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

Yasuharu Abe\textsuperscript{a,\textbullet}, Aya Nambu\textsuperscript{d}, Sachiko Yamaguchi\textsuperscript{d}, Ayako Takamori\textsuperscript{d}, Hajime Suto\textsuperscript{e}, Sachiko Hirose\textsuperscript{f}, Tadashi Yokosuka\textsuperscript{a}, Susumu Nakae\textsuperscript{d,\textbullet,\textcircled{}}\textsuperscript{g}, Katsuko Sudo\textsuperscript{b,\textbullet,\textcircled{}}

\textsuperscript{a} Department of Immunology, Tokyo Medical University, Tokyo 160-8402, Japan
\textsuperscript{b} Animal Research Center, Tokyo Medical University, Tokyo 160-8402, Japan
\textsuperscript{c} Department of Pharmacy, Toyohashi Medical Center, National Hospital Organization, Aichi 440-8510, Japan
\textsuperscript{d} Laboratory of Systems Biology, Center for Experimental Medicine and Systems Biology, The Institute of Medical Science, The University of Tokyo, Tokyo 108-8639, Japan
\textsuperscript{e} Atopy Research Center, Juntendo University, Tokyo 113-8412, Japan
\textsuperscript{f} Toin Human Science and Technology Center, Department of Biomedical Engineering, Toin University of Yokohama, Yokohama 225-8502, Japan
\textsuperscript{g} Precursory Research for Embryonic Science and Technology, Japan Science and Technology Agency, Saitama 332-0012, Japan

\textsuperscript{\textbullet} Corresponding author at: Laboratory of Systems Biology, Center for Experimental Medicine and Systems Biology, The Institute of Medical Science, The University of Tokyo, 4-6-1 Shirokanedai, Minato, Tokyo 108-8639, Japan.
\textsuperscript{\textcircled{}} Corresponding author.

E-mail addresses: snakae@ims.u-tokyo.ac.jp (S. Nakae), ksudo@tokyo-med.ac.jp (K. Sudo).

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\textsuperscript{+} F4/80\textsuperscript{+} 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\textsuperscript{-/-}) 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
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 μg/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% CO2 incubator. Cell proliferative responses were determined by pulsing with 0.25 μCi/ml [3H]-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 [3H]-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. 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 immune diseases such as experimental autoimmune encephalitis in mice [13, 14]. 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. Since IL-25 can inhibit IL-17-producing Th17 cells in this model. Taken together, 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.

Acknowledgments

We thank Ayano Ishii for her skilled technical assistance and Dr. Yoichirow Ikawaka (Tokyo University of Biomedical Sciences, Chiba, Japan) for providing IL-1Ra−/− mice. We are grateful to Lawrence W. Stiver (Tokyo, Japan) for his critical reading of the manuscript. This work was supported by PRESTO, JST (S.N.).

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.

References

[1] Y. Ikawaka, S. Nakae, S. Saijo, H. Ishigame, The roles of IL-17A in inflammatory immune responses and host defense against pathogens, Immunol. Rev. 226 (2008) 57–79.
[2] W.E. Paul, J. Zhu, How are T(H)2-type immune responses initiated and amplified? Nat. Rev. Immunol. 10 (2010) 225–235.
[3] S.A. Saez, B.C. Taylor, D. Artis, Welcome to the neighborhood: epithelial cell-derived cytokines license innate and adaptive immune responses at mucosal sites, Immuno. Rev. 226 (2008) 172–190.
[4] J.M. Reynolds, P. Angkasekwinna, C. Dong, IL-17 family member cytokines: regulation and function in innate immunity, Cytokine Growth Factor Rev. 21 (2010) 413–423.
[5] C.S. Kloe, D. Artis, Innate lymphoid cells as regulators of immunity, immunization and tissue homeostasis, Nat. Immunol. 17 (2016) 765–774.
[6] A.M. Owyang, C. Zaph, E.H. Wilson, K.J. Guild, T. McLellan, H.R. Miller, D.J. Cua, M. Goldschmidt, C.A. Hunter, R.A. Kastelein, D. Artis, Interleukin 25 regulates type 2 cytokine-dependent immunity and limits chronic inflammation in the gastrointestinal tract, J. Exp. Med. 203 (2006) 843–849.
[7] P.G. Fallon, S.J. Ballantine, N.L. Mangan, J.L. Barlow, A. Davvarma, D.R. Hewett, A. Mcgirm, H.E. Jolin, A.N. McKenzie, Identification of an interleukin (IL)-25-dependent cell population that provides IL-4, IL-5, and IL-13 at the onset of helminth expulsion, J. Exp. Med. 203 (2006) 1105–1116.
[8] A. Zhao, J.F. Urban Jr., R. Sun, J. Stitziel, M. Morimoto, L. Notari, K.B. Madden, Z. Yang, V. Grinchuk, T.R. Ramalingam, T.A. Wynn, T. Shea-Donohue, Critical role of IL-25 in nematode infection-induced alterations in intestinal function, J. Immunol. 185 (2010) 6921–6929.
[9] S.D. Hurst, T. Muchamuel, D.M. Gorman, J.M. Gilbert, T. Clifford, S. Kwan, S. Menon, B. Seymour, C. Jackson, T.T. Rung, J.K. Breitbard, R.W. Chapman, G. Zurawski, R.L. Coffman, New IL-17 family members promote Th1 or Th2 responses in the lung: in vivo function of the novel cytokine IL-25, J. Immunol. 169 (2002) 443–452.
[10] M. Suzuki, H. Morita, A. Nambu, K. Arai, E. Shimura, A. Shibui, S. Yamaguchi, K. Suzukiwa, W. Nakakishi, K. Oboki, N. Kajiwara, T. Ohno, A. Ishii, H. Korner, D.J. Cua, H. Suto, T. Yoshimoto, Y. Ikawaka, T. Yasokata, K. Ohtani, K. Sudo, H. Saijo, K. Okumura, D.H. Broide, K. Matsumoto, S. Nakae, Epithelial cell-derived IL-25, but not Th17 cell-derived IL-17 or IL-17F, is crucial for murine asthma, J. Immunol. 189 (2012) 3641–3652.
[11] S.J. Ballantine, J.L. Barlow, H.E. Jolin, P. Nath, A.S. Williams, K.F. Chung, G. Sturton, S.H. Wong, A.N. McKenzie, Blocking IL-25 prevents airway hyper-reactivity and/or pathogenic effects on development of IL-17-mediated spontaneous arthritis in IL-1Ra−/− mice.

65