Cutting Edge: Generation of IL-18 Receptor-Deficient Mice: Evidence for IL-1 Receptor-Related Protein as an Essential IL-18 Binding Receptor

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*J Immunol* 1999; 162:5041-5044;
http://www.jimmunol.org/content/162/9/5041

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IL-18 is a proinflammatory cytokine that plays an important role in NK cell activation and Th1 cell response. Recently IL-1R-related protein (IL-1Rrp) has been cloned as the receptor for IL-18. However, the functional role of IL-1Rrp is still controversial due to its low affinity to IL-18 as well as the possibility of the presence of another high-affinity binding receptor. In the present study, we have generated and characterized IL-1Rrp-deficient mice. The binding of murine rIL-18 was not detected in Th1-developing splenic CD4+ T cells isolated from IL-1Rrp-deficient mice. The activation of NF-κB or c-Jun N-terminal kinase were also not observed in the Th1 cells. NK cells from IL-1Rrp-deficient mice had defects in cytolytic activity and IFN-γ production in response to IL-18. Th1 cell development was also impaired in IL-1Rrp-deficient mice. These data demonstrate that IL-1Rrp is a ligand-binding receptor that is essential for IL-18-mediated signaling events. The Journal of Immunology, 1999, 162: 5041–5044.
fragment with the neomycin resistance gene (neo) from pMC1-neo (Stratagene). An HSV-thymidine kinase gene (HSV-TK) was inserted into the 3’ end of the vector. The targeting vector was electroporated into E14.1 embryonic stem (ES) cells. The generation of chimeric mice and mutated mice was essentially as described previously (10).

Analysis of NK cell activity and Th1 cell development

NK cell activity was analyzed as described previously (4). In vivo and in vitro induction of Th1 cell differentiation was performed essentially as described previously (4). Splenic CD4+ T cells were enriched by positive selection on LS+ columns using a high-gradient magnetic cell separation system (Miltenyi Biotec, Bergisch Gladbach, Germany) and were used for additional experiments.

Binding assay

Radiiodination of murine rIL-18 (5 μg) was performed using 1.85 MBq of Bolton-Hunter reagent (DuPont/New England Nuclear, Boston, MA) according to the manufacturer’s instructions. The specific radioactivity of the preparations was 20,700 cpm/ng protein.

Naive splenic CD4+ T cells were purified as described above and cultured for 4 days in the presence of 100 ng/ml anti-CD3 Ab and 2 ng/ml IL-12. Next, the cells were washed twice and suspended in RPMI 1640 medium containing 0.1% BSA, 0.1% NaN3, and 100 mM HEPES (pH 7.2) (binding medium) before use. A standard binding assay was performed as described previously (3). The dissociation constant (Kd) and the number of binding sites were calculated from Scatchard plots.

Gel-mobility shift assay (GMSA) and in vitro kinase assay

Th1-developing splenic CD4+ T cells were prepared as described above and stimulated with or without IL-18. A GMSA and an in vitro kinase assay were performed as described previously (8).

Results and Discussion

Generation of IL-1Rrp+/− mice

The mouse IL-1Rrp gene was disrupted by homologous recombination in E14.1 ES cells. A targeting vector was designed to replace an exon, which corresponded to amino acid residue 296–350, encoding the transmembrane region of the IL-1Rrp with neo. Targeted ES clones successfully transmitted the disrupted IL-1Rrp gene through germline (Fig. 1A). The mice remained healthy as they grew and showed no obvious abnormalities until they reached 21 wk of age. RT-PCR analysis confirmed the normal composition in 6-wk-old IL-1Rrp+ mice (data not shown). The DX5-positive NK cell population in the spleen and liver was not altered on IL-1Rrp+/− mice (data not shown).

Impaired in vivo Th1 development in IL-1Rrp+/− mice

It has been shown recently that IL-18 does not drive Th1 development but does potentiate IL-12-induced Th1 development (2). Like IL-18-deficient mice, naive T cells from IL-1Rrp+/− mice developed into IFN-γ-producing Th1 cells in response to IL-12 in vitro (Fig. 2A) (4). It has been shown that IL-12Rβ2 mRNA is expressed only in Th1 cells (12). The expression of IL-12Rβ2 mRNA in both wild-type (wt) and IL-1Rrp+/− T cells was upregulated in response to IL-12 in vitro (Fig. 2B). However, when mice were injected i.p. with heat-killed P. acnes, IFN-γ production from the T cells of IL-1Rrp+/− mice was reduced compared with wt T cells. IL-18 augmented IFN-γ production from activated wt T cells (Fig. 2C). However, IFN-γ production was not enhanced in IL-1Rrp+/− T cells by stimulation with IL-18. Similar results were obtained from two other independent experiments. These data show that IL-1Rrp+/− mice have a defect in Th1 cell development in vivo, as is the case for IL-18-deficient mice (4).

Lack of IL-18 binding in splenocytes from IL-1Rrp+/− mice

To examine whether the Th1-developing splenecy CD4+ T cells obtained from IL-1Rrp+/− mice could bind IL-18, we performed binding experiments. Because Th1 cells preferentially express IL-18R (3), naive splenic CD4+ T cells obtained from IL-1Rrp+/− and wt mice were cultured in the presence of anti-CD3 Ab plus IL-12 for 4 days to induce Th1 cell development. As shown in Fig. 2, A and B, both wt and IL-1Rrp+/− T cells displayed Th1 phenotypes. The Th1-developing cells obtained from wt mice showed specific binding to 125I-labeled murine rIL-18. The Th1 cells from wt mice displayed both high- (Kd = 1.9 nM with 13,500 sites/cell) and low- (Kd = 27 nM with 13,500 sites/cell) affinity binding sites. In contrast, no specific binding sites for IL-18 were found on Th1-developing cells from IL-1Rrp+/− mice (Fig. 3). These results demonstrate that IL-1Rrp is a receptor component that is essential for IL-18 binding on the surface of Th1 cells.

Loss of IL-18-induced NF-κB DNA binding activity and JNK activation in IL-1Rrp+/− mice

IL-18 has been shown to induce the activation of NF-κB DNA binding activity in Th1 cells (2, 8). We subsequently investigated whether an IL-18-induced activation of NF-κB was observed in IL-1Rrp+/− mice. Th1-developing splenecy CD4+ T cells were starved for 3 h and then stimulated with 100 ng/ml IL-18. Nuclear extracts from the stimulated cells were analyzed by GMSA using a specific probe containing NF-κB binding site. IL-18-induced
The concentration of IFN-γ in the presence or absence of 20 ng/ml IL-18 for 24 h. cDNA was used for PCR analysis using specific primers for IL-12Rβ2 mRNA and reverse-transcribed before and after Th1 induction. The resulting cDNA was used for PCR analysis using specific primers for IL-12Rβ2 and β-actin (11). Primer sequences are available upon request.

NF-κB DNA binding activity was detected in the nuclear extract from wt cells but not in that from IL-1Rrp−/− cells (Fig. 4A). In addition to the induction of NF-κB activation, IL-18 has also been shown to activate JNK (8). We conducted an in vitro kinase assay using GST-c-Jun fusion protein as a substrate. Treatment with IL-18 induced JNK activation in Th1-developing CD4+ T cells from wt mice. However, IL-18-induced JNK activation was not observed in Th1 cells from IL-1Rrp−/− mice (Fig. 4B). These data demonstrate that IL-1Rrp is essential for the IL-18-induced activation of both NF-κB and JNK in Th1 cells.

**Impaired NK cell activity in IL-1Rrp−/− mice**

IL-18 has been shown to be a potent activator of NK cells (2). We analyzed the function of NK cells by NK lytic activity against YAC-1 cells in IL-1Rrp−/− mice. Splenocytes were incubated with 51Cr-labeled YAC-1 target cells at the indicated E:T ratios, and 51Cr release from YAC-1 target cell was measured. The killing activities of NK cells obtained from IL-1Rrp−/− mice were at least one-fourth those from wt mice (Fig. 5A). We subsequently examined in vitro activation of NK cell activity in IL-1Rrp−/− mice. An in vitro culture of splenocytes with IL-18 dramatically enhanced lytic activity in wt mice (Fig. 5C). However, IL-18 did not enhance NK lytic activity in IL-1Rrp−/− mice. Stimulating splenocytes with IL-12 or IL-12 led to almost equal levels of NK lytic activity in IL-1Rrp−/− mice compared with wt mice (Fig. 5A, D and E). Therefore, these data suggest that the IL-18-mediated activation of NK cells is specifically impaired in IL-1Rrp−/− mice.

IL-18 and IL-12 have also been shown to synergistically induce IFN-γ production from spleen cells (2). Indeed, in vitro stimulation of splenocytes with IL-18 and IL-12 synergistically augmented IFN-γ production in wt mice (Fig. 5F). However, synergistic IFN-γ production was not observed in IL-1Rrp−/− mice. These results suggest that the splenocytes of IL-1Rrp−/− mice have a defect in their ability to produce IFN-γ in response to IL-18.

Although IL-1Rrp is actually involved in IL-18 signaling, the role of IL-1Rrp in IL-18 signaling is still controversial. Similar to other cytokine receptor systems, the IL-1R complex is composed of two chains, a ligand-binding subunit, IL-1R1, and a signal transducing subunit, the IL-1R accessory protein (IL-1RACP) (13). IL-1RACP is essential for signaling but lacks ligand-binding ability by itself. IL-1 binds first to the IL-1R with a low affinity. The docking of IL-1Rrp in IL-18 signaling is still controversial.
and/or IL-12 for 24 h. The concentration of IFN-γ was analyzed.

F. Splenocytes from IL-1Rrp−/− and wt mice were prepared, and NK lytic activity against YAC-1 target cells was analyzed immediately. Splenocytes were cultured for 24 h with medium alone (D), 20 ng/ml IL-18 (C), 2 ng/ml IL-12 (D), or 500 U/ml IL-2 (E). Next, NK lytic activity against YAC-1 cells was analyzed. F. Splenocytes were cultured with or without IL-18 and/or IL-12 for 24 h. The concentration of IFN-γ in the culture supernatants was measured by ELISA. Indicated values are means ± SDs of duplicates. N. D., not detected.

IL-1RaCP to the IL-1RI/IL-1 complex gives rise to a high-affinity binding capacity of the IL-1R complex (14). Therefore, it is speculated that the IL-18R complex is composed of a ligand binding chain and a second signal transducing chain such as IL-1RaCP. In fact, the $K_d$ values for both IL-18 binding to the L248 cell line used in the purification process and IL-18 binding to IL-1Rrp-transfected COS-1 cells are high, which further strengthens speculation of the existence of a second chain providing a higher affinity (5).

Recently, AcPL, a novel receptor subunit homologous to IL-1RaCP, has been obtained (15). Therefore, IL-1Rrp could be placed in the same position with IL-1R1 in the IL-1R system. Further study will be required to determine whether the docking of IL-1Rrp and AcPL gives rise to a high-affinity binding capacity of the IL-18R complex.

Acknowledgments

We thank Drs. T. Taga and T. Yoshimoto for the protocol on the binding assay; Drs. T. Kaisho and H. Okamura for helpful discussions; A. Maekawa for technical assistance; T. Aoki, M. Hyuga, and E. Nakatani for secretarial assistance; and all members of our laboratory for their helpful advice regarding the preparation of this manuscript.

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