Selective Expression and Functions of Interleukin 18 Receptor on T Helper (Th) Type 1 but not Th2 Cells

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Summary

Interleukin (IL)-18 induces interferon (IFN)-γ synthesis and synergizes with IL-12 in T helper type 1 (Th1) but not Th2 cell development. We report here that IL-18 receptor (IL-18R) is selectively expressed on murine Th1 but not Th2 cells. IL-18R mRNA was expressed constitutively and consistently in long-term cultured clones, as well as on newly polarized Th1 but not Th2 cells. IL-18 sustained the expression of IL-12Rβ2 mRNA, indicating that IL-18R transmits signals that maintain Th1 development through the IL-12R complex. In turn, IL-12 upregulated IL-18R mRNA. Antibody against an IL-18R–derived peptide bound Th1 but not Th2 clones. It also labeled polarized Th1 but not Th2 cells derived from naive ovalbumin–T cell antigen receptor–ab transgenic mice (D011.10). Anti–IL-18R antibody inhibited IL-18–induced IFN-γ production by Th1 clones in vitro. In vivo, anti–IL-18R antibody reduced local inflammation and lipopolysaccharide-induced mortality in mice. This was accompanied by shifting the balance from Th1 to Th2 responses, manifest as decreased IFN-γ and proinflammatory cytokine production and increased IL-4 and IL-5 synthesis. Therefore, these data provide a direct mechanism for the selective effect of IL-18 on Th1 but not Th2 cells. They also show that the synergistic effect of IL-12 and IL-18 on Th1 development may be due to the reciprocal upregulation of their receptors. Furthermore, IL-18R is a cell surface marker distinguishing Th1 from Th2 cells and may be a therapeutic target.

Key words: T helper type 1 cells • T helper type 2 cells • interleukin 18 receptor • inflammation • septic shock

Functional heterogeneity of CD4+ T cells was recognized in the 1970's (1). Subsequently, Th1- and Th2-type T cell responses have been classified by virtue of IFN-γ/IL-2 and IL-4/IL-5 production, respectively (2). It is now generally accepted that the balance between these two T cell subsets determines the phenotype and progression of numerous experimental and clinical diseases (3–5). Therefore, elucidating their preferential induction and regulation is of considerable importance both in facilitating potential clinical immunoregulatory applications and understanding fundamental immunology. Thus, there has been an intensive search for genes and/or cell surface molecules selectively expressed on Th1 or Th2 cells which are implicated in selectively directing and regulating their biological functions.

Using differential display PCR, we have recently identified a gene encoding a cell surface molecule, originally designated ST2L/T1/DER4/Fit, expressed constitutively and stably on murine Th2 but not Th1 cells, even after stimulation with a range of immunological stimuli (6). Anti-ST2L antibody can decrease Th2 functions in vitro and in vivo. ST2L is a member of the IL-1R family with 28% amino acid sequence homology with the type I IL-1R (7). Although IL-1α acts as a cofactor in IL-12–induced Th1 development in BALB/c mice, IL-1α responsiveness is lost by committed Th1 cells and clones (8). Thus, members of the IL-1R family may be differentially expressed and thereby regulate the sequential functions of Th1 and Th2 cells. Therefore, we investigated other members of the IL-1R family for their expression and function in Th1 and Th2 cells. We have found that a gene known as IL-1R–related protein (IL-1Rrp) (9)
with previously unknown function was selectively expressed in Th1 but not Th2 cells. During the course of this work, it was reported that human IL-18R shared complete amino acid sequence homology with IL-1Rrp and has 30% homology with ST2L (10). IL-18 is a cytokine produced by monocytes cells capable of promoting proliferation and IFN-γ production by Th1, CD8+, and NK cells in mice and in humans (11). It shares some of the biological activities of IL-12 but without significant structural homology, and serves as a costimulatory factor in the activation of Th1 but not Th2 cells (12). It appears not to drive Th1 development but synergizes with IL-12 for IFN-γ production (8). Consistent with this is the recent report that mice deficient in IL-18 gene are defective in Th1 and NK cell activities (13).

In this study, we show that IL-18R is preferentially expressed on the surface of Th1 compared with Th2 cells IL-18 and IL-12 reciprocally upregulated each other’s receptors. Furthermore, anti–IL-18R antibody decreased the functions of Th1 cells in vitro and in vivo. Therefore, these findings provide a mechanism for the synergistic effect of IL-12 and IL-18 in the development of Th1 but not Th2 cells. They also demonstrate that IL-18R is not only a selective cell surface marker for Th1 cells, distinguishing them from Th2 cells but may also be an immunotherapeutic target.

**Materials and Methods**

**Mice.** BALB/c and CBA mice were obtained from Harlan U.K. Ltd. (Bicester, Oxon, UK). OVA-TCR-α transgenic mice (D011.10) of the BALB/c background were provided by Dr. Ken Murphy (W Ashington University, St. Louis, MO).

**Cell Lines and Cell Culture.** Murine T cell clones were as described previously (6). Dorris (Th1, specific for hen egg lysozyme, H-2k) and D10 (Th2, specific for conalbumin, H-2k) were obtained from American Type Culture Collection (Rockville, MD). X4 (Th1) and X12 (Th2) were both H-2k-restricted and specific against group A streptococcal M protein (14). The cells were maintained by periodic antigen stimulation with appropriate irradiated spleen cells from Th1 or Th2 line, or IL-4 (250 U/ml) plus anti–IL-12 (1/400, rabbit anti–IL-12 antiserum, R73.1) and anti–IFN-γ (5 μg/ml, R46A2) antibodies for Th2 line, for 7 d (16). IL-2 (10 U/ml) was added during the last 2 h. Cells were then fixed with paraformaldehyde (2%), and, after washing, permeabilized at room temperature (IgG2a; PharMingen) and PE-conjugated anti–mouse IL-4 (IgG2a; Pharmingen, San Diego, CA). Total IgG was purified from the immune serum and preimmunized serum by ammonium sulphate precipitation.

**Flow Cytometric Analysis.** Cells were incubated with anti-IL-18R or normal rabbit serum followed by biotinylated goat anti–rabbit IgG (DAKO Corp., Carpinteria, CA) and then developed with PerCP-streptavidin (Becton Dickinson). After two washes with FACS wash buffer (PBS containing FCS [5%] and NaN₃ [0.1%]), and all antibodies were centrifuged (11,000 g for 5 min) immediately before use. For intracellular staining, cells were suspended at 10⁶–10⁷ cells/ml and stimulated with PMA (50 ng/ml; Sigma Chemical Co.) and ionomycin (500 ng/ml; Sigma Chemical Co.) for 4 h. Brefeldin A (10 ng/ml; Sigma Chemical Co.) was added during the last 2 h. Cells were then fixed with paraformaldehyde (2%), and, after washing, permeabilized at room temperature with 0.5% saponin (Sigma Chemical Co.) in PBS and FCS (5%), then stained with FITC-conjugated anti–mouse IFN-γ (IgG2a; Pharmingen) and PE-conjugated anti–mouse IL-4 (IgG2a; Pharmingen), or FITC- and PE-conjugated isotype control antibodies (Becton Dickinson). After two washes with FACS wash buffer containing 0.1% saponin, samples were analyzed on a FACScan™ flow cytometer (Becton Dickinson).

Local Inflammation. Groups of BALB/c mice were injected daily with 0.5 mg i.p. of anti-IL-18R antibody or normal rabbit IgG. They were injected in the right hind footpad with 300 μg of IL-18R and Th1 Cells
lambda carrageenin (Sigma Chemical Co.) in 50 μl PBS 24 h after the first injection of antibody. Footpad swelling was measured over the next 3 d with a dial calliper (Kroeplin GmbH, Munich, Germany), and data are expressed as mean footpad thickness increase (right footpad — left footpad) ± SEM. Some mice were killed 24 h after carrageenin injection, and footpad and draining lymph nodes (DLN) were removed. Footpad was fixed in formaldehyde, and histology was examined for cellular infiltration after staining with hematoxylin and eosin. DLN cells were cultured with immobilized anti-CD3 antibody (Sigma Chemical Co.) for 48 h. Supernatants were collected for cytokine assay by ELISA using paired antibodies (PharMingen). T cell proliferation was analyzed by [3H]thymidine uptake during the last 6 h of culture. In some experiments, mice were killed 24 h after carrageenin injection, and peripheral blood was collected in heparin.

LPS-induced shock. Groups of BALB/c mice were injected daily with 0.5 mg i.p. of anti–IL-18R antibody or normal rabbit IgG. They were then challenged intraperitoneally, 24 h after the first antibody injection, with 18 mg/kg body wt of LPS (Salmonella enteritidis, Sigma Chemical Co.) in 0.2 ml PBS. Animals were observed every 6 h for general health and mortality. Experiments were terminated on day 4 after LPS injection as required by the guidelines for animal experimentation, Home Office, U.K. Mice were killed 24 h after LPS injection. The sera were pooled, and concentrations of IFN-γ, TNF-α, IL-4, IL-5, IL-6, and IL-12 were determined by ELISA using paired antibodies (PharMingen).

Statistical Analysis. Statistical analysis was performed using Minitab software for Macintosh. Comparison between groups was by Mann-Whitney test or Student’s t test except for lethality data, which were analyzed by the two-tailed log-rank test.

Results

IL-18R Message Is Consistently Expressed in Th1 but not Th2 Cells. A panel of cloned Th1 and Th2 cells were stimulated with antigens and irradiated APCs 5 d later. RNA was extracted, and the expression of IL-18, IL-12Rβ2, and ST2L was detected by the highly sensitive RT-PCR Southern blot analysis. Data presented in Fig. 1 show that, as expected (6), Th2 but not Th1 cells expressed ST2L. In contrast, Th1 but not Th2 cells expressed IL-12Rβ2 and IL-18R. IL-18R expression in Th1 cells was persistent because its message was clearly expressed for at least 28 d when the cells were maintained in culture medium containing IL-2. It should also be noted that T cell clones derived from BALB/c X4, X12 or CBA (Dorris, D10) mice show a similar pattern of expression of IL-18R, ST2L, and IL-12Rβ2 message (Fig. 1b).

We then investigated whether selective expression of IL-18R in Th1 cells also applied to newly polarized T cell lines, and whether this was due to a selective loss of the receptor in Th2 cells during development. CD4+ T cells were negatively selected from the splenic cell population of OVA-TCR-αβ transgenic mice (D011.10). They were cultured in vitro in the presence of IL-12 plus anti–IL-4 (Th1 line) or IL-4 plus anti–IL-12 and anti-IFN-γ (Th2 line). Cells were harvested on days 3, 5, and 7. Some cells were driven for a second round and harvested 5 d later. The differential expression pattern of IL-18R, IL-12Rβ2, and ST2L was evident even as early as day 3. The Th1 line progressively lost ST2L message and expressed strong IL-18R and IL-12Rβ2 messages. In contrast, the Th2 line expressed strong ST2L message but little or no IL-18R or IL-12Rβ2 mRNA (Fig. 1b). This pattern became more polarized with prolonged culture. By day 5 of the second round of driving, IL-18R and IL-12Rβ2 message was clearly present in Th1 lines but not detectable in Th2 lines, whereas ST2L was present in Th2 but not in Th1 lines.

In parallel experiments, we investigated the relative stability of the expression of IL-18R, IL-12Rβ2, and ST2L in cloned T cell lines. The clones were stimulated in vitro with their respective antigen (peptide) and irradiated APCs, and RNA was extracted at regular intervals. Although the expressions of ST2L and IL-18R were stable beyond day 9, that of IL-12Rβ2 began to decline on day 5 and was not detectable by day 9 (Fig. 1c).

Interactions of IL-18 and IL-12 in Th1 Cells. Since IL-18 synergizes with IL-12 in the development of Th1 cells (12) and the production of IFN-γ (8), we investigated the possibility that these two cytokines may influence each other’s receptor expression. Th1 clones were cultured with APCs and antigen in the presence of IL-12, IL-18, or a combination of IL-12 and IL-18. Cells were harvested on days 3, 5, and 7.
Northern blot analysis showed that IL-12Rβ2 was sustained and increased by the presence of IL-18, IL-12, and particularly a combination of IL-18 and IL-12, compared with cells cultured with medium alone. In contrast, IL-18R was markedly decreased by IL-18 but increased by IL-12. A combination of IL-12 and IL-18 induced a slight decrease of IL-18R (Fig. 2). The effect of IL-12 and IL-18 on IL-12Rβ2 was reflected in IFN-γ production by the treated Th1 cells. IL-18, IL-12, and especially a combination of the two cytokines significantly increased IFN-γ synthesis compared with cells cultured with medium alone (data not shown). Therefore, binding of IL-18 to IL-18R appears to transmit signals for a sustained expression of IL-12R complex and the production of IFN-γ. Conversely, binding of IL-12 to IL-12R increased the expression of IL-18R even in the presence of IL-18. This may account for the synergistic effect of IL-18 and IL-12 in Th1 expansion.

IL-18R is expressed on the surface of Th1 but not Th2 cells. A rabbit polyclonal antibody was raised against a peptide corresponding to the hydrophilic region of the extracellular domain of IL-18R. The antibody stained Th1 but not Th2 clones by flow cytometric analysis (Fig. 3 a). The expression was persistent, because Th1 cells remained IL-18R–positive after antigenic stimulation and prolonged culture in medium containing IL-2 alone (data not shown).

Figure 2. Effect of IL-18 and IL-12 on the expression of IL-18R, IL-12Rβ2, and Th1 functions. A representative Th1 clone (Dorris) was stimulated with irradiated APCs and antigen in the presence of IL-18, IL-12, a combination of IL-18 and IL-12, or medium alone. RNA was extracted on day 5 and analyzed by Northern blot. The relative level of the message was compared with reference to HPRT. Message expressed by cells cultured with medium alone was normalized as 1.0. Similar results were obtained with cells cultured for 3 or 7 d.

Figure 3. Flow cytometric analysis of cell surface expression of IL-18R. (a) Th1 (Dorris) and Th2 (D10) were stained with rabbit anti-IL-18R or pre-immune serum (1/25 dilution) followed by biotinylated goat anti-rabbit IgG and were developed with PerCP-streptavidin. Similar results were obtained with X4 (Th1) and X12 (Th2) (not shown). (b) CD4+ T cells from OVA-TCR-αβ transgenic mice (D011.10) were driven to Th1 or Th2 lines for 6 d with APCs and antigen in the presence of IL-12 and anti-IL-4 antibody (Th1 line) or IL-4 (Th2 line). They were stained for cell surface IL-18R (with PerCP) and intracellular IFN-γ (with FITC) and IL-4 (with PE). All cells in b were activated with PMA/ionomycin for 4 h and Brefeldin A added in the last 2 h. Unfilled histograms, Staining with control preimmune serum. Similar results were obtained with cells driven for up to five rounds of culture.

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with anti–IL-18R antibody or normal rabbit IgG. Mice treated with anti–IL-18R antibody developed significantly less footpad swelling within the first 48 h compared with normal IgG-treated controls (Fig. 4 a). Histological examination revealed marked mononuclear and polymorphonuclear cellular infiltration in the control footpad. This was significantly reduced in the footpad of the anti–IL-18R antibody-treated mice (Fig. 4, b–d). DLN T cells from the antibody-treated mice also produced considerably less IFN-γ and IL-6 but more IL-4 and IL-5 than those from the control mice when stimulated with anti-CD3 antibody in vitro (Fig. 4 e). Together, these findings are consistent with the notion that IL-18 is associated with local inflammation and that anti–IL-18R antibody can downregulate Th1 activity within such inflammatory responses.

Anti–IL-18R Antibody Reduces LPS-induced Shock. We next determined whether anti–IL-18R antibody could influence LPS-induced septic shock. BALB/c mice were injected intraperitoneally with LPS and treated with anti–IL-18R antibody or normal rabbit IgG. While the control IgG-treated mice developed substantial mortality, the disease was markedly reduced in the group treated with the anti–IL-18R antibody (Fig. 5 a). The antibody-treated mice also recovered from body weight loss more quickly (Fig. 5 b). The beneficial effect of the anti–IL-18R antibody was accompanied by reduced levels of serum IFN-γ, TNF-α, and IL-6 after LPS injection (Fig. 5 c). Thus, anti–IL-18R antibody could significantly ameliorate LPS-induced shock and in so doing could reduce the production of proinflammatory cytokines.

Figure 4. Effect of anti–IL-18R antibody on local inflammation. BALB/c mice were injected in the footpad with carrageenin (300 μg at 50 μl/mouse). They were also injected intraperitoneally with anti–IL-18R antibody or normal rabbit IgG (0.5 mg/mouse/d) daily for 4 d, starting 24 h before the footpad injection. (a) Footpad swelling was measured daily. Data are mean ± SEM; n = 5, *P < 0.01. Footpad thickness of the antibody-treated group was indistinguishable from the control at 72 h after injection. Thus, the antibody delayed the onset of local inflammation. (b) Inflammatory score of histological examination shows extensive cellular infiltration in the footpad of the control IgG–treated group at 24 h after carrageenin injection. This was significantly reduced after (d) anti–IL-18R injection. Sections of the carrageenin–injected footpads were stained with hematoxylin and eosin (original magnification: ×10). The number of infiltrating cells was counted and expressed as inflammatory score (0, no inflammation; 1, patchy mild inflammation; 2, patchy extensive inflammation; 3, continuous inflammation; 4, continuous inflammation with loss of architecture; n = 5, *P < 0.001). (e) DLN were pooled (five mice per group) 24 h after carrageenin injection and stimulated in vitro with immobilized anti-CD3 antibody. Supernatant was collected 48 h later, and cytokine concentration was determined by ELISA. Data are mean ± SD; n = 3, *P < 0.05, **P < 0.01. There was no significant difference in the T cell proliferative response between the two groups of mice (data not shown). Results are representative of three experiments.
**Discussion**

Data presented here demonstrate that IL-18R is selectively and persistently expressed on Th1 but not Th2 cells. Thus, IL-18R not only serves as a cell surface marker distinguishing Th1 from Th2 cells, but also provides an explanation for the selective biological effect of IL-18. In addition, our data provide a mechanism by which IL-18 synergizes with IL-12 in the expansion of Th1 cells through reciprocal modification of receptor expression, leading directly to enhanced production of IFN-γ. The persistent expression of IL-18R on Th1 cells suggests that IL-18 may play a dominant role in Th1 expansion and function. Furthermore, antibody against IL-18R affects Th1 functions in vitro and in vivo.

It is of interest that IL-18 downregulated but did not abrogate IL-18R (Fig. 2). This is consistent with a recent report that IL-18-deficient mice expressed elevated IL-18R message (13). The mechanism for this is currently unknown, but may represent a self-limiting feedback circuit to curtail overexpansion of Th1 cells, which have been implicated in a range of immunopathologies (17, 18). This possibility is currently being addressed. It should also be noted that Th1 cells from both BALB/c and CBA origin expressed substantial levels of IL-18R and IL-12Rb2 (Fig. 1) and that IL-18 could upregulate IL-12Rb2 (Fig. 2). This suggests that the observed sustained expression of IL-12R in CBA mice, which may account for the resistance of this mouse strain to intracellular infection (19), may be associated with innate IL-18 expression.

IL-18 is produced by monocytic cells after pathogenic infections (11). Since IL-18R is selectively present on a distinct subset of T cells, IL-18 likely plays an important role beyond providing a link between innate and adaptive immune response. The ability of IL-18 to support Th1 expansion is determined by the selective expression of IL-18R on Th1 cells. Therefore, it would be of considerable interest to determine the mechanism whereby IL-18R is preferentially expressed on Th1 but not Th2 cells during their dichotomous development from a common precursor. However, since NK cells (11, 20) and neutrophils (our unpublished data) also express IL-18R, the rapid downregulation of proinflammatory cytokines during LPS-induced shock by anti-IL-18R antibody (2 h after LPS injection) may reflect a combination of effects of the antibody on Th1 cells as well as NK cells and neutrophils. However, the antibody treatment did not significantly affect the percentage of these cells in vivo (data not shown).

It is interesting that both ST2L and IL-18R, which are preferentially and persistently expressed on Th2 and Th1 cells, respectively, belong to the type I IL-1R family. Although it is now well established that cytokines play a major role in the polarization of Th1 and Th2 cells, other factors such as MHC, antigen dose, and antigenic affinity also appear to be important (for a review, see reference 21). The precise mechanisms by which these factors interact to preferentially induce Th1 and Th2 cells remain obscure. Differential activation of the genes of the type I IL-1R family may thus provide an answer to a central question in immunology. There is considerable amino acid sequence homology between the cytoplasmic domains of human IL-1R family and the *Drosophila* Toll protein (22), which controls the induction of potent antimicrobial factors in the adult fly (23). Thus, the IL-1R family may govern an evolutionary ancient immune response in both insects and vertebrates. Interestingly, all members of the IL-1R family signal...
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