IL-18 induction of IgE: dependence on CD4+ T cells, IL-4 and STAT6

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Overproduction of immunoglobulin E (IgE) and T helper cell type 2 (T_H2) cytokines, including interleukin 4 (IL-4), IL-5 and IL-13, can result in allergic disorders. Although it is known that IL-4 is critical to the polarization of naïve CD4+ T cells to a T_H2 phenotype, both *in vitro* and in many *in vivo* systems, other factors that regulate *in vivo* IL-4 production and T_H2 commitment are poorly understood. IL-18, an IL-1–like cytokine that requires cleavage with caspase-1 to become active, was found to increase IgE production in a CD4+ T cells–, IL-4– and STAT6–dependent fashion. IL-18 and T cell receptor–mediated stimulation could induce naïve CD4+ T cells to develop into IL-4–producing cells *in vitro*. Thus, caspase-1 and IL-18 may be critical in regulation of IgE production *in vivo*, providing a potential therapeutic target for allergic disorders.

Interleukin 4 (IL-4) is the most important determinant of immunoglobulin (Ig) class switching to IgE[1,2]. IL-13 may also induce class switching to IgE in human B cells[3,4]. In addition to regulating IgE production, both cytokines directly elicit allergic inflammatory responses such as mucus production by bronchiolar endothelial cells[5,6]. IL-4 and IL-13 both bind to the α chain of the IL-4 receptor (IL-4R)[7] and therefore the factors that induce IL-4 or IL-13 production are intimately associated with the pathogenesis of allergic disorders.

IL-18, originally designated as interferon (IFN)-γ–inducing factor (IGIF), is a pleiotropic cytokine secreted by activated macrophages and Kupffer cells[8–11]. Its major action is induction of IFN-γ by T_H1 cells and NK cells, especially in combination with IL-12[12–16]. Along with IL-12, IL-18 also induces anti-CD40–activated B cells to produce IFN-γ, which inhibits IL-4–dependent IgE and IgG1 production[17]. Administration of IL-12 and IL-18 in helminth-infected mice inhibits both IgE production and production of IL-4 and IL-13 by basophils and mast cells in an IFN-γ–dependent manner, thus offering a different approach for the treatment of allergic disorders[17,18].

However, it has also been reported that IL-18 enhances eosinophil recruitment into the airways[19] and, in combination with IL-2, increases IL-13 secretion by NK cells and T cells[20]. We have shown that in the presence of IL-3, IL-18 causes basophils and mast cells to express high levels of IL-4, IL-13 and histamine[18]. Although in combination IL-18 and IL-12 suppress IgE production in helminth-infected mice, injection of IL-18 alone into such mice actually increases IgE levels and enhances production of IL-4 and IL-13 by basophils, mast and CD4+ T cells[18]. These results taken together suggest that IL-18 may induce IL-4–producing CD4+ T cells or condition cells to make IL-4 in response to antigenic stimulation. Here we demonstrate that, when administered to mice alone, IL-18 induces high IgE expression by B cells. Expression is dependent on CD4+ T cells, IL-4 and STAT6. Caspase-1 transgenic mice[21], with elevated levels of IL-18 in their sera, display high IgE in their serum—which is entirely dependent on STAT6. In addition, when cultured in dishes coated with anti-CD3 and anti-CD28, naïve CD4+ T cells stimulated with IL-18 develop into cells that produce IL-4 in response to *in vitro* T cell receptor (TCR) engagement. Finally, we show significantly increased serum IL-18 levels in patients with lepromatous leprosy but not those with tuberculoid leprosy.

Results

IgE production in BALB/c mice injected with IL-18

To examine the capacity of IL-18 to induce IL-4–dependent IgE production *in vivo*, we gave daily injections of IL-18 (0.1–5 μg) to wild-type BALB/c mice for 13 days. IL-18 caused a dose-dependent increase in serum IgE, reaching ~8 μg/ml in response to IL-18 doses of 5 μg/day—an ~80-fold increase compared with serum IgE observed in control mice (**Fig. 1a**). In mice that received IL-18 doses of 5 μg/day, both IL-4 and IL-13 were detectable in serum. Normally these cytokines are below the level of detection but in the treated mice peak concentrations of IL-4 (280 pg/ml) and IL-13 (480 pg/ml) were observed on days 8 and 11, respectively (**Fig. 1b**). Induction of serum IL-4 and IL-13 was also dependent on the IL-18 dose administered. IL-18 doses of 1 μg/day moderately induced these cytokines (40 pg/ml of IL-4 and 110 pg/ml of IL-13 in sera on days 8 and 11, respectively).

To identify whether, in response to IL-18, increases in IgE and in serum IL-4 and IL-13 were dependent on CD4+ T cells, mice were treated with either anti-CD4 or a control antibody and injected daily with 5 μg IL-18. Anti-CD4–treatment diminished IgE levels by ~90% on days

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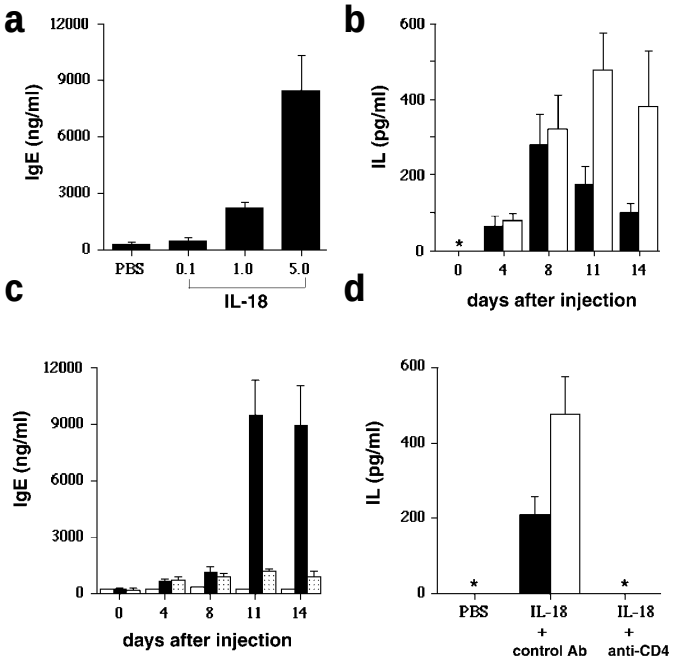

Figure 1. IL-18–induced IgE, IL-4 and IL-13 production *in vivo* is dependent on CD4⁺ T cells. (**a**) BALB/c mice (5 mice per group) were daily injected with PBS buffer or IL-18 for 13 days. They were bled on 14 day and serum IgE was measured by ELISA. (**b**) BALB/c mice (5 mice per group) were injected daily with PBS buffer or IL-18 (5 μg/day). Serum IL-4 (filled bars) and IL-13 (open bars) were measured by ELISA as indicated. Results are geometric means ± s.d. (**c,d**) BALB/c mice were injected daily with PBS buffer (open bars in **c**) or IL-18 (5 μg/day) for 13 days and also received anti-CD4 (GK1.5; 0.5 mg/day, five mice per group, shaded bars in **c**) or control antibody (rat IgG2b; 0.5 μg/day, 5 mice per group, filled bars in **c**) 4 and 7 days before IL-18 treatment and 0, 3 and 7 days after treatment. (**c**) IgE expression in sera was measured on indicated days. (**d**) IL-4 (filled bars) and IL-13 (open bars) on day 11 was measured. Results are geometric means ± s.d. (*, <0.5 ng/ml.)

11 and 14 and inhibited the induction of serum IL-4 and IL-13, whereas control antibody treatment did not (**Fig. 1c,d**). This indicated that increased expression of both IgE and serum IL-4 and IL-13, in response to IL-18, was dependent on CD4⁺ T cells.

IgE production in IL-18–injected IL-4Rα$^{-/-}$ or IL-4$^{-/-}$ mice

The importance of the T_H2 cytokines IL-4 and IL-13 in inducing IgE expression was examined by treating BALB/c IL-4Rα chain-deficient (IL-4Rα$^{-/-}$) mice[22] with IL-18. These mice failed to produce IgE in response to IL-18 (**Fig. 2a**). As the IL-4Rα chain is required for responses to both IL-4 and IL-13[7], we also examined IgE production by IL-4-deficient (IL-4$^{-/-}$) BALB/c mice[23] and by mice that had been treated with a soluble IL-13Rα2-IgGFc fusion protein (sIL-13Rα2-Fc), which neutralizes IL-13[24], in response to IL-18 injection. IL-4$^{-/-}$ mice showed no increase in IgE (**Fig. 2a**), whereas mice treated with sIL-13Rα2-Fc mounted a normal IgE response (**Fig. 2b**). This established that IgE responses to IL-18 are dependent on IL-4 but not on IL-13. We also examined responsiveness of STAT6$^{-/-}$ mice[25] to IL-18 treatment and found that IgE expression was not induced (data not shown), which further substantiated these observations.

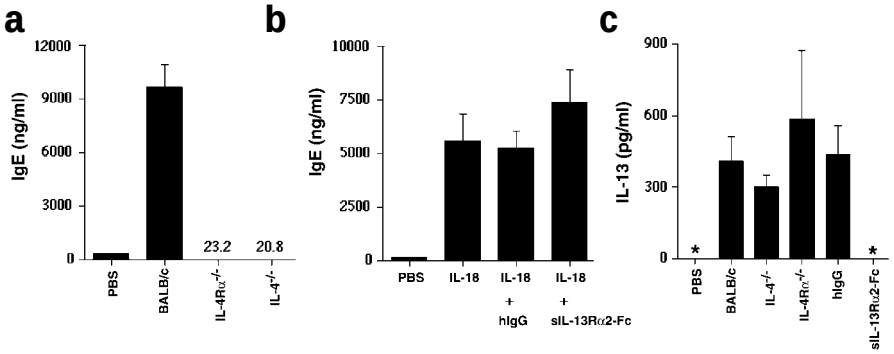

Figure 2. IL-18–induced IgE production *in vivo* is dependent on IL-4. BALB/c mice and BALB/c-background IL-4Rα$^{-/-}$ and IL-4$^{-/-}$ mice (5 mice per group) were injected daily with PBS buffer or IL-18 (5 μg/day) for 13 days. IL-18–injected BALB/c mice (5 mice per group) either received no further treatment or received additional i.p. injections of control human IgG (1 μg) or sIL-13Rα2–Fc (1 mg) every two days. (**a,b**) Serum IgE was measured on day 14 and (**c**) serum IL-13 on day 11. Results are geometric means ± s.d. (*, <10 pg/ml.)

Based on these results, it seems reasonable to conclude that CD4⁺ T cells and IL-4 are essential for IL-18–mediated induction of IgE and that the *in vivo* production of IL-4 and IL-13 also depends upon CD4⁺ T cells. This is consistent with, but does not fully establish, the conclusion that IL-18–treatment results in the appearance of IL-4– and IL-13–producing CD4⁺ T cells that are required for IgE production. However, both IL-4$^{-/-}$ and IL-4Rα$^{-/-}$ mice showed undiminished increases in serum IL-13 in response to treatment with IL-18 (**Fig. 2c**) indicating that the capacity of CD4⁺ T cells to express or cause expression of IL-13 (and presumably IL-4) is not IL-4–dependent.

IgE production in caspase-1 transgenic mice

Next we tested whether endogenously accumulated IL-18 resulted in high serum IgE. Transgenic mice in which caspase-1 is overexpressed in keratinocytes (caspase-1 Tg mice) have high serum IL-18 (5.2 ± 1.0 ng/ml at 6-weeks-old and 7.8 ± 0.7 ng/ml at 12-weeks-old, where n = 5) through intracellular processing of pro–IL-18 by caspase-1[21]. We found these mice had high serum IgE and IgG1 at 8 weeks (**Fig. 3a**), which increased further by 12-weeks-old. This IgE response does not occur in caspase-1 Tg mice lacking STAT6 (**Fig. 3b**), although these mice had high IL-18 (8.0 ± 1.5 ng/ml). As caspase-1 Tg mice also produce other cytokines (such as IL-1β), we deleted their *Il18* gene by crossing with IL-18$^{-/-}$ mice[26]. The caspase-1 Tg IL-18$^{-/-}$ mice (**Fig. 3c**) had significantly diminished serum IgE compared to their IL-18$^{+/-}$ littermates (**Fig. 3c**). Residual IgE may be induced by the action of cytokines other than IL-18 and IL-1β because those transgenic mice in which IL-1α, the biological functions of which are same as those of IL-1β, is overexpressed by keratinocytes do not constitutively produce IgE (H. Mizutani, unpublished observation). Thus, IL-18 is principally responsible for inducing IgE in caspase-1 Tg mice, although other factors may be involved in induction of IgE production.

CD40L expression changes on CD4⁺ T cells

We characterized CD4⁺ T cells derived from mice that had been injected daily with 5 μg of IL-18 for 13 days. These cells expressed concentrations of CD40 ligand (CD40L) mRNA comparable to those observed by CD4⁺ T cells isolated from mice treated with helminth the *Nippostrongylus brasiriensis* a week earlier (data not shown).

As we could induce IL-4–dependent IgE production by administration of IL-18 alone into BALB/c mice (**Fig. 1**), we examined whether

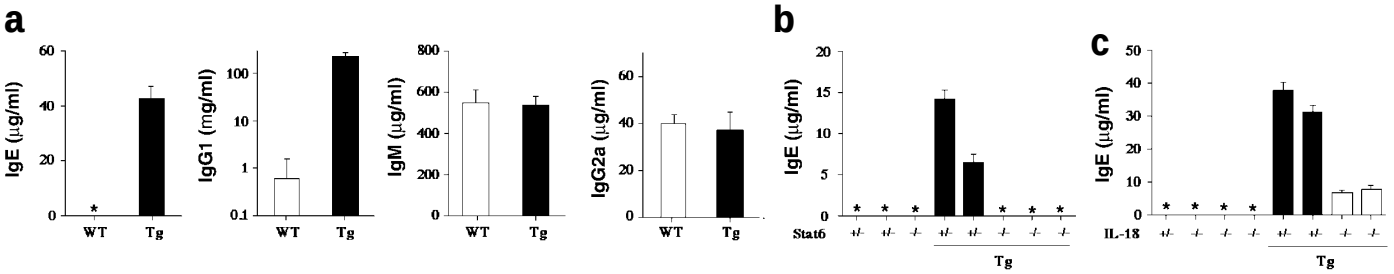

Figure 3. IL-18 secreted in caspase-1 Tg mice induced IgE production *in vivo* by activation of STAT6. (**a**) Serum immunoglobulins in caspase-1 Tg mice (n = 3) were measured at 8-weeks-old. We established the caspase-1 Tg STAT6$^{-/-}$ mice (**b**) and caspase-1 Tg IL-18$^{-/-}$ mice (**c**) by crossing the knock-out mice with caspase-1 Tg mice. Serum IgE in mice was measured at 8-week-old. (*, <0.5ng/ml.) (In **b**, +/– is heterozygous for STAT6; –/– is homozygous for STAT6. In **c**, +/– is heterozygous for IL-18; +/– is homozygous for IL-18.

IL-18 can act directly on naïve T cells to induce the capacity to express IL-4 and to help in IgE induction. As a source of naïve CD4$^+$ T cells, we used CD4$^+$ T cells derived from ovalbumin (OVA)-specific TCR transgenic BALB/c mice (DO11.10)[27]. We treated them with IL-18 in the absence of OVA for 4 days *in vitro*. CD40L was modestly induced on these cells; induction was enhanced by the addition of IL-2 (**Fig. 4a**). Addition of even low doses of IL-18 (5 ng/ml) along with IL-2 induced a substantial increase in CD40L-positive T cells (16.4%). CD4$^+$ T cells stimulated using higher doses IL-18 (50 ng/ml) and IL-2 (200 pM) expressed detectable but relatively modest amounts of IL-4 (380 pg/10^6 cells/ml; data not shown) and were able to induce B cells to secrete IgE (260 ng/ml) if IFN-γ were neutralized (**Fig. 4b**). Naïve CD8$^+$ T cells failed to express IL-4 or IL-13 in response to IL-2 and IL-18 (data not shown).

These results leave some questions unanswered. Does IL-18–treatment of normal mice induce IL-4 and IL-13 production and IgE synthesis by causing CD4$^+$ T cells to become T$_H$2 cells that help B cells to switch, both by expressing CD40L and producing IL-4? Or does this treatment enhance the capacity of basophils (or some other cell type) to produce IL-4 in response to T cell–dependent IL-3 production? Indeed we have already reported that mice treated with IL-18 develop populations of basophils with heightened capacity to produce IL-4[18]. The optimal stimulant of IL-4–production by these cells was IL-3 plus IL-18. However, an enigma remains—no obvious stimulant for IL-3 production by the CD4$^+$ T cells has been discovered.

IL-18 causes IL-4–dependent *in vitro* T$_H$2 polarization

We examined the effect of IL-18 on T$_H$2 polarization *in vitro* (**Fig. 5a**). CD4$^+$ T cells were cultured in anti-CD3 and anti-CD28 coated dishes for 4 days with IL-2 alone, IL-2 plus IL-4, or with IL-2 plus IL-18 and/or anti-IL-4. The cells were challenged with phorbol 12-myristate 13-acetate (PMA) and ionomycin and analyzed by fluorescence-activated cell sorting (FACS) for cytosolic IL-4 and IFN-γ. T cells cultured with IL-2 and IL-4 were positive for cytoplasmic IL-4 (11.6%) and IFN-γ (4.2%). When cultured with IL-2 alone, 3.5% T cells expressed cytoplasmic IL-4 and 7.1% IFN-γ. However, cytoplasmic IL-4 and IL-4 "priming" was inhibited by anti–IL-4 indicating that it depended on *in situ* IL-4 production. Culturing naïve CD4$^+$ T cells with IL-2 and IL-18 (2 ng/ml) for 4 days led to an increase in cytoplasmic IL-4–positive cells (to 10.4%) but a decrease in cytoplasmic IFN-γ–positive cells from 7.1% to 3.1%. Higher concentrations of IL-18 (20 ng/ml) modestly increased the proportion of cytoplasmic IL-4-positive cells (to 12.1%), but this could be completely blocked by the addition of anti–IL-4 (to 0.4%). Simultaneously, we examined the capacity of these stimulated T cells to produce IL-4 and IFN-γ in response to anti-CD3 and anti-CD28 *in vitro* challenge (**Fig. 5b**). Like T cells primed with IL-4, T cells cultured with IL-18 produced IL-4, which was completely inhibited by addition of anti–IL-4. This treatment disclosed the original capacity of IL-18 to act on T$_H$1 cells to increase IFN-γ production.

Thus, IL-18 induction of T cell capacity to produce IL-4 is IL-4–dependent. IL-18 may either increase the amount of IL-4 produced

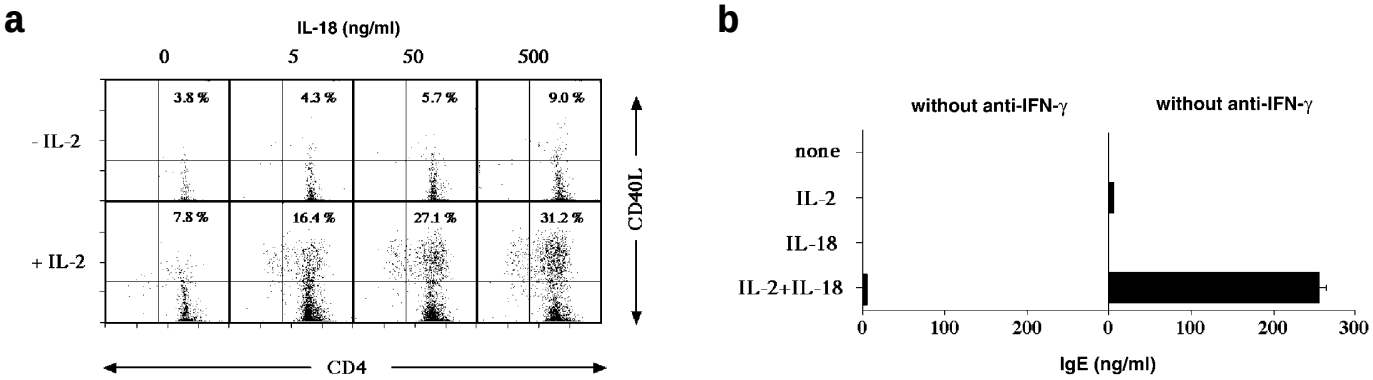

Figure 4. IL-18 stimulated CD4$^+$ T cells to up-regulate CD40L expression and to cause B cells to produce IgE *in vitro*. (**a**) Naïve CD4$^+$ T cells (1 × 10^5/0.2 ml/well) from DO11.10 transgenic mice were cultured with various concentrations of IL-18 (0–500 ng/ml) in the presence or absence of IL-2 (200 pM). After 4 days of culture, surface expression of CD40L was analyzed by flow cytometry. The percentages shown represent the proportion of CD40L positive cells among CD4$^+$ T cells. (**b**) Naïve CD4$^+$ T cells (1 × 10^5/0.2 ml/well) from DO11.10 transgenic mice were cultured with medium alone, or IL-18 (50 ng/ml) and/or IL-2 (200 pM). After 4 days of culture freshly purified BALB/c splenic B cells (1 × 10^5/0.2 ml/well) were added with or without anti-IFN-γ (10 μg/ml). After an additional 10 days of incubation, supernatants were collected and IgE contents measured by ELISA. Results are geometric means ± s.e.m.

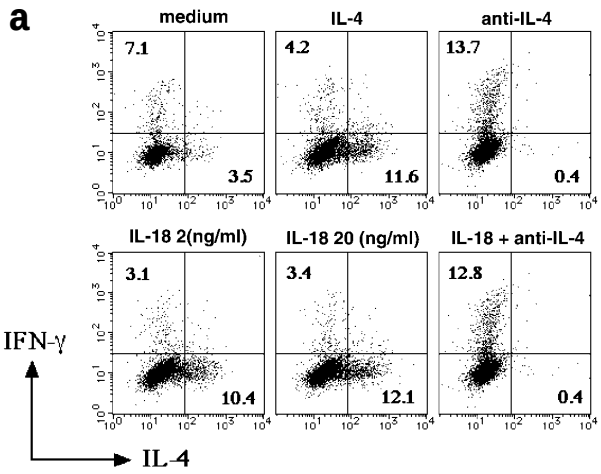

Figure 5. IL-18 stimulates naïve CD4⁺ T cells to develop into T_H2 cells in the presence of TCR engagement *in vitro*. Naïve CD4⁺ T cells (1×10^6) from C57BL/6 mice were cultured with 200 pM of IL-2 plus immobilized anti-CD3 and anti-CD28 (each 5 μg/ml) in the presence of IL-4 (1000 U/ml) or IL-18 (0, 2 and 20 ng/ml) with or without anti–IL-4 (11B11; 10 μg/ml) in 24-well plate in a total of 1 ml. After initial priming for 4 days, cells were washed and recultured with PMA (50 ng/ml) plus ionomycin (500 ng/ml) for 4 h and analyzed by FACS for (**a**) cytosolic IL-4 and IFN-γ or (**b**) recultured with immobilized anti-CD3 and anti-CD28 (each 5 μg/ml for coating) for 24 h, for induction of cytokine secretion.

early in the culture, which acts with anti-CD3 and anti-CD28 to induce T_H2 differentiation, or it may increase the sensitivity of these cells to IL-4.

High serum IL-18 in lepromatous leprosy

Finally we investigated IL-18–dependent T_H2 responses in naturally occurring human infectious diseases caused by *Mycobacterium leprae*. There are two polar forms of the disease: tuberculoid leprosy (TL) and lepromatous leprosy (LL). T_H1-deviated (in TL) and T_H2-deviated (in LL) immune responses to intracellular microbes play a critical role in determination of the lesions[28], although many patients belong to a less clear, intermediate group (IG). We measured serum IL-12 and IL-18 in patients with LL before (n = 33) or after (n = 16) treatment with anti-leprosy drugs. We also tested serum IL-18 in patients with TL (n = 6) or IG (n = 6). As shown in **Fig. 6**, serum IL-18 was significantly increased in patients with LL, but was almost equivalent between patients with TL and healthy individuals. The IG had intermediate serum IL-18. Although we measured serum IL-12 simultaneously, it was undetectable or remained within normal range (data not shown). Effective treatment caused a significant reduction in serum IL-18, suggesting that expression of IL-18 can also be used as a good parameter for measuring activity of the disease. These results indicated that IL-18 may be involved in T_H2 immune responses to *M. leprae*.

Discussion

Here we show that elevation of IL-18, either by administration or as a result of endogenous production, resulted in increased serum IgE. Production of IgE was STAT6-dependent and, at least in the case of IL-18 administration, IL-4–dependent. In addition, injected IL-18 resulted in the appearance of serum IL-4 and IL-13. The increase in IL-13 (and presumably IL-4) was independent of IL-4 indicating that IL-18 provides a potential mechanism for generating the IL-4 needed to induce T_H2 responses.

IL-18 is produced by many cell types, most notably Kupffer cells[9] and keratinocytes[29]; stimulants such as lipopolysaccharide (LPS) induce its secretion, possibly by the induction of enzymes capable of cleaving pro–IL-18[9,30]. Induced IL-18–production provides a potential mechanism through which the innate immune response can mobilize IL-4 and IL-13 production.

The nature of cells that produce IL-4 in response to IL-18 is still unclear, although IL-4 and IL-13 production is T cell dependent. Indeed the simplest interpretation of these results is that IL-4 is produced by CD4⁺ T cells because treatment with anti-CD4 prevents the appearance of serum IL-4 and IL-13. However, it is known that IL-3 produced by CD4⁺ T cells can induce or enhance the production of IL-4 by basophils and mast cells[31], so that the role of CD4⁺ T cells in IL-4 production could be indirect.

The observation that administration of IL-18 resulted in detectable serum IL-4 and IL-13 is provocative. Whatever the cellular source of IL-4 produced in response to IL-18, it appears to be induced in sufficient amounts that concentrations of IL-4 in the extracellular fluid may be adequate to polarize CD4⁺ T cells responding to an immunologic stimulus to a T_H2 phenotype. This is important as it has generally been believed that IL-4 acts locally and that the IL-4–producing cells important in T_H2 polarization need to be in the immediate vicinity of the developing T cells. This view has implicated T cells themselves as being the most important source of such IL-4.

We have demonstrated that IL-18, either administered or accumulated, could induce IgE production *in vivo* without antigenic stimulation. However, for induction of *in vivo* IgE production we had to administer relatively high doses of IL-18 (~5 μg/day per mouse). Moreover, the capacity of CD4⁺ T cells, which had been stimulated with IL-18 (50 ng/ml) and IL-2 (200 pM) for 4 days, to induce class switching to IgE in B cells was modest. These results may raise the question of a pathophysiological role for IL-18 in the IgE response.

To address this issue we examined the capacity of IL-18 to promote T_H2 polarization *in vitro* and showed that relatively low concentrations of IL-18 (2 ng/ml) strongly promoted *in vitro* T_H2 development. As IL-18 failed to induce T_H2 development in the presence of anti–IL-4, there are two possible conclusions. IL-18 may increase the early production of IL-4 and in combination with anti-CD3 and anti-CD28, causes naïve T cells to develop into T_H2 cells. Or IL-18 enhances the sensitivity of cells to a fixed amount of IL-4. Thus, IL-18 with IL-12 selectively

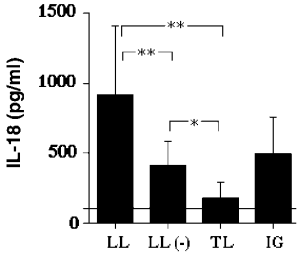

Figure 6. IL-18 expression in patients suffering from leprosy. Serum IL-18 in LL patients before (n = 33) or after (n = 16) treatment with anti-leprosy drugs, patients suffering from TL (n = 6) or belonging to the IG (n = 6) were measured. Results are geometric means ± s.d. **, <0.0005; *; not significant. IL-18 levels in the IG are not significantly different from those in the other groups. Horizontal line indicates level of IL-18 in the sera of healthy volunteers (n = 20).

stimulates T_H1 cells to produce IFN-γ and IL-2[13,14], but IL-18 by itself induces a T_H2 response.

These results imply that an infectious agent or an allergenic challenge that causes IL-18 secretion without IL-12 production may induce sufficiently robust and generalized IL-4 production so that developing T cells, even if they are not in the immediate vicinity of the IL-4 producing cells, can receive a sufficiently strong IL-4 stimulus to develop into T_H2 cells. As we originally discovered IL-18 in the sera of mice sequentially treated with *Propionibacterium acnes* and LPS[8-11], IL-18 production has been intimately associated with IL-12 production, leading to synergistic induction of IFN-γ production[8-11]. However, we demonstrated that patients with lepromatous leprosy T_H2-polarized disease[28] have significantly higher serum IL-18 than patients with tuberculoid leprosy T_H1-polarized disease[28]. Thus, IL-18 may be an important determinant in the development of T_H2 cells, lesions and the clinical course of *M. Leprae* infections. At the very least IL-18 expression correlates well to the activity of leprosy. Although this disease is not an example of diseases caused by IgE-mediated immediate type hypersensitivity, our observations suggest that there are some circumstances in which IL-18 is secreted independently of IL-12.

Our results indicate the potential involvement of IL-18 (without IL-12) in the pathogenesis of allergic disorders. It is striking that IL-18 induced IL-4 and IL-13 production and increases in serum IgE without the need for overt immunization. Thus it may be that by inducing a vigorous polyclonal IL-4 response, IL-18 production at critical times in the life of an individual (such as in the neonatal period) may thereafter contribute to the skewing of the immune response to allergic types of inflammatory responses. This could be one of the causes of an atopic state.

IL-18 may also play an important role in the effector limb of allergic responses. Thus, both airway epithelial cells[32] and keratinocytes[29]—important targets in asthma and atopic dermatitis, respectively—constitutively express IL-18. Stimulation of IL-18–processing enzymes such as caspase-1 could cause the release of IL-18. In turn, this could result in production of IL-4 and IL-13 in the airways and the skin in an IgE-independent manner, where they may directly induce allergic inflammatory responses. These findings suggest that caspase-1 and IL-18 may be potential targets in the effort to develop agents that regulate allergic inflammatory responses and provide a new insight into the potential mechanisms through which T_H2 responses may become dominant in an individual.

Methods

Animals. Specific pathogen-free (SPF) 8-week-old female C57BL/6 and BALB/c mice and BALB/c IL-4$^{-/-}$ and IL-4Rα$^{-/-}$ mice generated as described[22,23] and bred under SPF conditions in National Institute of Allergy and Infectious Diseases Animal Care Unit (Rockville, MD) were used. Generation of STAT6$^{-/-}$ or IL-18$^{-/-}$ mice was detailed previously[25,26]. Mice transgenic for an αβ TCR recognizing OVA(323-339) (DO11.10; BALB/c genetic background)[27] were provided by D. Loh (Washington University, MO). Keratinocyte-specific human caspase-1 transgenic mice were established[21]. Offspring were screened for the incorporation of the transgene by polymerase chain reaction (PCR) analysis and Southern blot analysis using DNA from the tail skin. All experiments were performed on the heterozygous transgenic line. All mice were bred under SPF conditions at the animal facilities of Hyogo College of Medicine (Nishinomiya, Japan) and were used when aged 6- to 10-weeks-old. All animal experiments were conducted according to the Guideline for Animal Experiments, Hyogo College of Medicine.

***In vivo* treatment of mice.** BALB/c normal, BALB/c IL-4$^{-/-}$ or IL-4Rα$^{-/-}$ mice were injected on a daily basis with PBS buffer or IL-18 (0.1–5 μg/day) for 13 days. They were bled 0, 4, 8, 11 and 14 days later and serum IgE, IL-4 and IL-13 were measured by enzyme-linked immunosorbent assay (ELISA) as described[18]. To deplete CD4$^+$ T cells, BALB/c mice were injected with monoclonal antibody to CD4 by the intraperitoneal (i.p.) route (clone, GK1.5; 0.5 mg/day) (ATCC: TIB-207) or control antibody (rat IgG2b; 0.5 μg/day) (Pharmingen) on 7 and 4 days before and 0, 3, 7 days after IL-18 injection. For the *in vivo* blockade of IL-13, 1 mg of sIL-13Rα2-Fc or 1 mg of control human IgG were administered by the i.p. route every two days. Establishment and purification of sIL-13Rα2-Fc was as described[24]. *In vitro* ID_{50}, as determined by ability to neutralize 3 ng/ml of murine IL-13 in the B9 proliferation assay, was approximately 10 ng/ml[24].

***In vitro* culture.** Splenic CD4$^+$ T cells were purified by MicroBeads (Miltenyi Biotec, Auburn CA). Naïve CD4$^+$ T cells (1×10^5/0.2 ml per well) from DO11.10 transgenic mice were cultured with various doses of IL-18 (0–500 ng/ml) in the presence or absence of IL-2 (200 pM) for 4 days in RPMI 1640 supplemented with 10% fetal bovine serum, β–mercapto-ethanol (2-ME) (50 μM), l-glutamine (2 mM), penicillin (100 U/ml) and streptomycin (100 μg/ml). Supernatants were collected and tested for IL-4 and/or IL-13 contents by ELISA. The collected T cells were also examined for their capacity to induce B cell to express IgE by incubation with the highly purified B cells[17] for an additional 10 days in the presence or absence of 10 μg/ml of antibody to IFN-γ (R4-6A2, rat IgG1. ATCC: HB-170).

Induction of T_H1 or T_H2 cells. T_H1 and T_H2 cells were induced by stimulation of naïve splenic CD4$^+$ T cells (1×10^6) from C57BL/6 mice with 200 pM of IL-2 plus immobilized anti-CD3 and anti-CD28 (each 5 μg/ml for coating) in the presence of IL-4 (1000 U/ml) or IL-18 (0, 2 and 20 ng/ml) with or without anti-IL-4 (11B11[33]); 10 μg/ml) in 24-well plate in a total 1 ml[14,34]. After 4 days of priming, cells (10^5/0.2 ml per well) were challenged with immobilized anti-CD3 and anti-CD28 for 24 h. Supernatants were examined for IL-4 and IFN-γ content by ELISA. For intracellular cytokine staining, after priming cells (1×10^6 /ml) were restimulated with PMA (50 ng/ml) plus ionomycin (500 ng/ml) for 4 h with a pulse of 1 μg/ml of Brefeldin A (Pharmingen) during the final 2 h to inhibit cytokine secretion. Cells were washed, fixed with 4% (w/v) paraformaldehyde in PBS buffer, and the cell membrane permeated with ice-cold PBS containing 1% FCS + 0.1% saponin. Resultant cells were further stained with phycoerythrin (PE)-rat anti-mouse IL-4 (0.5 μg) plus fluorescein isothiocyanate (FITC)-rat anti-mouse IFN-γ (0.5 μg) antibodies or PE-rat IgG1 plus FITC-rat IgG1 isotype-matched antibodies (Pharmingen) for 30 min. Samples were analyzed on a FACS Calibur (Becton Dickinson).

CD40L staining. DO11.10 CD4$^+$ T cells cultured with IL-18 (0–500 ng/ml) in the presence or absence of IL-2 (200 pM) for 4 days were first treated with 10 μg/ml anti-FcγRII/III for 30 min at 4 °C and then biotinylated anti-CD40L (Pharmingen) for 30 min at 4 °C in staining buffer (PBS, 1% FCS). Cells were then washed twice and stained with FITC-anti-CD4 and PE-labeled avidin (Pharmingen) for 30 min. Samples were analyzed on a FACS Calibur (Becton Dickinson, San Jose).

Patients. 33 patients had LL, six had TL and six belonged to the IG. All patients gave informed consent to this study, which was approved by our institutional board. We measured serum IL-12 and IL-18 in LL patients before (n = 33) or after (n = 16) treatment with anti-leprosy drugs and in the patients suffering from TL (n = 6) or belonging to the IG (n = 6). We also measured IL-12 and IL-18 in sera from healthy subjects (n = 20).

Assay for serum IL-12 and IL-18. Serum samples were collected by venepuncture and were stored at –80 °C until analysis. Serum IL-12 and IL-18 concentrations were measured by commercially available IL-12 ELISA kit (Endogen, Woburn MA) and IL-18 ELISA kit (MBL, Nagoya, Aichi, Japan).

Statistical analysis. Results are geometric means ± s.d. Student's t-test and regression analysis were used. P-values less than 0.05 were accepted as significant.

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