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Role of interleukin-25 in development of spontaneous arthritis in interleukin-1 receptor antagonist-deficient mice

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ABSTRACT

Interleukin (IL)-25, which is a member of the IL-17 family of cytokines, induces production of such Th2 cytokines as IL-4, IL-5, IL-9 and/or IL-13 by various types of cells, including Th2 cells, Th9 cells and group 2 innate lymphoid cells (ILC2). On the other hand, IL-25 can suppress Th1- and Th17-associated immune responses by enhancing Th2-type immune responses. Supporting this, IL-25 is known to suppress development of experimental autoimmune encephalitis, which is an IL-17-mediated autoimmune disease in mice. However, the role of IL-25 in development of IL-17-mediated arthritis is not fully understood. Therefore, we investigated this using IL-1 receptor antagonist-deficient (IL-1Ra$^{-/-}$) mice, which spontaneously develop IL-17-dependent arthritis. However, development of spontaneous arthritis (incidence rate, disease severity, proliferation of synovial cells, infiltration of PMNs, and bone erosion in joints) and differentiation of Th17 cells in draining lymph nodes in IL-25$^{-/-}$ IL-1Ra$^{-/-}$ mice were similar to in control IL-25$^{+/+}$ IL-1Ra$^{-/-}$ mice. These observations indicate that IL-25 does not exert any inhibitory and/or pathogenic effect on development of IL-17-mediated spontaneous arthritis in IL-1Ra$^{-/-}$ mice.

1. Introduction

Interleukin (IL)-25 is a member of the IL-17 family of cytokines and binds to IL-17 receptor (IL-17R) A and IL-17RB [1]. IL-25 is produced by epithelial cells, Th2 cells, macrophages and mast cells [2,3]. It induces production of such Th2 cytokines as IL-4, IL-5 and/or IL-13 by various types of cells—including Th2 cells, Th9 cells, CD11c^{+} F4/80^{+} macrophages, natural killer T (NKT) cells and/or group 2 innate lymphoid cells (ILC2) [4,5]—that are involved in host defense against such pathogens as *Trichuris muris* and *Nippostrongylus brasiliensis* [6–8]. On the other hand, inappropriate/excessive activation of IL-25 leads to development of certain Th2-type allergic diseases such as asthma. Expression of IL-25 mRNA/protein is elevated in specimens from asthmatics [4]. In addition, inhalation of recombinant IL-25 by mice resulted in development of airway inflammation accompanied by accumulation of eosinophils in the lungs [9]. By contrast, IL-25-deficient (IL-25$^{-/-}$) mice and/or mice treated with anti-IL-25–neutralizing Ab showed attenuation of allergic airway inflammation induced by ovalbumin [10,11]. On the other hand, IL-25 is thought to suppress Th1- and Th17-associated immune responses by enhancing Th2-type immune responses [12,13]. For example, IL-25 suppressed development of experimental autoimmune encephalitis [12], colitis [14–16] and type I diabetes [17] in mice.

Mice deficient in IL-1 receptor antagonist (IL-1Ra), which binds to IL-1R but does not induce cellular signal transduction, are known to spontaneously develop certain diseases such as arthritis [18], aortitis [19] and dermatitis [20]. In particular, the spontaneous arthritis seen in IL-1Ra$^{-/-}$ mice is known to be caused by excessive Th17-associated

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immune responses [21,22]. These observations suggested that IL-25 also may inhibit development of Th17-associated autoimmune arthritis. Therefore, in the present study we investigated the role of IL-25 in development of spontaneous autoimmune arthritis in IL-1Ra$^{-/-}$ mice.

2. Materials and methods

2.1. Mice

IL-25$^{-/-}$ IL-1Ra$^{-/-}$ mice were generated by crossing IL-25$^{-/-}$ mice [23] and IL-1Ra$^{-/-}$ mice [24] on the BALB/cA background (more than N8). Gender- and age-matched littermates (IL-25$^{+/+}$ IL-1Ra$^{-/-}$ mice) were used as controls. All mice were housed in a specific pathogen-free environment at The Institute of Medical Science, The University of Tokyo. The animal protocol for experiments was approved by the Institutional Review Board of The Institute (A14-11) and met the ethical and safety guidelines of The Institute.

2.2. Scoring of severity of arthritis

For each mouse paw, the severity of arthritis was scored on a scale of 0–3 based on the macroscopic degree of redness and swelling, as described previously [18]. Grade 0 = normal, grade 1 = light swelling of the joint and/or redness of the footpad, grade 2 = obvious swelling of the joint, and grade 3 = severe swelling and fixation of the joint. The total severity score was calculated for the four limbs of each mouse (maximum score of 12 per mouse). The scoring was performed by an investigator who was blinded to the mouse genotypes.

2.3. Histology

Joints of mice were fixed in 10% neutral buffered formalin, decalcified in 10% EDTA-4Na and embedded in paraffin. Sections were prepared from the paraffin-embedded tissues and stained with hematoxylin and eosin.

2.4. Cell culture

Popliteal lymph nodes (LNs) were collected, and LN cells were suspended in RPMI1640 (Sigma-Aldrich) supplemented with 10% heat-inactivated FBS (Invitrogen), 50 μM 2-mercaptoethanol (Invitrogen), 50 μg/ml streptomycin and 50 U/ml penicillin (Invitrogen). The suspended LN cells (2×10^5 cells/well in 0.2 ml in 96-well flat-bottom plates (IWAKI)) were cultured in the presence and absence of 0.1 μg/ml anti-mouse CD3 mAb (145-2C11; BioLegend) and anti-mouse CD28 mAb (37.51; BioLegend) at 37 °C for 48 h in a 5% CO_2 incubator. Cell proliferative responses were determined by pulsing with 0.25 μCi/ml [^{3}H]-labeled thymidine for 6 h. The cells in each well were then harvested on a glass filter with a Micro 96 cell harvester (Skatron), and the [^{3}H]-thymidine radioactivity on the filter was measured with a Micro Beta counter (Pharmacia Biotech).

2.5. ELISA

Forty-eight hours after LN cell cultivation, the culture supernatants were collected, and the levels of IFN-γ, IL-4, IL-10 and IL-17A in the culture supernatants were determined with ELISA kits according to the manufacturer's instructions (BioLegend).

2.6. Statistics

The chi-square test and two-way ANOVA were used for statistical evaluation of the incidence and score, respectively. For other results, the unpaired Student's *t*-test, two-tailed, was used. The statistical analyses were performed using Prism (GraphPad Software, Inc.).

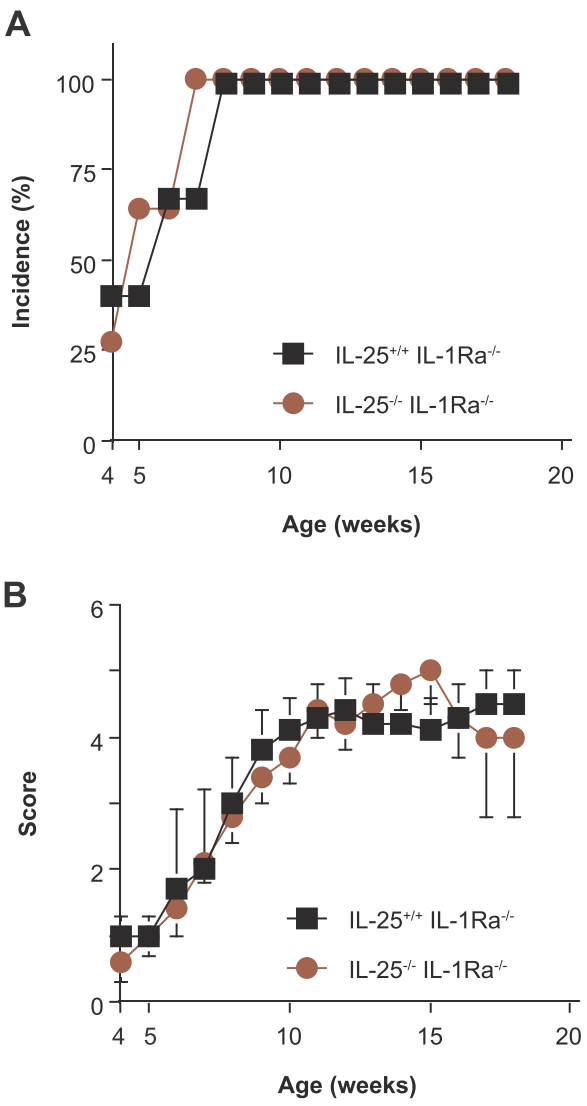

Fig. 1. IL-25 is not essential for development of spontaneous arthritis in IL-1Ra$^{-/-}$ mice. (A) The incidence rate and (B) the disease severity score were compared between IL-25$^{+/+}$ IL-1Ra$^{-/-}$ mice (n = 9–15) and IL-25$^{-/-}$ IL-1Ra$^{-/-}$ mice (n = 11–16). Data show the mean ± SEM.

3. Results

As reported previously [18], IL-1Ra$^{-/-}$ mice (IL-25$^{+/+}$ IL-1Ra$^{-/-}$ mice) spontaneously developed autoimmune-like arthritis (Fig. 1). Spontaneous development of arthritis in IL-1Ra$^{-/-}$ mice was shown to be dependent on excessive activation of T cells and production of IL-17 and TNF [22,25,26]. IL-25 suppressed Th17 cell differentiation and thereby contributed to suppression of Th17-mediated autoimmune diseases such as experimental autoimmune encephalitis in mice [12,13]. Those findings suggested that IL-25 might suppress IL-17-mediated spontaneous arthritis in IL-1Ra$^{-/-}$ mice. However, the incidence rate and disease severity of spontaneous arthritis were the same in IL-25$^{-/-}$ IL-1Ra$^{-/-}$ mice and IL-25$^{+/+}$ IL-1Ra$^{-/-}$ mice (Fig. 1). In the histological analysis, proliferation of synovial cells, infiltration of PMNs and bone erosion were also similar in both groups (Fig. 2). It was reported that LN cells from IL-1Ra$^{-/-}$ mice excessively produced IL-17 compared with LN cells from wild-type mice [21]. The proliferative responses and IL-17 production, as well as IFN-γ, IL-4 and IL-10 production, by LN cells from IL-25$^{-/-}$ IL-1Ra$^{-/-}$ mice were comparable to those by LN cells from IL-25$^{+/+}$ IL-1Ra$^{-/-}$ mice (Fig. 3), suggesting that IL-25 does not influence differentiation of Th17 cells or Th1 and Th2

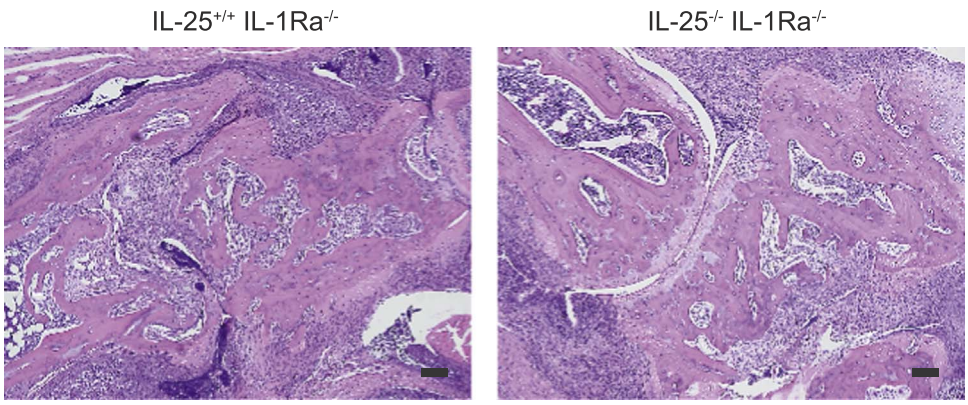

Fig. 2. IL-25-deficiency did not affect local inflammation in IL-1Ra$^{-/-}$ mice. Histology of the ankle joints of IL-25$^{+/+}$ IL-1Ra$^{-/-}$ mice and IL-25$^{-/-}$ IL-1Ra$^{-/-}$ mice. Representative results for the mice in Fig. 1 are shown. Scale bar = 100 μm.

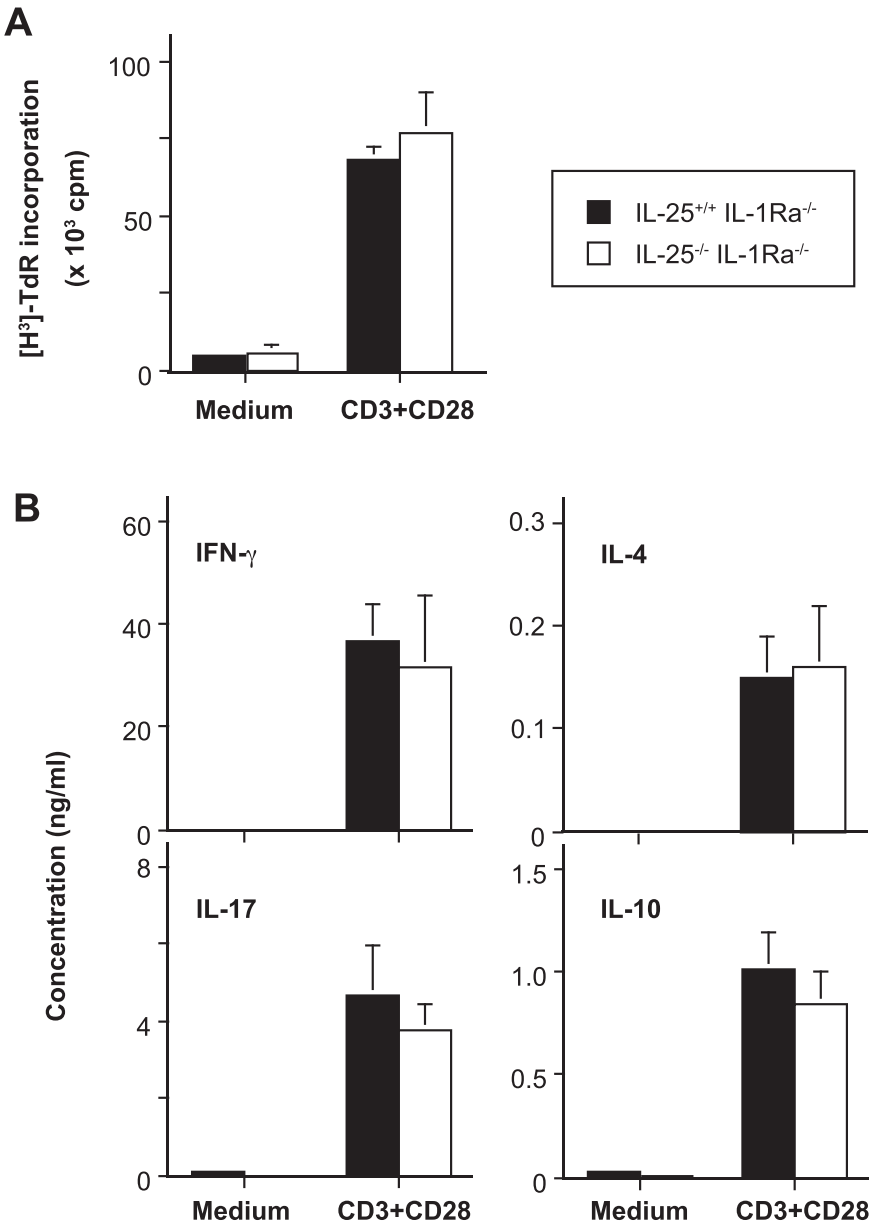

Fig. 3. IL-25-deficiency did not affect differentiation of helper T-cell subsets in draining LNs. Popliteal LN cells were cultured in the presence and absence of anti-CD3 and anti-CD28 mAbs for 48 h. (A) Proliferative responses and (B) cytokine production of LN cells from IL-25$^{+/+}$ IL-1Ra$^{-/-}$ mice (n = 5) and IL-25$^{-/-}$ IL-1Ra$^{-/-}$ mice (n = 5). Data show the mean ± SEM. Representative results of three independent experiments.

cells in this model. Taken together, our findings suggest that IL-25 exerts no inhibitory and/or pathogenic effects on development of IL-17-mediated spontaneous arthritis in IL-1Ra$^{-/-}$ mice.

4. Discussion

Recently, IL-25 levels were shown to be elevated in specimens from patients with rheumatoid arthritis [27], suggesting that IL-25 somehow contributes to development of rheumatoid arthritis. Since IL-25 can inhibit IL-17-producing Th17 cell differentiation that is dependent on IL-13, thereby contributing to suppression of IL-17-mediated autoimmune diseases such as experimental autoimmune encephalitis in mice [13], it was thought that IL-25 might also suppress IL-17-mediated arthritis. Indeed, it was recently reported that administration of recombinant IL-25 resulted in amelioration of development of collagen-induced arthritis, which is one murine model of IL-17-mediated arthritis, by inhibiting Th17 cell differentiation in an IL-13–dependent manner [27]. On the other hand, we demonstrated that IL-25-deficiency did not exert any effect on the incidence rate, disease severity or Th17 cell differentiation in another IL-17-mediated spontaneous arthritis model in IL-1Ra$^{-/-}$ mice. In the setting, IL-25 mRNA could not be detected in the animals' inflamed joints by quantitative PCR (data not shown). Therefore, in contrast to collagen-induced arthritis and experimental autoimmune encephalitis, our results suggest that IL-25 does not play any inhibitory role in development of IL-17-mediated spontaneous arthritis in IL-1Ra$^{-/-}$ mice.

5. Conclusion

IL-25 does not play any inhibitory and/or pathogenic role in development of IL-17-mediated spontaneous arthritis in IL-1Ra$^{-/-}$ mice.

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Appendix A. Transparency document

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.bbrep.2017.08.006.

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