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-αβ 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)1

1Abbreviations used in this paper: DLN, draining lymph node(s); HPRT, hypoxanthine phosphoribosyltransferase; IL-1R rp, IL-1 receptor–related protein; R T, reverse transcription.
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-1R rp and has 30% homology with ST2L (10). IL-18 is a cytokine produced by monocyctoc cells capable of promoting proliferation and IFN-γ production by Th1, CD8 T, 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 UK Ltd. (Bicester, Oxon, UK). OVA-TCR(a) of the BALB/c background (D011.10) and anti–IL-12 (40 U/ml) plus anti–IL-4 antibody (10 ng/ml) were provided by Dr. Ken Murphy (Washington University, St. Louis, MO).

Cell Lines and Cell Culture. Murine Th1 cell clones were described previously (6). D. and Weissman, specific for hen egg lysozyme, H-2k and D.10 (Th2, specific for conalbumin, H-2b) 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 BALB/c spleen cells and antigen, or with immobilized anti-murine CD3 antibody (Sigma Chemical Co., Poole, UK). To this was added recombinant murine IL-18 (10 ng/ml) and a serial dilution of the rabbit anti–IL-18R antibody. Culture supernatant was harvested at 48 h, and concentrations of IFN-γ were determined by ELISA, using paired antibodies (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. They were injected in the right hind footpad with 300 μg of IL-18 and served as a control. The mixture was kept at 4°C in PBS 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 analyzed on a FACScan flow cytometer (Becton Dickinson).
of IL-12 and anti–IL-4 antibody (Th1 line), or IL-4, anti–IL-12, and anti–IFN-γ (Th2 line). The cells were harvested 3, 5, and 7 d after start of the culture or 5 d (*5) after the second round of culture. R N A was extracted and analyzed by 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. 1 a).

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–T C R-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 m R N A (Fig. 1 b). 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 A P C s, and R N A 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. 1 c).

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 A P C s and antigen in the presence of IL-12, IL-18, or a combination of IL-12 and IL-18. Cells were harvested on days 3–7, 5–9, and 18.

Figure 1. Selective expression of IL-18R message in Th1 compared with Th2 cells. (a) Cloned Th1 (Dorris X4) and Th2 cells (D10, X12) were stimulated with irradiated spleen cells (APCs) and antigen. R N A was extracted 5 d later and analyzed by R T–PCR Southern blot with probes of IL-18R, IL-12Rβ2, and ST2L. In contrast, Th1 but not Th2 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. 1 a).

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Similar results were obtained with cells driven for up to five rounds of culture. 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). To determine whether this staining pattern of IL-18R also occurs in cells derived from naive T cells, we polarized the splenic CD4⁺ T cells from OVA-TCR-αβ transgenic mice (D011.10) with IL-12 and anti–IL-4 (Th1 line) or IL-4 (Th2 line) in vitro in a 6-d culture and stimulated the cells with PMA and ionomycin for 4 h (16). They were then stained for cell surface IL-18R and intracellular IL-4 and IFN-γ. Th1 lines stained positive for IL-18R and IFN-γ, but negative for IL-4. In contrast, the Th2 line was negative for IL-18R and IFN-γ, but positive for IL-4 (Fig. 3 b). Titration studies showed that cell surface expression of IL-18R by Th1 cells was positively related to the concentration of IL-12 used in the culture; 40 U/ml of IL-12 induced stronger IL-18R expression than 20 or 10 U/ml. This is consistent with the results reported in Fig. 2 above.

To determine whether the anti–IL-18R antibody can affect Th1 functions, Th1 cells were cultured with APCs and antigen in vitro in the presence of IL-18 (15 ng/ml) plus IL-12 (10 ng/ml), and anti–IL-18R antibody (700 ng/ml) or normal IgG. The antibody significantly inhibited the production of IFN-γ by Th1 cells (normal IgG versus anti–IL-18R [mean ± SD]: 1,140 ± 130 vs. 640 ± 8 ng/ml, representative of four experiments). However, the antibody had little or no effect on Th1 cell proliferation (data not shown).

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-12R β2, 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 preimmune 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.
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.

Figure 4. Effect of anti–IL-18R antibody on local inflammation. BALB/c mice were injected in the footpad with carrageenin (300 μg/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 infiltrations in the footpad of the (c) normal 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 < 0.01). (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.
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-12Rβ2 (Fig. 1) and that IL-18 could upregulate IL-12Rβ2 (Fig. 2). This suggests that the observed sustained expression of IL-12Rβ2 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
through the IL-1R–associated kinase–nuclear factor-κB (IRAK-NF-κB) pathway (8, 24) yet lead to differential expression and regulation of distinct T cell subsets. Unraveling these detailed signaling pathways and their transcriptional control represents an important challenge. It will also be important to determine whether the homologues of these molecules are also differentially expressed on human Th1 and Th2 cells as well as the CD8+ subsets.

Knowledge of Th1 and Th2 biology was advanced considerably by the demonstration that Th1 and Th2 cells can be polarized by culturing CD4+ T cells from transgenic mice recognizing a single peptide (25, 26), marked by differential intracellular staining of cytokines produced by the subsets (16). However, identification of stable cell surface markers, analogous to the CD4/CD8 molecules, capable of recognizing live cells and contributing towards the functions of Th1 and Th2 cells, would be of considerable importance in advancing this field. CD30 was reported to be important to determine whether the homologues of these receptors may have important implications for the migratory and activation patterns and susceptibility to HIV infection of Th1 and Th2 cells. However, these receptors are rapidly lost upon T cell activation by IL-2, anti-CD3, and anti-CD28 (31, 32). Data presented here demonstrate that IL-18R is a selective and consistent cell surface marker for Th1 cells, distinguishing them from Th2 cells.

The finding of persistent Th1 and Th2 cell surface markers directly demonstrates the validity of the Th1/Th2 classification. These markers will also facilitate investigation into the interaction between Th1 and Th2 cells, using pure populations of cells ex vivo. The antibodies will enable in vivo tracking of the migratory pattern and selective interactions of Th1 and Th2 cells with other cell types, including APCs and B cells, in the induction of humoral responses. Finally, antibodies, agonists, and antagonists for these cell surface molecules may have important clinical applications during infectious and autoimmune diseases in which the balance between Th1 and Th2 cells is known to play a critical role.

This work was supported by the Wellcome Trust, the Medical Research Council, and the Arthritis Research Council of the United Kingdom.

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Received for publication 30 April 1998 and in revised form 7 August 1998.
    gamma-inducing factor (IGIF) is a costimulatory factor on the activation of TH1 but not TH2 cells 
    8:383-390.
    streptococci leads to major histocompatibility complex class II presentation of T cell epitopes from 
    Heterogeneity of intracellular cytokine synthesis at the single-cell level in polarized TH1 helper 1 and 
    Cloning of the cDNA for human IFN-gamma-inducing factor, expression in Escherichia coli, and studies 
20. Ushio, S., M. Namba, T. Okaura, K. Hatori, Y. Nakada, K. Akita, F. Tanabe, K. Konishi, M. Micallef, 
    86:973-983.
    244:183-186.
    4 (IL-4)-producing cells in vivo and in vitro: IL-2 and IL-4 are required for in vitro generation of IL-4-producing 
    TH2-type cytokines. FASEB J. 9:81-86.
    expression in developing T helper 1 (TH1) and TH2 cells. J. Exp. Med. 185:817-824.
    expression of an interleukin-12 receptor component by human T helper 1 cells. J. Exp. Med. 185:825-831.
    Scheffold, A. Radbruch, and A. Hamann. 1997. P- and E-selectin mediate recruitment of TH1-helper 1 
33. Bonecchi, R., G. Bianchi, P.P. Bordignon, D. D’Ambrosio, R. Lang, A. Borsatti, S. Sozzani, P. 
    receptors and chemotactic responsiveness of type 1 T helper cells (Th1s) and Th2s. J. Exp. Med. 187:129-134.
34. Salustro, F., D. Lenig, C.R. Mackay, and A. Lanzavecchia. 1998. Flexible programs of chemokine 