of mononuclear cells in the lamina propria (LP) of the colon of RAG-$2^{-/-}$ mice transferred with WT $CD4^+CD45RB^{high}$ T cells (Fig. 2E). In contrast, the inflammation was mostly abrogated and only a few mononuclear cells were observed in the LP of the colon of RAG-$2^{-/-}$ mice transferred with IL-$2^{-/-}$ $CD4^+$ $CD45RB^{high}$ T cells as well as in RAG-$2^{-/-}$ mice transferred with WT $CD4^+CD45RB^{high}$ T cells and Treg (Fig. 2E). This difference was confirmed by histological scoring of colon sections (Fig. 2F). Further quantitative evaluation of LP $CD4^+$ T-cell infiltration was done using flow cytometry (Fig. 2G). The number of LP $CD4^+$ cells recovered from the colon of RAG-$2^{-/-}$ mice transferred with WT $CD4^+CD45RB^{high}$ T cells far exceeded the number originally

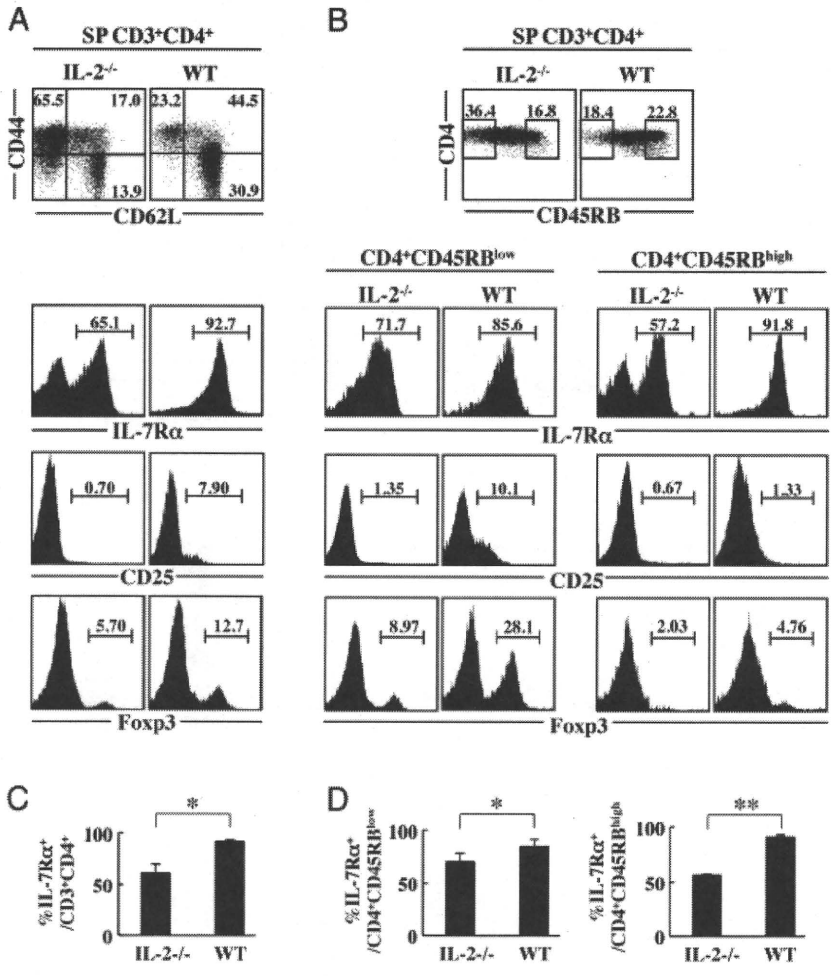


**Figure 1.** Phenotypic characteristics of IL-2$^{-/-}$ CD4$^+$CD45RB$^{high}$ T cells. (A) Identification and characterization of SP CD4$^+$ T cells in young IL-2$^{-/-}$ and WT mice. FACS analysis shows the expression of CD44 and CD62L, IL-7Rα, CD25, or Foxp3 on/in SP CD4$^+$ T cells. (B) Characterization of SP CD4$^+$CD45RB$^{high}$ and CD45RB$^{low}$ T cells in young IL-2$^{-/-}$ and WT mice. (C) The percentage of IL-7Rα$^+$ cells in SP CD3$^+$CD4$^+$ T cells was determined using a FACSCalibur. (D) The percentage of IL-7Rα$^+$ cells in the CD4$^+$CD45RB$^{low}$ or CD45RB$^{high}$ T-cell populations was determined using a FACSCalibur. Data are representative of six mice *per* group. (A and B) or show mean+SEM (C and D; $n = 6$ *per* group). *$p = 0.011$ (Mann-Whitney *U*) and **$p = 0.011$ (Student's *t*).

injected, indicating extensive T-cell proliferation and survival in the inflamed colon, which was mostly abrogated in RAG-2$^{-/-}$ mice transferred with WT CD4$^+$CD45RB$^{high}$ T cells and Treg or RAG-2$^{-/-}$ mice transferred with IL-2$^{-/-}$ CD4$^+$CD45RB$^{high}$ T cells (Fig. 2G).

As shown in Fig. 1B, however, there were two populations of IL-2$^{-/-}$CD4$^+$CD45RB$^{high}$ T cells, IL-7Rα$^{high}$ and IL-7Rα$^{low}$. Thus, it is possible that IL-7Rα$^{low}$ cells suppress IL-7Rα$^{high}$ cells when they are transferred into RAG-2$^{-/-}$ mice as IL-2$^{-/-}$CD4$^+$ CD45RB$^{high}$ T cells. To rule out this possibility, we performed another *in vivo* experiment. CD4$^+$CD45RB$^{high}$ T cells from the SP of 4- to 5-wk-old IL-2$^{-/-}$ mice were divided into two populations, IL-7Rα$^{high}$ and IL-7Rα$^{low}$ by cell sorting, and each population was separately transferred into RAG-2$^{-/-}$ hosts. As a positive control, a same number of WT CD4$^+$CD45RB$^{high}$ T cells were again transferred into RAG-2$^{-/-}$ mice (Fig. 3A). Mice transferred with IL-7Rα$^{high}$ or IL-7Rα$^{low}$ did not develop clinical or histological aspects of colitis, whereas mice transferred with WT CD4$^+$ CD45RB$^{high}$ T cells did develop severe colitis (Figs. 3B–D). However, a proportion of IL-7Rα$^{high}$ IL-2$^{-/-}$CD4$^+$CD45RB$^{high}$ T cells converted to IL-7Rα$^{low}$ and *vice versa* (data not shown) after they were transferred to RAG-2$^{-/-}$ mice, which shows that the expression of IL-7Rα on IL-2$^{-/-}$CD4$^+$CD45RB$^{high}$ T cells is flexible, rather than fixed on naïve cells.

To determine the effect of these transfers on Th1/Th17 development, we next measured IFN-γ and IL-17 production by anti-CD3/CD28-stimulated CD4$^+$ LP T cells. As shown in Fig. 4A, the production of IFN-γ and IL-17 by anti-CD3/CD28-stimulated CD4$^+$ LP T cells from RAG-2$^{-/-}$ mice transferred with IL-2$^{-/-}$ CD4$^+$CD45RB$^{high}$ T cells or from RAG-2$^{-/-}$ mice transferred with WT CD4$^+$CD45RB$^{high}$ T cells and Treg was significantly lower than that from RAG-2$^{-/-}$ mice transferred with WT CD4$^+$ CD45RB$^{high}$ T cells. To assess the cell surface markers on isolated SP and LP cells in each group, we then performed flow cytometric analysis. As shown in Fig. 4B, the transferred IL-2$^{-/-}$ SP and LP CD4$^+$ T cells may differentiate into CD44$^{high}$ memory T cells in

 

the absence of colitis, as was the case with the paired $CD4^+$ T cells in colitic RAG-$2^{-/-}$ mice transferred with WT $CD4^+$ $CD45RB^{high}$ T cells and non-colitic RAG-$2^{-/-}$ mice transferred with WT $CD4^+CD45RB^{high}$ T cells and WT Treg. It is noteworthy that the percentages of the central memory type of $CD44^{high}CD62L^+$ T cells ($T_{CM}$) in the SP (Fig. 4B and C, left) and

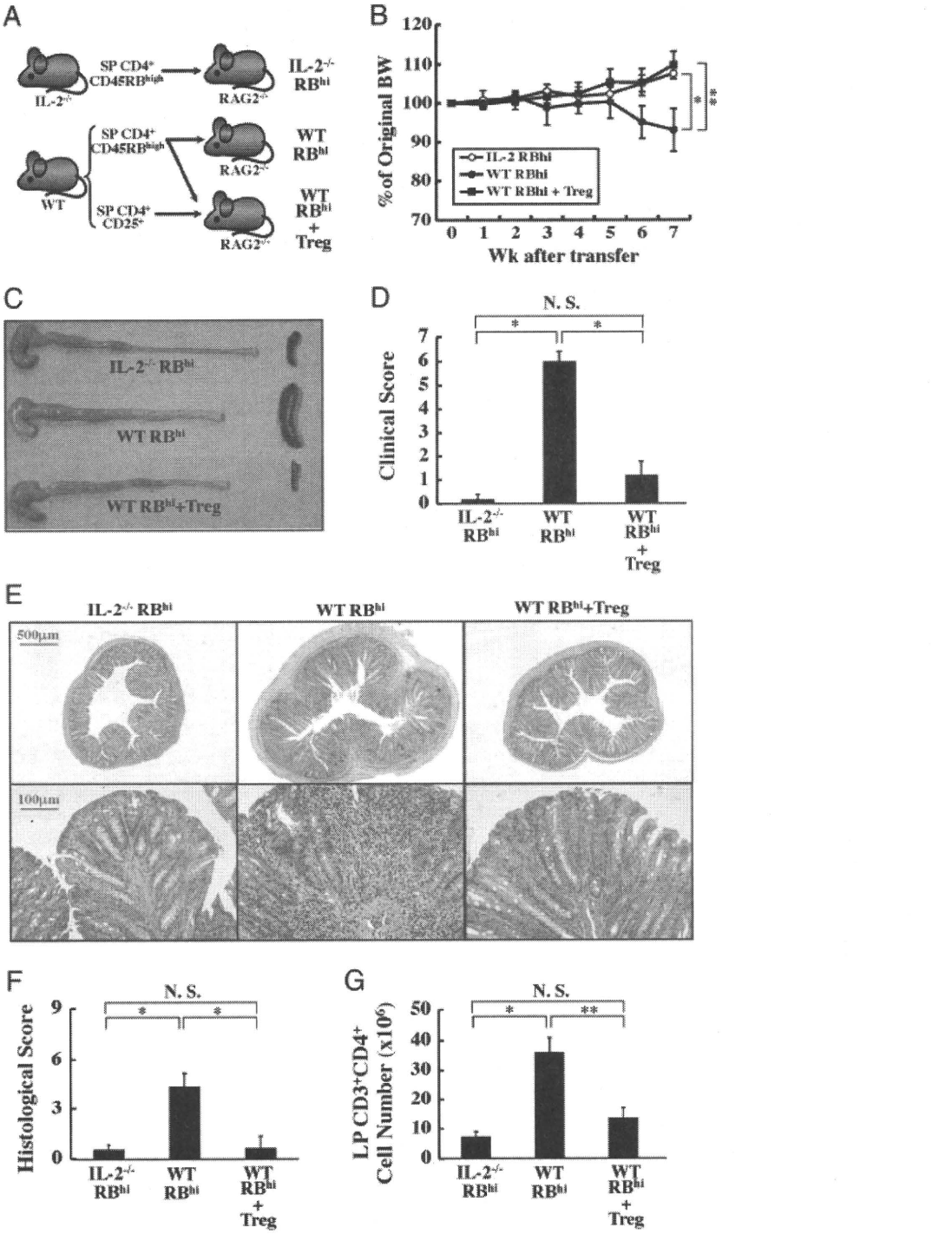


**Figure 2.** RAG-$2^{-/-}$ mice transferred with IL-$2^{-/-}$ $CD4^+CD45RB^{high}$ T cells do not develop colitis. We performed two independent experiments. (A) Experimental design. RAG-$2^{-/-}$ mice were transferred with SP WT or IL-$2^{-/-}$ $CD4^+CD45RB^{high}$ T cells ($3 \times 10^5$ cells *per* mouse) or a mixture of WT $CD4^+$ $CD45RB^{high}$ T cells ($3 \times 10^5$) and WT $CD4^+CD25^+$ Treg ($1 \times 10^5$). (B) The change in body weight over time is expressed as a percentage of the original weight. $^*p = 0.049$ (Welch's *t*), and $^{**}p = 0.049$ (Student's *t*). (C) Gross appearance of the colon and spleen. (D) Clinical scores. Data are expressed as the mean ± SEM of six mice *per* group. $^*p = 0.014$ (Mann-Whitney *U*.) (E) Histological results for the colons of each group. Original magnification, × 40 (upper) and × 200 (lower). (F) Histological scoring. $^*p = 0.014$ (Mann-Whitney *U*). (G) The number of $CD4^+CD3^+$ cells in colonic LP for each group. Cell number was determined using FACS. $^*p = 0.0049$ (Welch's *t*), and $^{**}p = 0.014$ (Mann-Whitney *U*). Data are expressed as the mean ± SEM of eight mice *per* group.

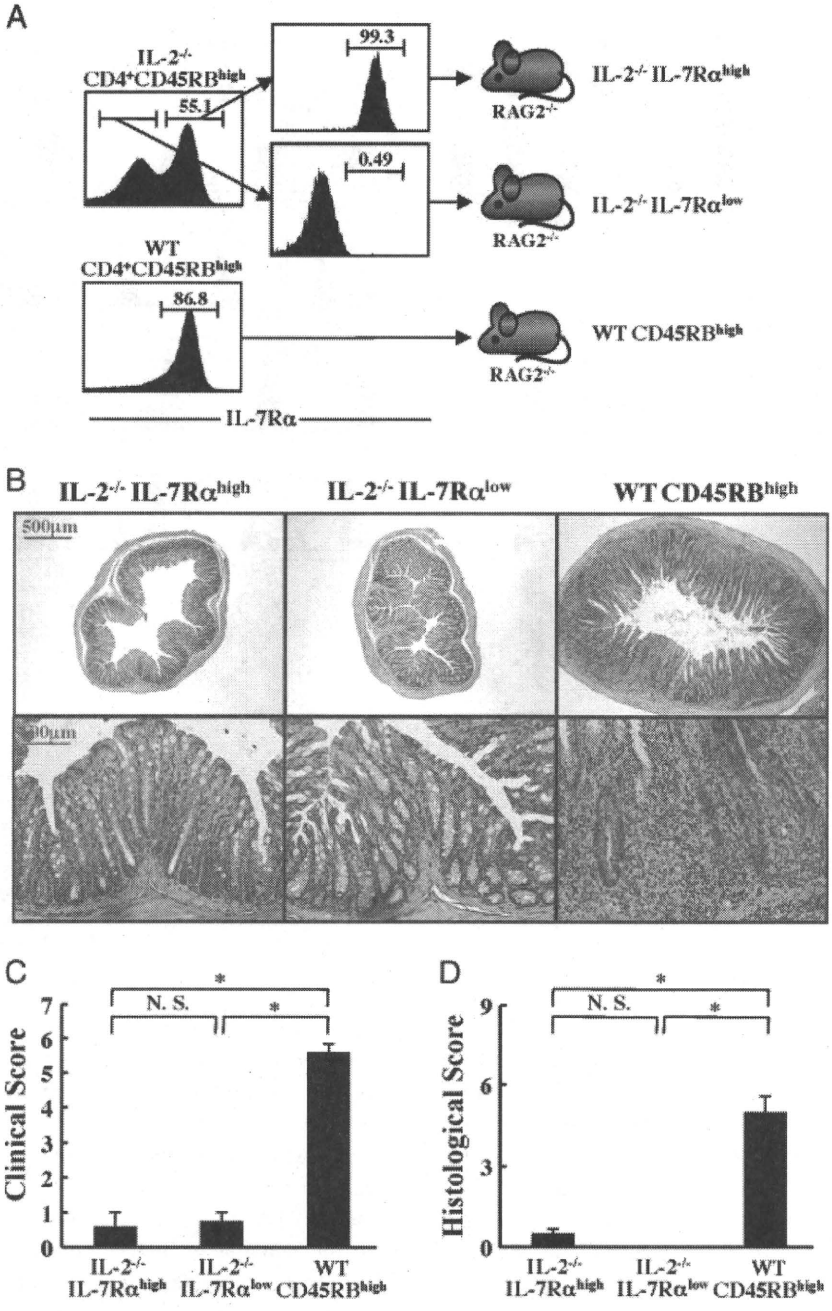


**Figure 3.** Either RAG-$2^{-/-}$ mice transferred with IL-$2^{-/-}$ $CD4^+CD45RB^{high}$IL-$7R\alpha^{high}$ T cells or those transferred with IL-$2^{-/-}$ $CD4^+CD45RB^{high}$ IL-$7R\alpha^{kow}$ T cells do not develop colitis. We performed one independent experiment. (A) Experimental design. RAG-$2^{-/-}$ mice were transferred with IL-$7R\alpha^{high}CD4^+CD45RB^{high}$ T cells ($3 \times 10^5$) or IL-$7R\alpha^{low}CD4^+CD45RB^{high}$ T cells ($3 \times 10^5$) from the spleen of IL-$2^{-/-}$ mice. As a positive control, we transferred the same number of WT $CD4^+CD45RB^{high}$ T cells into RAG-$2^{-/-}$ mice. (B) Histological results for the colons of each group. Original magnification, × 40 (upper) and × 200 (lower). (C) Clinical scores. (D) Histological scoring. Data are expressed as the mean ± SEM of six (C) and five (D) mice *per* group. N.S., not significant. *$p = 0.014$ (Mann-Whitney *U*). N.S., not significant.

MLN (data not shown) IL-$2^{-/-}$ $CD4^+$ T cells were significantly higher than those in the SP and MLN of colitic RAG-$2^{-/-}$ mice transferred with WT $CD4^+CD45RB^{high}$ T cells or non-colitic RAG-$2^{-/-}$ mice transferred with WT $CD4^+CD45RB^{high}$ T cells and WT Treg. In contrast, LP $CD4^+$ T cells in all groups exhibited characteristics of effector-memory type of $CD44^{high}CD62L^-$ ($T_{EM}$) cells, which is consistent with the non-lymphoid nature of the LP (Fig. 4B and C, right) [28, 29]. Furthermore, the positive frequency of IL-7Rα expression on SP and LP IL-$2^{-/-}$ $CD4^+$ T cells also significantly reduced, as compared with the paired cells from colitic RAG-$2^{-/-}$ mice transferred with WT $CD4^+$ $CD45RB^{high}$ T cells or non-colitic RAG-$2^{-/-}$ mice transferred with

 

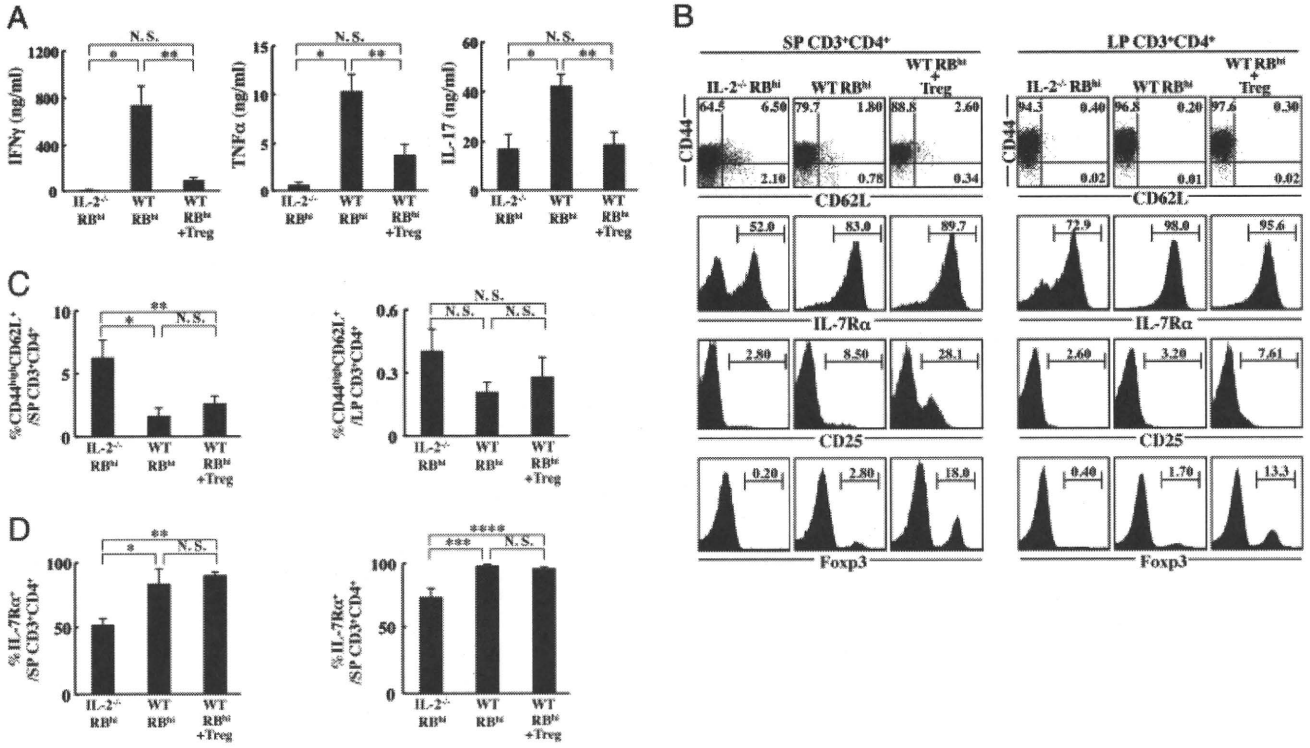


**Figure 4.** IL-7Rα expression of memory $CD4^+$ T cells in IL-$2^{-/-}$ $CD4^+CD45RB^{high}$ T-cell-transferred RAG-$2^{-/-}$ mice was impaired. We performed two independent experiments. (A) Cytokine production by LP $CD4^+$ T cells. LP $CD4^+$ T cells were stimulated with plate-coated anti-CD3 mAb and soluble anti-CD28 mAb for 48 h. Cytokine concentration in the supernatants were measured using ELISA. Data are expressed as the mean ± SEM of eight mice *per* group. $*p = 0.021$ (Mann-Whitney *U*), and $**p = 0.014$ (Mann-Whitney *U*). (B) Expression of various cell surface markers on SP and LP $CD3^+CD4^+$ T cells was determined by FACS. Representative results from eight mice *per* group for CD44, CD62L, IL-7Rα, CD25, and Foxp3 are shown. (C) The percentage of $T_{CM}$ cells was determined using FACS. Data are expressed as the mean ± SEM of eight mice. $*p = 0.0001$ (Student's *t*), $**p = 0.047$ (Student's *t*), and N.S., not significant. (D) The percentage of $CD3^+CD4^+$ T cells positive for IL-$7R\alpha^+$ is shown. Data are expressed as the mean ± SEM of eight mice *per* group. $*p = 0.0001$ (Student's *t*). $**p = 0.0009$ (Mann-Whitney *U*), $***p = 0.001$ (Mann-Whitney *U*), $****p = 0.017$ (Mann-Whitney *U*), and N.S., not significant.

WT $CD4^+CD45RB^{high}$ T cells and WT Treg (Fig. 4B and D). In addition, $CD4^+CD25^+Foxp3^+$ Treg did not emerge in the SP or LP of RAG-$2^{-/-}$ mice transferred with IL-$2^{-/-}$ $CD4^+CD45RB^{high}$ T cells, which is in contrast to RAG-$2^{-/-}$ mice transferred with WT $CD4^+CD45RB^{high}$ T cells, which contained a substantial number of inducible Treg (Fig. 4B), albeit fewer than RAG-$2^{-/-}$ mice transferred with WT $CD4^+CD45RB^{high}$ T cells and WT Treg, which contained a mixture of naturally occurring and inducible Treg (Fig. 4B).

## Paracrine IL-2 from WT $CD4^+$ T cells enables IL-$2^{-/-}$ LP $CD4^+$ T cells to induce mild colitis in an IL-7-dependent manner

To further assess the role of IL-2 signalling in the expansion of $CD4^+$ donor cells, we performed *in vivo* competition experiments. First, the same number of $CD4^+CD45RB^{high}$ donor cells from $Ly5.1^+$ WT and $Ly5.2^+$ IL-$2^{-/-}$ mice were co-injected intraperitoneally into RAG-$2^{-/-}$ mice (Fig. 5A). As expected, recipient mice developed severe colitis 6 wk after co-transfer (data not shown), and a significantly lower proportion of $Ly5.2^+$ IL-$2^{-/-}$ $CD4^+$ T cells were observed in the inflamed LP and SP compared with the paired $Ly5.1^+$ WT $CD4^+$ T cells (Fig. 5B). Furthermore, the positive frequency of IL-7Rα expression on IL-$2^{-/-}$ LP $CD4^+$ cells was markedly reduced, as compared with that on WT LP $CD4^+$ cells (Fig. 5C).

We next addressed the question of whether LP IL-$2^{-/-}$ $CD4^+$ T cells sustained in colitic RAG-$2^{-/-}$ mice transferred with a mixture of WT and IL-$2^{-/-}$ $CD4^+CD45RB^{high}$ T cells (Fig. 5A) have the potential to induce colitis when transferred to new RAG-$1^{-/-}$ mice, because it was considered possible that a small but substantial number of IL-$2^{-/-}$ LP $CD4^+$ T cells in those mice (Fig. 5) would gain colitogenicity by using paracrine IL-2 from surrounding WT LP $CD4^+$ T cells. If they were colitogenic, it would also be necessary to examine whether IL-7 is also needed for the development of colitis by those cells to assess whether they are effector or memory T cells (Fig. 5A), as is the case with colitic WT LP $CD4^+$ T cells as previously demonstrated by our group [27]. To this end, we isolated LP WT ($Ly5.1^+$) and IL-$2^{-/-}$ ($Ly5.2^+$) $CD4^+$ T cells from colitic RAG-$2^{-/-}$ mice previously transferred with the same number of WT and IL-$2^{-/-}$ $CD4^+$ $CD45RB^{high}$ T cells and then separately retransferred them into new IL-$7^{+/+}$ × RAG-$1^{-/-}$ or IL-$7^{-/-}$ × RAG-$1^{-/-}$mice (Fig. 5A). As expected, IL-$7^{+/+}$ × RAG-$1^{-/-}$ recipients transferred with WT $CD4^+$ T cells (WT→IL-$7^{+/+}$) developed severe colitis 4–6 wk

 

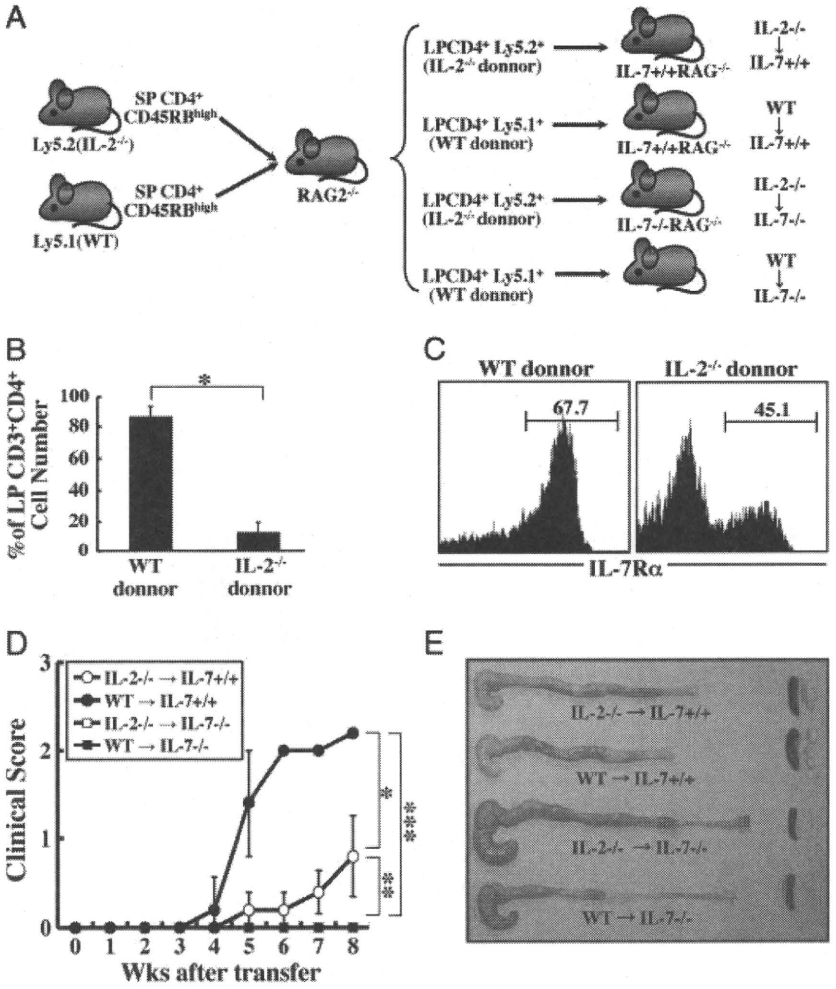


**Figure 5.** IL-7 is essential for the development of colitis in mice transferred with IL-$2^{-/-}$ LP $CD4^+$ T cells obtained from colitic mice previously transferred with a mixture of WT and IL-$2^{-/-}$ $CD4^+CD45RB^{high}$ T cells. We performed one independent experiment. (A) Experimental design. RAG-$2^{-/-}$ mice were co-injected i.p. with a mixture of WT ($Ly5.1^+$) and IL-$2^{-/-}$ ($Ly5.2^+$) $CD4^+CD45RB^{high}$ T cells. Eight weeks after transfer, WT and IL-$2^{-/-}$ LP $CD4^+$ T cells were isolated and separately retransferred into IL-$7^{+/+}$ × RAG-$1^{-/-}$ or IL-$7^{-/-}$ × RAG-$1^{-/-}$ mice. (B) Numbers of WT ($Ly5.1^+$) and IL-$2^{-/-}$ ($Ly5.2^+$) LP $CD3^+CD4^+$ T cells recovered from colitic RAG-$2^{-/-}$ donor mice. Data are expressed as the mean ± SEM of five mice *per* group. *$p = 0.049$ (Mann-Whitney *U*). (C) Expression of IL-7Rα on SP WT and IL-$2^{-/-}$ $CD4^+$ T cells of colitic RAG-$2^{-/-}$ donor mice was determined using FACS. (D) Clinical score after retransfer into new IL-$7^{+/+}$ × RAG-$1^{-/-}$ or IL-$7^{-/-}$ × RAG-$1^{-/-}$ mice. Data are expressed as the mean ± SEM of five mice *per* group. *$p = 0.025$, **$p = 0.034$, ***$p = 0.034$ (Mann-Whitney *U*). (E) Gross appearance of the colon, SP, and MLN 8 wk after retransfer.

after transfer, which was characterized by significant weight loss, diarrhoea, higher total clinical scores (Fig. 5D), and thickening of the colonic wall with inflammation (Fig. 5E). In contrast, IL-$7^{-/-}$ × RAG-$1^{-/-}$ recipients transferred with WT $CD4^+$ T cells (WT → IL-$7^{-/-}$) appeared healthy, exhibited no signs of colitis until 8 wk after transfer (Fig. 5D), and exhibited no apparent thickening of the colonic wall (Fig. 5E). As expected, IL-$7^{-/-}$ × RAG-$1^{-/-}$ recipients transferred with IL-$2^{-/-}$ $CD4^+$ T cells (IL-$2^{-/-}$ → IL-$7^{-/-}$) did not develop colitis. However, IL-$7^{+/+}$ × RAG-$1^{-/-}$ recipients transferred with IL-$2^{-/-}$ $CD4^+$ T cells (IL-$2^{-/-}$ → IL-$7^{+/+}$) exhibited clinical signs of colitis and a thickened colonic wall (Fig. 5E) 8 wk after transfer, albeit less severely than WT → IL-$7^{+/+}$ mice (Fig. 5E).

Histological examinations revealed no evident pathological changes in the colons of WT → IL-$7^{-/-}$ or IL-$2^{-/-}$ → IL-$7^{-/-}$ mice in contrast to colitic WT → IL-$7^{+/+}$ or IL-$2^{-/-}$ → IL-$7^{+/+}$ mice, which showed prominent epithelial hyperplasia with massive infiltration of mononuclear cells (Fig. 6A). This was confirmed by assessing each histological score (Fig. 6B). Furthermore, the score of IL-$2^{-/-}$ → IL-$7^{+/+}$ mice was significantly less than that of WT → IL-$7^{+/+}$ mice (Fig. 6B). Consistent with this, the average number of LP $CD4^+$ T cells recovered from colitic IL-$2^{-/-}$ → IL-$7^{+/+}$ mice was significantly less than that of WT → IL-$7^{+/+}$ mice (Fig. 6C). The number of LP $CD4^+$ cells in IL-$2^{-/-}$ → IL-$7^{-/-}$ or WT → IL-$7^{-/-}$ mice was almost zero (Fig. 6C). Furthermore, the positive frequency of IL-7Rα expression on SP $CD4^+$ T cells obtained from IL-$2^{-/-}$ $CD4^+$ T-cell-transferred mice was markedly reduced in both IL-$7^{+/+}$ and IL-$7^{-/-}$ recipients, whereas that from WT $CD4^+$ T-cell-transferred mice was not impaired in IL-$7^{+/+}$ or IL-$7^{-/-}$ recipients (Fig. 6D).

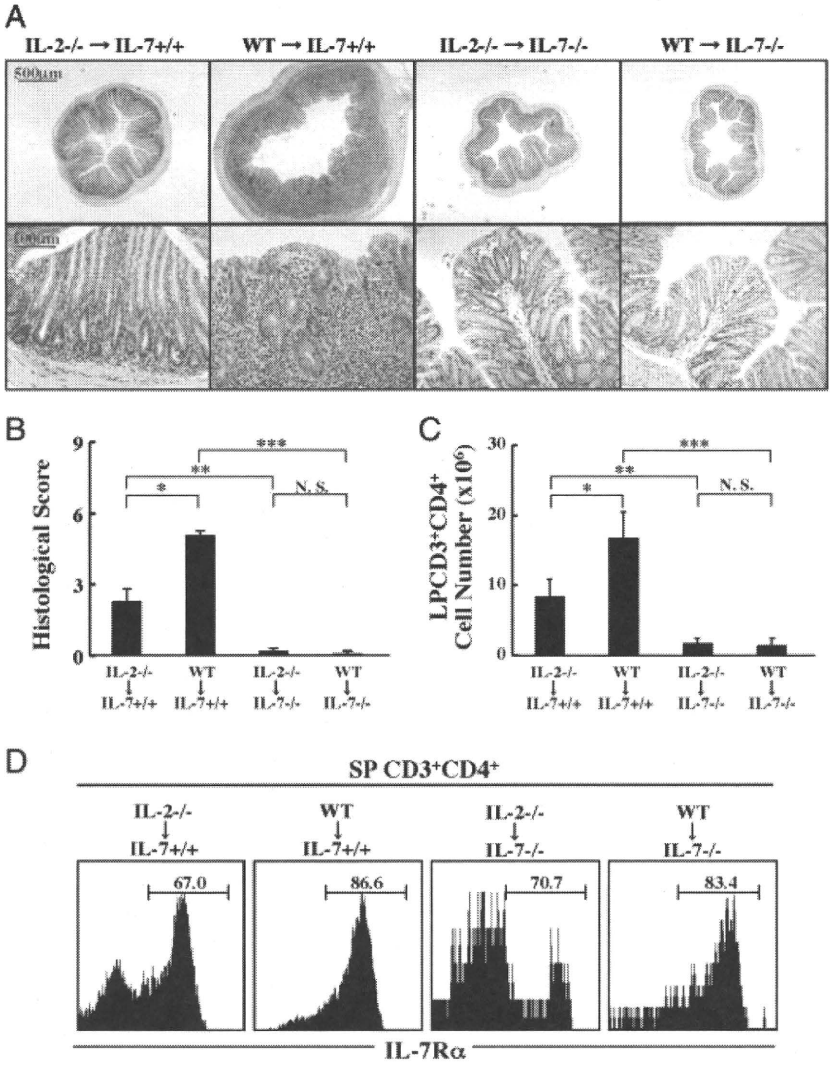


**Figure 6.** IL-7 is absolutely essential for the development of colitis in mice transferred with IL-$2^{-/-}$ LP $CD4^+$ T cells despite down-modulated IL-7Rα expression. We performed one independent experiment. (A) Histopathology of the distal colon 8 wk after retransfer. Original magnification, × 40 (upper) and × 200 (lower). (B) Histological score 8 wk after retransfer. Data are expressed as the mean ± SEM of five mice *per* group. $^*p = 0.009$, $^{**}p = 0.016$, $^{***}p = 0.009$ (Mann-Whitney *U*). (C) Number of LP $CD3^+CD4^+$ T cells recovered 8 wk after retransfer. Data are expressed as the mean ± SEM of five mice *per* group. $^*p = 0.049$ (Student's *t*), $^{**}p = 0.033$ (Welch's *t*), and $^{***}p = 0.014$ (Mann-Whitney *U*). (D) Expression of IL-7Rα on SP $CD3^+CD4^+$ T cells was determined using FACS. Representative FACS data of five mice are shown.

## IL-$2^{-/-}$ $CD4^+CD45\ RB^{high}$ T cells do not induce colitis, even if they were developed in IL − $2^{-/-}$ and WT BM chimaeric mice

To precisely prepare similar naïve WT and IL-$2^{-/-}$ $CD4^+$ $CD45RB^{high}$ T cells in the same *in vivo* setting, we generated mixed BM chimeras. To this end, irradiated GFP mice were first transplanted with a mixture of the same number of BM cells obtained from young WT and IL-$2^{-/-}$ mice (Fig. 7A). Six weeks after the BM transplantation, various cell markers were compared between the transplanted WT and IL-$2^{-/-}$ BM-derived $GFP^-$ $CD4^+$ cells. As shown in Fig. 6B, almost all the cell markers, including IL-7Rα, were identical in the transplanted WT and IL-$2^{-/-}$ BM-derived $CD4^+$ cells. Thus, $GFP^-$ $Ly5.1^+$ WT and $Ly5.2^+$ IL-$2^{-/-}$ $CD4^+CD45RB^{high}$ T cells were isolated, and then the separated cells or a mixture of the same number of the two cell types was transferred into RAG-$2^{-/-}$ mice. Again, we found that RAG-$2^{-/-}$ mice transferred with $GFP^-$ $Ly5.1^+$ WT $CD4^+$ $CD45RB^{high}$ T cells or a mixture of $GFP^-$ $Ly5.1^+$ WT and $Ly5.2^+$ IL-$2^{-/-}$ $CD4^+CD45RB^{high}$ T cells showed similar signs of wasting disease (Fig. 7B), clinical symptoms (Fig. 7D), and clinical and histological scores (Fig. 7E and F) 8 wk after transfer, which differs from the findings for non-colitic RAG-$2^{-/-}$ mice transferred with $GFP^-$ $Ly5.2^+$ IL-$2^{-/-}$ $CD4^+CD45RB^{high}$ T cells (Fig. 7C–F). The number of LP $CD4^+$ T cells recovered from RAG-$2^{-/-}$ mice transferred with $GFP^-$ $Ly5.1^+$ WT $CD4^+CD45RB^{high}$ T cells or a mixture of $GFP^-$ $Ly5.1^+$ WT and $Ly5.2^+$ IL-$2^{-/-}$ $CD4^+CD45RB^{high}$ T cells was significantly higher than that recovered from mice transferred with $Ly5.2^+$ IL-$2^{-/-}$ $CD4^+CD45RB^{high}$ T cells (Fig. 8A). The production of IFN-γ,

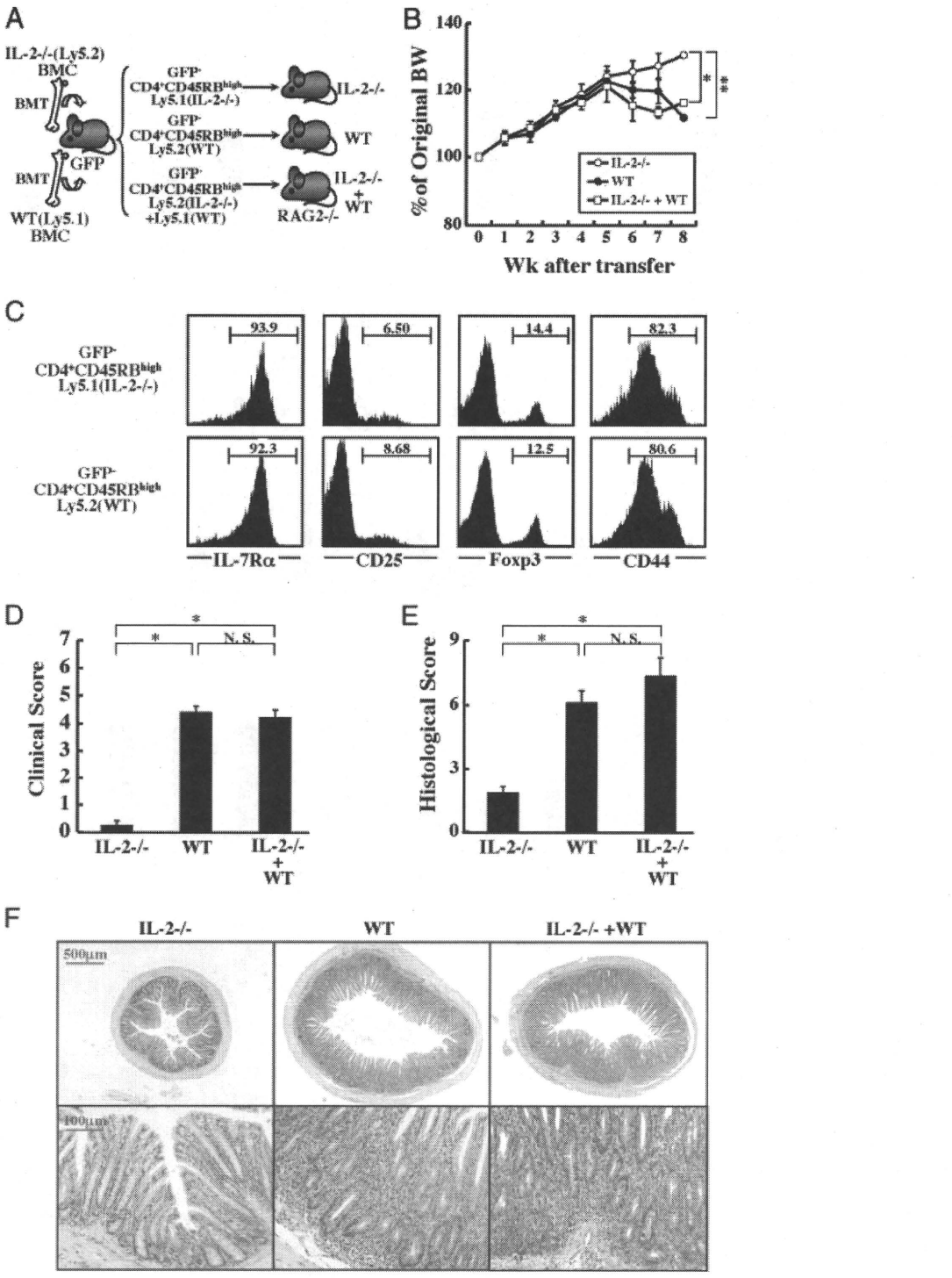


**Figure 7.** RAG-$2^{-/-}$ mice transferred with SP IL-$2^{-/-}$ $CD4^+CD45RB^{high}$ T cells from GFP mice previously transplanted with WT and IL-$2^{-/-}$ BM cells do not develop colitis. We performed one independent experiment. (A) Experimental design. Irradiated (7.5 Gy) GFP mice were transplanted with a mixture of the same number ($5 \times 10^6$ cells) of CD3-depleted BM cells from WT ($Ly5.1^+$) and IL-$2^{-/-}$ ($Ly5.2^+$) mice. Eight weeks after the BM transplantation, WT ($Ly5.1^+$) and IL-$2^{-/-}$ ($Ly5.2^+$) $GFP^-$ $CD4^+CD45RB^{high}$ T cells were isolated using FACSAria. The cells ($3 \times 10^5$ cells *per* mouse) or cell mixtures ($3 \times 10^5$ cells of each) were transferred into new RAG-$2^{-/-}$ mice ($n = 5$). Mice were sacrificed 8 wk after transfer. (B) The change in body weight over time is expressed as a percentage of original weight. Data are expressed as the mean ± SEM of five mice *per* group. $^*p = 0.008$, and $^{**}p = 0.041$ (Mann-Whitney *U*). (C) Phenotypic characterization of SP $GFP^-$ WT and IL-$2^{-/-}$ $CD4^+CD45RB^{high}$ donor T cells after BM transplantation. (D) Clinical score 8 wk after transfer. Data are expressed as the mean ± SEM of five mice *per* group. $^*p = 0.0143$ (Mann-Whitney *U*). (E) Histological score 8 wk after transfer. Data are expressed as the mean ± SEM of five mice *per* group. $^*p = 0.0143$ (Mann-Whitney *U*). (F) Histopathology of the distal colon 8 wk after transfer. Original magnification, × 40 (upper) and × 200 (lower).

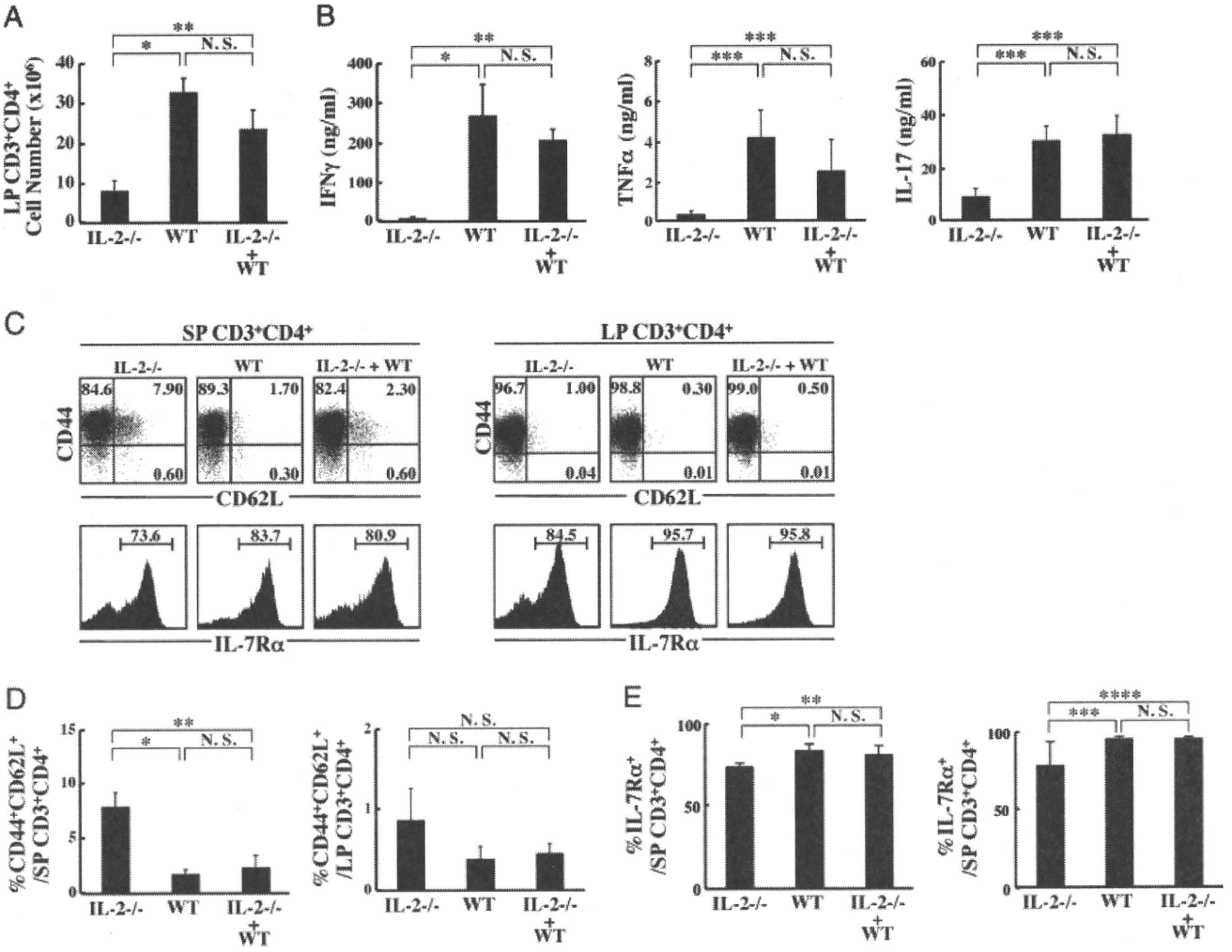


**Figure 8.** Marked down-modulation of IL-7Rα expression in non-colitic RAG-2$^{-/-}$ mice transferred with SP IL-2$^{-/-}$ CD4$^+$CD45RB$^{high}$ T cells. (A) Numbers of LP CD3$^+$CD4$^+$ T cells recovered from each mouse 8 wk after transfer. Data are expressed as the mean ± SD of five mice *per* group. $^*p = 0.037$ (Student's *t*), $^{**}p = 0.05$ (Mann-Whitney *U*), and N.S., not significant (B) Cytokine production (IFN-γ, TNF-α, and IL-17) by LP CD4$^+$ T cells. Data are expressed as the mean ± SEM of five mice *per* group. $^*p = 0.003$, $^{**}p = 0.007$, $^{***}p = 0.0143$, and N.S., not significant. (C) Expression of various cell surface markers on SP (left) and LP (right) CD3$^+$CD4$^+$ T cells of each group was assessed using FACS. (D) The percentage of SP (left) and LP (right) $T_{CM}$ cells was determined using FACS. Data are expressed as the mean ± SEM of five mice. $^*p = 0.0015$, $^{**}p = 0.0017$ (Mann-Whitney *U*), and N.S., not significant. (E) Percentage of SP (left) and LP (right) CD3$^+$CD4$^+$ T cells positive for IL-7Rα$^+$. Data are expressed as the mean ± SEM of five mice *per* group. $^*p = 0.0002$ (Welch's *t*), $^{**}p = 0.0065$ (Mann-Whitney *U*), $^{***}p = 0.0027$ (Mann-Whitney *U*), $^{****}p = 0.011$ (Mann-Whitney *U*), and N.S., not significant.

TNF-α, and IL-17 by anti-CD3/CD28 mAb-stimulated LP CD4$^+$ T cells obtained from RAG-2$^{-/-}$ mice transferred with WT CD4$^+$ CD45RB$^{high}$ T cells or a mixture of WT and IL-2$^{-/-}$ CD4$^+$ CD45RB$^{high}$ T cells was significantly higher than that produced in RAG-2$^{-/-}$ mice transferred with IL-2$^{-/-}$ CD4$^+$CD45RB$^{high}$ T cells (Fig. 8B).

We next examined cell surface markers in terms of $T_{CM}/T_{EM}$ and IL-7Rα after transfer. Again, the proportion of $T_{CM}$ in the SP of RAG-2$^{-/-}$ mice transferred with IL-2$^{-/-}$ CD4$^+$CD45RB$^{high}$ T cells was significantly higher than that in RAG-2$^{-/-}$ mice transferred with WT CD4$^+$CD45RB$^{high}$ T cells or a mixture of WT and IL-2$^{-/-}$ CD4$^+$CD45RB$^{high}$ T cells (Fig. 8C and D). In contrast, LP CD4$^+$ T cells were exclusively $T_{EM}$ cells irrespective of the cells transferred (Fig. 8C and D). It is noteworthy that the positive frequency of IL-7Rα expression on the SP and LP of RAG-2$^{-/-}$ mice transferred with IL-2$^{-/-}$ CD4$^+$CD45RB$^{high}$ T cells significantly reduced, as compared with that in the paired cells of other groups, irrespective of the presence or absence of colitis (Fig. 8C and E).

## Discussion

In the present work, we demonstrated that IL-2 is essential for the development and perpetuation of chronic colitis. First, IL-2 is needed for the normal expression of IL-7Rα on naïve CD4$^+$ T cells during the development of mature naïve CD4$^+$ T cells. Second, IL-2 is required for retaining IL-7Rα expression on colitogenic

 

$CD4^+$ T cells during conversion from effector to memory $CD4^+$ T cells, resulting in the acquisition of IL-7 dependency for the perpetuation of chronic colitis.

IL-$2^{-/-}$ mice, as well as IL-2Rα $(CD25)^{-/-}$, IL-2Rβ $(CD122)^{-/-}$, and IL-2Rγ $(CD132)^{-/-}$ mice [12], all of which are severely defective in the IL-2/IL-2R signalling pathway, are known to spontaneously develop chronic colitis. Thus, it was considered possible that the abnormal naïve $CD4^+$ T cells in IL-$2^{-/-}$ mice themselves might be critically involved in the development of colitis in such colitis models. It was also considered possible that IL-2 itself may not be needed for the development of pathogenic effector and memory $CD4^+$ T cells, but is solely essential for the development and maintenance of Treg, as was recently emphasized [16–18]. To solve these issues, we used an adoptive transfer model of colitis using WT or IL-$2^{-/-}$ $CD4^+$ $CD45RB^{high}$ T cells, which are almost all naïve T cells, excluding Treg. Surprisingly, we found that RAG-$2^{-/-}$ mice transferred with IL-$2^{-/-}$ $CD4^+CD45RB^{high}$ T cells did not develop colitis until 8 wk after transfer. Furthermore, IL-$2^{-/-}$ $CD4^+CD45RB^{high}$ T-cell-derived cells differentiated into $CD44^{high}$ memory T cells in the LP and SP, similar to WT T-cell-derived cells (Figs. 2 and 3). It should be noted that inducible $CD4^+CD25^+Foxp3^+$ Treg were present in RAG-$2^{-/-}$ mice transferred with WT, but not IL-$2^{-/-}$, $CD4^+CD45RB^{high}$ T cells. These results clearly demonstrate that IL-2 is positively involved in the development of colitis without the effect of naturally occurring Treg, although IL-2 supports the development of inducible Treg, which are generally thought to suppress the development of colitis. However, an unresolved discrepancy remains between the present finding that IL-2 is positively important for the development and perpetuation of colitis and the previous finding that IL-$2^{-/-}$ mice spontaneously develop colitis together with various autoimmune diseases. Indeed, we showed that adoptive retransfer of IL-$2^{-/-}$ LP $CD4^+$ T cells obtained from colitic RAG-$2^{-/-}$ mice previously transferred with a mixture of WT and IL-$2^{-/-}$ $CD4^+CD45RB^{high}$ T cells induced colitis, albeit less severely than adoptive retransfer of WT LP $CD4^+$ T cells, suggesting that IL-2 is not absolutely essential for the development of colitis, but is a fine tuner for the full development of colitis. Indeed, we confirmed that RAG-$2^{-/-}$ mice transferred with IL-$2^{-/-}$ $CD4^+CD45RB^{high}$ T cells develop mild colitis 20 wk after transfer (data not shown). It is also possible that other cells, such as B cells and NK T cells may affect the development of colitis in IL-$2^{-/-}$ mice. Further investigation of this issue is needed.

The positive frequency of IL-7Rα expression on LP IL-$2^{-/-}$ $CD4^+CD44^{high}$ T cells was significantly reduced, as compared with the paired WT cells, indicating that IL-2 was needed for the expression of IL-7Rα on some subpopulation of $CD4^+$ T cells during the differentiation of memory $CD4^+CD44^{high}$ T cells in all the adoptive transfer experiments. Importantly, since we demonstrated that mice transferred with isolated IL-$7R\alpha^{high}$ or IL-$7R\alpha^{low}$ $CD4^+CD45RB^{high}$ T cells obtained from young IL-$2^{-/-}$ mice did not develop colitis (Fig. 3), it is not likely that IL-$7R\alpha^{low}$ cells suppress IL-$7R\alpha^{high}$ cells when they are co-transferred into RAG-$2^{-/-}$ mice. Rather, IL-2 may be critically involved in the retainment of IL-7Rα expression on colitogenic $CD4^+$ T cells. In other words, the lack of IL-2 may induce the exhaustion of colitogenic $CD4^+$ T cells, resulting in the apoptosis of those cells. Furthermore, it is a matter of controversy whether IL-2 positively or negatively controls IL-7Rα expression [13, 30]. One group demonstrated that IL-2 is a negative regulator of IL-7Rα expression during *in vitro* stimulation with anti-CD3/anti-CD28 mAb [30], whereas another group argued that IL-2 promotes the generation of $CD4^+$IL-$7R\alpha^+$ memory T cells based on findings from a physiologically relevant *in vitro* and *in vivo* priming system with antigen and antigen-presenting cells [15]. Our result from adoptive transfer in the *in vivo* colitis model supports the latter result. In our previous study with the same model of colitis using IL-2-sufficient $CD4^+CD45RB^{high}$ T cells [27], we observed that the expression of IL-7Rα on $CD4^+$ T cells was down-modulated approximately 1 wk after transfer and was then up-regulated until 4 wk after transfer. Thus, our current result suggests that IL-2 is needed for re-expression of IL-7Rα during the differentiation of functionally colitogenic memory $CD4^+$ T cells. In accordance with this, we showed that IL-$7^{-/-}$ × RAG-$2^{-/-}$ mice retransferred with IL-$2^{-/-}$ LP $CD4^+$ T cells obtained from colitic RAG-$2^{-/-}$ mice previously transferred with a mixture of WT and IL-$2^{-/-}$ $CD4^+CD45RB^{high}$ T cells did not develop colitis, whereas IL-$7^{+/+}$ × RAG-$2^{-/-}$ mice retransferred with those cells did develop colitis, albeit to a lesser extent than IL-$7^{+/+}$ × RAG-$2^{-/-}$ mice retransferred with WT $CD4^+CD45RB^{high}$ T-cell-derived colitic LP $CD4^+$ T cells. IL-2 may play a crucial role in the re-expression of IL-7Rα on colitogenic memory $CD4^+$ T cells, which may be critical for the acquisition of their IL-7 dependency in chronic colitis. Furthermore, since we showed that mice transferred with IL-$2^{-/-}$ $CD4^+CD45RB^{high}$ T cells retained IL-$7R\alpha^{high}$ $CD4^+$ T-cell population partly as well as IL-$7R\alpha^{low}$ population, but did not develop colitis, it is possible that non-colitogenic $CD4^+$ memory T cells could re-express IL-7Rα highly in IL-2-independent manner. Further investigation of this issue is needed.

Nevertheless, our model is open to criticism concerning the role of IL-2 in the development of functional memory $CD4^+$ T cells when impaired IL-$2^{-/-}$ $CD4^+CD45RB^{high}$ T cells are used because their IL-7Rα expression is down-modulated before transfer. To this end, we next used donor WT and IL-$2^{-/-}$ $CD4^+$ $CD45RB^{high}$ T cells after preparing a mixed BM chimera that received a mixture of WT and IL-$2^{-/-}$ BM cells. As mentioned previously, the phenotypic characteristics of the IL-$2^{-/-}$ $CD4^+$ T cells in the BM chimera mice, including IL-7Rα expression, were similar to those of WT $CD4^+$ T cells, indicating that paracrine IL-2 secretion from WT $CD4^+$ T cells supports the normal development of naïve IL-$2^{-/-}$ $CD4^+CD45RB^{high}$ T cells in the BM and/or thymus and prevents preclinical autoimmunity *via* Treg that develop from WT precursor cells. However, we found that RAG-$2^{-/-}$ mice transferred with the IL-$2^{-/-}$ $CD4^+CD45RB^{high}$ T cells sufficiently expressing IL-7Rα- did not develop colitis along with the down-modulated expression of IL-7Rα on both LP and SP $CD4^+$ T cells after the transfer, which is in contrast to mice transferred with the WT $CD4^+CD45RB^{high}$ T cells, which

developed severe Th1/Th17-mediated colitis. These results reinforce our evidence that IL-2 is needed for the development of colitogenic memory $CD4^+$ T cells.

Finally, it should be noted that our study might not reflect the pathogenesis of human IBD directly because our colitis model is in the extreme "lymphopenic" environment for the induction of colitis, in which IL-7 and IL-2 might be important for rapid proliferation of T cells. Further studies will be needed to address this point using other colitis models in non-lymphopenic conditions.

Collectively, our findings indicate that, at least in the absence of Treg, IL-2 is critically involved in the development and perpetuation of colitis in three ways: (i) in the normal development of naïve $CD4^+$ T cells, (ii) in the re-expression of IL-7Rα on colitogenic memory $CD4^+$ T cells, and (iii) in the conversion from $T_{CM}$ to $T_{EM}$/effector T cells. Although it will be necessary to consider the double-edged role of IL-2 in colitogenic $CD4^+$ T cells and Treg in other models of colitis, which may affect the balance between these two cell types, the current study appears to shed light on the positive involvement of IL-2 in chronic colitis. Thus, it will be necessary to assess when strategies for blocking and promoting IL-2 for IBD treatment should begin.

## Materials and methods

### Mice

C57BL/6-Ly5.1 and C57BL/6-Ly5.2-RAG-$2^{-/-}$ mice were obtained from Taconic Laboratory (Hudson, NY) and the Central Experimental Animal Institute (Kawasaki, Japan). IL-$2^{-/-}$ mice were obtained from the Jackson Laboratory (Bar Harbor, ME). C57BL/6-Ly5.2- IL-$7^{-/-}$ × RAG-$1^{-/-}$ mice were kindly provided by Dr. Rosa Zamoyska (National Institute for Medical Research, London, UK) [31]. GFP transgenic mice were originally generated by Dr. Masaru Okabe (Osaka University, Japan) [32]. All mice were maintained under specific pathogen-free conditions at the Animal Care Facility of Tokyo Medical and Dental University. All experiments were approved by the regional animal study committees.

### Antibodies

Biotin-conjugated anti-IL-7Rα (A7R34) was obtained from Immuno-Biological Laboratories (Takasaki, Japan). The following mAb were obtained from BD PharMingen (San Diego, CA): 145–2C11, FITC- or PerCP- anti-CD3; RM4–5, FITC-, PE-, PerCP- or APC- anti-CD4; 16A, FITC- or APC- anti-CD45RB; IM7, PE-anti-CD44; PC61, PE-anti-CD25; MEL-14, FITC-anti-CD62L; H1.2F3, FITC-anti-CD69; A20, FITC- or PE-anti-Ly5.1 (CD45.1); and 104, FITC- or PE-anti-Ly5.2 (CD45.2). Biotinylated antibodies were detected with PE–streptavidin (BD Phar Mingen).

### *In vivo* adoptive transfer experiments

Adoptive transfers of WT or IL-$2^{-/-}$ $CD4^+CD45RB^{high}$ T cells into RAG-$2^{-/-}$ mice were performed to avoid the effect of $CD4^+CD25^+$ Treg. Exp. 1: Ly5.1-derived WT (IL-$2^{+/+}$) or Ly5.2-derived IL-$2^{-/-}$ ($n = 8$) $CD4^+CD45RB^{high}$ T cells ($3 \times 10^5$ cells) were injected intraperitoneally into RAG-$2^{-/-}$ mice. As a negative control, RAG-$2^{-/-}$ mice were transferred with $CD4^+CD45RB^{high}$ T cells ($3 \times 10^5$ cells) and Treg ($1 \times 10^5$ cells). Exp. 2: $CD4^+CD45RB^{high}$ T cells from SP of 4- to 5-wk-old IL-$2^{-/-}$ mice were divided into two populations, IL-7R$\alpha^{high}$ or IL-7R$\alpha^{low}$, by cell sorting and then each population was transferred into RAG-$2^{-/-}$ hosts. As a positive control, we transferred the same number of WT $CD4^+CD45RB^{high}$ T cells into RAG-$2^{-/-}$ mice. ($n = 5$). Exp. 3: First, SP $Ly5.1^+$ and $Ly5.2^+$ $CD4^+$ T cells were separately isolated from mice transferred with $Ly5.1^+$ WT and $Ly5.2^+$ IL-$2^{-/-}$ $CD4^+CD45RB^{high}$ T cells using FACSAria. Second, the isolated $Ly5.1^+$ or $Ly5.2^+$ T cells were separately transferred into new IL-$7^{+/+}$ × RAG-$2^{-/-}$ or IL-$7^{-/-}$ × RAG-$2^{-/-}$ mice. Exp. 4: First, preparations from the femurs and tibias of WT or IL-$2^{-/-}$ mice were incubated with biotin-conjugated anti-CD3 mAb and anti-biotin magnetic beads, followed by bead depletion using a MACS separation system (Miltenyi Biotec, Auburn, CA). Then $5 \times 10^6$ T-cell-depleted BM cells from each mouse were injected intravenously into lethally irradiated (7.5 Gy) GFP transgenic mice. Second, mice were sacrificed to isolate SP naïve $GFP^-$ $Ly5.1^+$ WT and $GFP^-$ $Ly5.2^+$ IL-$2^{-/-}$ $CD4^+$ $CD45RB^{high}$ T cells 8–12 wk post-transplantation. Third, the isolated $Ly5.1^+$ WT or $Ly5.2^+$ IL-$2^{-/-}$ $CD4^+CD45RB^{high}$ T cells were transferred into RAG-$2^{-/-}$ mice.

### Disease monitoring and histological examination

The recipient mice were weighed immediately after T-cell transfer and three times *per* week thereafter. They were observed for clinical signs of illness as previously described [9]. Histological examination was performed as described before [9].

### Flow cytometry

To detect the surface expression of a variety of molecules, isolated splenocytes or LP mononuclear cells were pre-incubated with an FcγR-blocking mAb (CD16/32; 2.4G2, BD PharMingen) for 20 min, followed by incubation with specific antibodies for 30 min on ice. Biotinylated antibodies were detected with PE–streptavidin. Intracellular Foxp3 staining was performed using a PE-anti-mouse Foxp3 staining set (eBioscience) according to the manufacturer's instructions. Background fluorescence was assessed by staining with control-irrelevant isotype-matched mAb.

### Cytokine ELISA

To measure cytokine production, $1 \times 10^5$ LP $CD4^+$ T cells were cultured in 200 μL of culture medium at 37°C in a humidified

atmosphere containing 5% $CO_2$ in 96-well plates (Costar, Cambridge, MA) precoated with 5 μg/mL of hamster anti-mouse CD3ε mAb (145–2C11, BD PharMingen) and 2 μg/mL of hamster anti-mouse CD28 mAb (37. 51, BD PharMingen) in PBS overnight at 4°C. Culture supernatants were removed after 48 h and assayed for cytokine production. Cytokine concentrations were determined by specific ELISA according to the manufacturer's recommendations (R&D Systems, Minneapolis, MN).

### Statistical analysis

We examined the normality of each group. If either group was not normally distributed, we assessed the difference between the two groups using the Mann–Whitney *U*-test. If both groups were normally distributed, we assessed the variance of the population to which each group belonged using the *f*-test. When homoscedasticity of both populations occurred, we assessed the difference between two groups using Student's *t*-test. In the absence of homoscedasticity, we assessed the difference using Welch's *t*-test. We used Statcell software for all statistical analyses. Results are expressed as the mean+SEM. Differences were considered statistically significant when $p<0.05$.

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## References

1 **Xavier, R. J. and Podolsky, D. K.,** Unravelling the pathogenesis of inflammatory bowel disease. *Nature* 2007. **448**: 427–434.

2 **Hibi, T. and Ogata, H.,** Novel pathophysiological concepts of inflammatory bowel disease. *J. Gastroenterol.* 2006. **41**: 10–16.

3 **Baumgart, D. C. and Sandborn, W. J.,** Inflammatory bowel disease: clinical aspects and established and evolving therapies. *Lancet* 2007. **369**: 1641–1657.

4 **Sartor, R. B.,** Mechanisms of disease: pathogenesis of Crohn's disease and ulcerative colitis. *Nat. Clin. Pract. Gastroenterol. Hepatol.* 2006. **3**: 390–407.

5 **Baumgart, D. C. and Carding, S. R.,** Inflammatory bowel disease: cause and immunobiology. *Lancet* 2007. **369**: 1627–1640.

6 **Mowat, A. M.,** Anatomical basis of tolerance and immunity to intestinal antigens. *Nat. Rev. Immunol.* 2003. **3**: 331–341.

7 **Strober, W., Fuss, I. J. and Blumberg, R. S.,** The immunology of mucosal models of inflammation. *Annu. Rev. Immunol.* 2002. **20**: 495–549.

8 **Tomita, T., Kanai, T., Totsuka, T., Nemoto, Y., Okamoto, R., Tsuchiya, K., Sakamoto, N. *et al.*,** IL-7 is essential for lymphopenia-driven turnover of colitogenic $CD4^+$ memory T cells in chronic colitis. *Eur. J. Immunol.* 2009. **39**: 2737–2747.

9 **Totsuka, T., Kanai, T., Nemoto, Y., Tomita, T., Tsuchiya, K., Sakamoto, N., Okamoto, R. and Watanabe, M.,** Immunosenescent colitogenic $CD4^+$ T cells convert to regulatory cells and suppress colitis. *Eur. J. Immunol.* 2008. **38**: 1275–1286.

10 **Dayer, S. J., Seibold, I., Saxer-Sekulic, N., Paredes, B. E., Saurer, L. and Mueller, C.,** Lack of TNFR2 expression by $CD4^+$ T cells exacerbates experimental colitis. *Eur. J. Immunol.* 2009. **39**: 1743–1753.

11 **Minami, Y., Kono, T., Miyazaki, T. and Taniguchi, T.,** The IL-2 receptor complex: its structure, function, and target genes. *Annu. Rev. Immunol.* 1993. **11**: 245–268.

12 **Malek, T. R.,** The biology of interleukin-2. *Annu. Rev. Immunol.* 2008. **26**: 453–479.

13 **Dooms, H., Wolslegel, K., Lin, P. and Abbas, A. K.,** Interleukin-2 enhances $CD4^+$ T cell memory by promoting the generation of IL-7R alpha-expressing cells. *J. Exp. Med.* 2007. **204**: 547–557.

14 **Sadlack, B., Merz, H., Schorle, H., Schimpl, A., Feller, A. C. and Horak, I.,** Ulcerative colitis-like disease in mice with a disrupted interleukin-2 gene. *Cell* 1993. **75**: 253–261.

15 **Suzuki, H., Kündig, T. M., Furlonger, C., Wakeham, A., Timms, E., Matsuyama, T., Schmits, R. *et al.*,** Deregulated T cell activation and autoimmunity in mice lacking interleukin-2 receptor beta. *Science* 1995. **268**: 1472–1476.

16 **Nelson, B. H.,** IL-2, regulatory T cells, and tolerance. *J. Immunol.* 2004. **172**: 3983–3988.

17 **Sakaguchi, S.,** Naturally arising $CD4^+$ regulatory T cells for immunologic self-tolerance and negative control of immune responses. *Annu. Rev. Immunol.* 2004. **22**: 531–562.

18 **Malek, T. R. and Bayer, A. L.,** Tolerance, not immunity, crucially depends on IL-2. *Nat. Rev. Immunol.* 2004. **4**: 665–674.

19 **Namen, A. E., Lupton, S., Hjerrild, K., Wignall, J., Mochizuki, D. Y., Schmierer, A., Mosley, B. *et al.*,** Stimulation of B-cell progenitors by cloned murine interleukin-7. *Nature* 1988. **333**: 571–573.

20 **Watanabe, M., Ueno, Y., Yajima, T., Iwao, Y., Tsuchiya, M., Ishikawa, H., Aiso, S. *et al.*,** Interleukin 7 is produced by human intestinal epithelial cells and regulates the proliferation of intestinal mucosal lymphocytes. *J. Clin. Invest.* 1995. **95**: 2945–2953.

21 **Fry, T. J. and Mackall, C. L.,** The many faces of IL-7: from lymphopoiesis to peripheral T cell maintenance. *J. Immunol.* 2005. **174**: 6571–6576.

22 **Bradley, L. M., Haynes, L. and Swain, S. L.,** IL-7: maintaining T-cell memory and achieving homeostasis. *Trends Immunol.* 2005. **26**: 172–176.

23 **Sprent, J. and Surh, C. D.,** T cell memory. *Annu. Rev. Immunol.* 2002. **20**: 551–579.

24 **Watanabe, M., Ueno, Y., Yajima, T., Okamoto, S., Hayashi, T., Yamazaki, M., Iwao, Y. *et al.*,** Interleukin 7 transgenic mice develop chronic colitis with decreased interleukin 7 protein accumulation in the colonic mucosa. *J. Exp. Med.* 1998. **187**: 389–402.

 

25 **Yamazaki, M., Yajima, T., Tanabe, M., Fukui, K., Okada, E., Okamoto, R., Oshima, S. *et al.***, Mucosal T cells expressing high levels of IL-7 receptor are potential targets for treatment of chronic colitis. *J. Immunol.* 2003. **171**: 1556–1563.

26 **Okada, E., Yamazaki, M., Tanabe, M., Takeuchi, T., Nanno, M., Oshima, S., Okamoto, R. *et al.***, IL-7 exacerbates chronic colitis with expansion of memory IL-7R$^{high}$ CD4$^{+}$ mucosal T cells in mice. *Am. J. Physiol. Gastrointest. Liver Physiol.* 2005. **288**: G745–G754.

27 **Totsuka, T., Kanai, T., Nemoto, Y., Makita, S. and Watanabe, M.**, IL-7 is essential for the development and the persistence of chronic colitis. *J. Immunol.* 2007. **178**: 4737–4748.

28 **Jameson, S. C.**, Maintaining the norm: T-cell homeostasis. *Nat. Rev. Immunol.* 2002. **2**: 547–556.

29 **Schluns, K. S. and Lefrancois, L.**, Cytokine control of memory T-cell development and survival. *Nat. Rev. Immunol.* 2003. **3**: 269–279.

30 **Xue, H. H., Kovanen, P. E., Pise-Masison, C. A., Berg, M., Radovich, M. F., Brady, J. N. and Leonard, W. J.**, IL-2 negatively regulates IL-7 receptor alpha chain expression in activated T lymphocytes. *Proc. Natl. Acad. Sci. USA* 2002. **99**: 13759–13764.

31 **Seddon, B., Tomlinson, P. and Zamoyska, R.**, Interleukin 7 and T cell receptor signals regulate homeostasis of CD4 memory cells. *Nat. Immunol.* 2003. **4**: 680–686.

32 **Kawakami, N., Sakane, N., Nishizawa, F.,Iwao, M., Fukada, S. I., Tsujikawa, K., Kohama, Y. *et al.***,Green fluorescent protein-transgenic mice: immune functions and their application to studies of lymphocyte development. *Immunol. Lett.* 1999. **70**: 165–171.

*Abbreviations:* **IBD:** inflammatory bowel disease · **LP:** lamina propria · **SP:** spleen · $T_{CM}$**:** central memory T cell · $T_{EM}$**:** effector memory T cell

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# Upregulated IL-7 Receptor α Expression on Colitogenic Memory CD4$^+$ T Cells May Participate in the Development and Persistence of Chronic Colitis

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**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$^{-/-}$ CD4$^+$CD25$^-$ T cells were differentiated to CD4$^+$ CD44$^{high}$CD62L$^-$ 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: 2623–2632.**

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 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-7Rα$^{high}$ T cells in RAG-2$^{-/-}$ mice in which colitis was induced by adoptive transfer of CD4$^+$CD45RB$^{high}$ T cells have characteristics of colitogenic memory T cells (20); 4) the selective elimination of CD4$^+$IL-7Rα$^{high}$ T cells by administrating toxin-conjugated anti–IL-7Rα mAb completely ameliorated ongoing colitis in TCR-α–deficient 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).

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Abbreviations used in this article: IBD, inflammatory bowel disease; LP, lamina propria; MFI, mean fluorescence intensity; SP, spleen; $T_{EM}$, effector-memory T; Treg, regulatory T cell; TSLP, thymic stromal lymphopoietin; WT, wild type.



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