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An Essential Role for IL-18 in CD8 T Cell-Mediated Suppression of IgE Responses

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The ability of CD8 T cells to suppress IgE responses is well established. Previously, we demonstrated that CD8 T cells inhibit IgE responses via the induction of IL-12, which promotes Th1 and suppresses Th2 responses. In this study, we show that IL-18 also plays an essential role in IgE suppression. In vitro, IL-18 synergized with IL-12 to promote Th1/T cytotoxic 2 differentiation. OVA-specific TCR transgenic (OT-1) CD8 cells induced both IL-12 and IL-18 when cultured with OVA257-264 peptide-pulsed dendritic cells. In vivo, IL-18−/− mice exhibited higher IgE and IgG1 levels compared with wild-type mice after immunization with OVA/alum. Furthermore, adoptive transfer of CD8 T cells from OVA-primed mice suppressed IgE responses in OVA/alum-immunized mice, but not in IL-18−/− mice. IgE suppression in IL-18−/− mice was restored if CD8 T cells were coadaptively transferred with IL-18-competent wild-type bone marrow dendritic cell progenitors, demonstrating an essential role of IL-18 in CD8 T cell-mediated suppression of IgE responses. The data suggest that CD8 T cells induce IL-18 production during a cognate interaction with APCs that synergizes with IL-12 to promote immune deviation away from the allergic phenotype. Our data identify IL-18 induction as a potentially useful target in immunotherapy of allergic disease.

R egulatory T cells were first described over three decades ago by Gershon et al. (1). Among the first described were CD8 T cells that inhibited IFN-γ responses (2, 3). This function since has been confirmed by subsequent studies (4–9). Treatment with the toxin ricin depletes CD8 T cells in vivo, resulting in decreased IFN-γ and increased IL-4 production, leading to elevated IgE responses (8). Using the prototype allergen OVA, similar effects were observed when CD8 T cells were depleted using anti-CD8 (10). IgE-suppressive CD8 T cells were OVA specific, expressed the αβ TCR, and were MHC class I restricted (11). CD8 T cells also down-regulated Th2 responses that were transiently induced through low dose Leishmania major infection (12), whereas CD8 T cell depletion abrogated protective Th1 responses (13). Similarly, in respiratory syncytial virus infection, depletion of CD8 T cells resulted in aberrant increases in IgA and the suppression of all other Ig isotypes (14). Furthermore, CD8 T cells have been shown to regulate airway hyperresponsiveness (15, 16).

The ability of CD8 T cells to down-regulate Th2 and IgE responses could be attributed to their ability to license dendritic cells (DC)5 for the effective priming of Th1 responses (17). Consistent with this, CD8 T cells have been reported to produce IFN-γ during early interactions with DC, which can cooperate with CD40L-expressing CD4 T cells to induce IL-12 for the development of Th1 responses (18). However, in an IFN-γ-independent process, it has been demonstrated that CD8 T cell-mediated suppression of IgE responses required bone marrow DC precursor (BMDCp)-derived IL-12 (19). In these experiments, the eventual suppression of IgE responses required CD4 T cell IFN-γ, although the initial induction of IL-12 was independent of CD8 T cell-derived IFN-γ (19).

IL-18 is a proinflammatory, pleiotropic cytokine with complex functions in vivo (20). Originally named IFN-γ-inducing factor (21), it is now established that IL-18 synergizes with IL-12 to induce IFN-γ production and promote Th1 responses (22, 23). However, IL-18 alone can also promote Th2 responses (24, 25). Previously, we had established the importance of IL-12 in CD8 T cell-mediated IgE suppression (19). However, given that IL-18 synergizes with IL-12 to promote Th1 responses, we hypothesized that IL-18 is also an important factor in CD8 T cell-mediated IgE suppression. In this study, we determined the effects of IL-18 ± IL-12 on Th1/Th2 and T cytotoxic 1 (Tc1)/Tc2 development in vitro. We then examined the role of IL-18 in vivo during allergic sensitization in the presence or absence of transferred CD8 IgE-suppressor cells. The results indicate that IL-18 induced after interaction with CD8 T cells is crucial to the regulation of IgE responses in vivo.

Materials and Methods

Mice

C57BL/6 mice (6–8 wk) and Wistar rats were obtained from Harlan Olac. Unless stated otherwise, all C57BL/6 mice were wild type. OVA peptide-specific, class I-restricted Vε2Vβ8 TCR transgenic mice (OT-I) were a gift from M. Merkenschlager (Royal Postgraduate Medical School, Imperial College, London, U.K.). IL-12−/−/H11546 knockout mice were a gift from K. Heeg (University of Heidelberg, Heidelberg, Germany). IL-12−/−/H11002 and IFN-γ−/−/H11002 knockout mice were obtained from The Jackson Laboratory. All knockout mice and OT-I mice were on a C57BL/6 background.
Reagents
RPMI 1640 and AIM V culture medium, PBS, and HBSS were purchased from Invitrogen Life Technologies. Complete medium comprised an equal mixture of AIM V serum-free medium and RPMI 1640 supplemented with t-glutamine (2 mM), nonessential amino acids (1%), streptomycin (100 ng/ml), penicillin (100 U/ml), sodium pyruvate (1 mM), and 2-ME (5 mM) (all from Invitrogen Life Technologies). FCS was purchased from Globe Phar.

Purified OVA (grade V) was purchased from Sigma-Aldrich. Rodent lymphoprep 1.077 was purchased from Nycomed. Anti-mouse IgE H chain (LO-ME-3), anti-IgE α-L chain, biotin (OX-20), and mouse rIL-4 were purchased from Sigma-Aldrich.

Sigma-Aldrich. IL-18 mAbs for ELISA and rIL-18 for cell cultures were purchased from BD Biosciences. Anti-IL-18 PE were added at 1 μg/ml in carbonate/bicarbonate (pH 9.6, 0.1 M) buffer overnight at 4°C. Plates were subsequently washed thrice with PBS with 0.05% Tween 20. Duplicate supernatant samples, diluted with assay diluent (PBS with 1% rat serum and 0.05% Tween 20), were added to coated plates for overnight incubation at 4°C. Plates were washed, and 1 μg/ml biotinylated detector Ab was added. After 2 h, plates were washed and 1 μg/ml streptavidin-conjugated alkaline phosphatase was added. After 45 min, the plates were washed and p-nitrophenyl phosphate substrate diluted to 1 mg/ml in diethanolamine buffer (0.1 M) was added. After 1 h, absorbance at 405 nm was measured with a plate reader (Molecular Devices), and the results were expressed as ng/ml or pg/ml with reference to a standard curve constructed using dilutions of recombinant cytokine.

Cytokine ELISAs
Culture supernatants were harvested and frozen at −40°C until analysis. Throughout, 50-μl volumes were used, and the assay was performed at 25°C. IL-12 (p40 chain) and IL-18 in supernatants were measured with capture and detector Ab pairs. Microtiter plates (Nunc Maxisorp; VWR) were coated with capture Ab at 1 μg/ml in carbonate/bicarbonate (pH 9.6, 0.1 M) buffer overnight at 4°C. Plates were subsequently washed thrice with PBS with 0.05% Tween 20. Duplicate supernatant samples, diluted with assay diluent (PBS with 1% rat serum and 0.05% Tween 20), were added to coated plates for overnight incubation at 4°C. Plates were washed, and 1 μg/ml biotinylated detector Ab was added. After 2 h, plates were washed and 1 μg/ml streptavidin-conjugated alkaline phosphatase was added. After 45 min, the plates were washed and p-nitrophenyl phosphate substrate diluted to 1 mg/ml in diethanolamine buffer (0.1 M) was added. After 1 h, absorbance at 405 nm was measured with a plate reader (Molecular Devices), and the results were expressed as ng/ml or pg/ml with reference to a standard curve constructed using dilutions of recombinant cytokine.

Assessment of OVA-specific IgE by passive cutaneous anaphylaxis (PCA)
PCA was used to measure the levels of biologically active OVA-specific IgE Abs in mouse serum. Four-fold serial dilutions of mouse serum ranging from 1/8 to 1/2048 in PBS were made, and 50 μl of up to 50 diluted samples was injected intradermally into the shaved back of an anesthetized Wistar rat. After 48 h, the rat was again anesthetized, and 500 μl of 10 mg/ml OVA in 1% Evans blue dye was injected into the tail vein. After 30 min further anesthesia, extravasation of blue dye into the skin was recorded. OVA-specific IgE Ab titers from mouse serum were measured at days 0, 7, 14, and 21 and are represented as the mean ± SD of the highest dilution of test serum that produced a positive mast cell-dependent PCA reaction.

Statistics
Data were analyzed using unpaired Student’s t test. A value of p < 0.05 was considered significant. Results are expressed as means ± SD.

Results
IL-18 synergizes with IL-12 to induce IFN-γ-producing CD4 and CD8 T cells
The effect of IL-18 on the generation of IFN-γ-producing CD4 and CD8 T cells was investigated in vitro. CD4 and CD8 T cells were isolated from C57BL/6 mice by positive selection using anti-CD4- and anti-CD8-coated magnetic beads and stimulated using plate-bound anti-CD3 and soluble anti-CD28 under type 1 (Fig. 1a) or type 2 (Fig. 1b) polarizing conditions (as described in Materials and Methods) together with IL-12, IL-18, or IL-12/IL-18. After 5 days, the cells were recovered, washed, and re-stimulated with PMA and ionomycin for 5 h in the presence of 5 μM monensin and stained for intracellular IFN-γ and IL-4. Results in text are mean ± SD of four independent experiments, and a representative example of one experiment is shown in Fig. 1. Under type 1 polarizing conditions, half of the CD8 T cells made IFN-γ 48.1 ± 9.0%, whereas 4.1 ± 1.5% of CD4 T cells made IFN-γ, and very few made IL-4 (Fig. 1a). Addition of IL-12 and IL-18 separately increased the percentage of IFN-γ+ CD8 T cells to 69.8 ± 5.8% and 66.8 ± 3.3%, respectively, whereas IL-12 and IL-18 together yielded 80.3 ± 1.5% IFN-γ+ cells (Fig. 1a).
IL-12 and IL-18 had a more dramatic effect on the number of IFN-γ-producing CD4 T cells. Separately, IL-12 and IL-18 increased IFN-γ+ CD4 T cells from 4.1 ± 1.5% to 18.4 ± 3.2% and 15.4 ± 2.0%, respectively, whereas IL-12 and IL-18 together acted synergistically to increase the percentage of CD4 T cells that stained positive for IFN-γ by 10-fold to 37.1 ± 3.5% (Fig. 1a). The effect of IL-12 and IL-18 on cytokine production by CD4 and CD8 T cells stimulated under type 2 polarizing conditions was also investigated. In the absence of IL-12 or IL-18, 14.2 ± 1.2% of CD8 and 17.2 ± 1.4% of CD4 T cells were positive for IFN-γ, and very few T cells were positive for IL-4. Addition of IL-12 alone reduced the percentage of IL-4+ positive CD4 and CD8 T cells to 1.2 ± 0.4% and 1.8 ± 1.5%, respectively, and increased the percentage of IFN-γ-producing CD4 and CD8 T cells to 7.5 ± 1.1% and 11.2 ± 2.8%, respectively (Fig. 1b). IL-18 alone was less effective, reducing IL-4+ CD4 T cells to 4.5 ± 2.5% and CD8 T cells to 8.1 ± 2.5%, and increasing the percentage of IFN-γ-producing CD4 and CD8 T cells to 1.4 ± 0.5% and 8.1 ± 2.5%, respectively. When added together, IL-12 and IL-18 reduced the percentage of IL-4+ CD8 and CD4 T cells to <2%, and increased the percentage of IFN-γ-producing CD4 and CD8 T cells to 8.2 ± 1.2% and 21.4 ± 2.0%, respectively (Fig. 1b).

Effect of IL-18 and IL-12 on CD4 and CD8 T cell cytokine secretion in vitro

In addition to measuring the percentage of IFN-γ- and IL-4-producing T cells by intracellular staining, we also studied the effects of IL-12 and IL-18 on the levels of IFN-γ and IL-4 secreted by CD4 T cells (Fig. 2, a and b) and CD8 T cells (Fig. 2, c and d) in vitro by ELISA. Following culture of CD4 and CD8 T cells isolated from C57BL/6 mice for 5 days under type 1 (Fig. 2, left) or type 2 (Fig. 2, right) polarizing conditions with or without IL-12 and/or IL-18, the T cells were restimulated for 24 h with PMA and ionomycin, and supernatants were harvested for measurement of IFN-γ and IL-4 by ELISA. Moderate levels of IFN-γ were secreted by type 1 polarized CD4 T cells, and this could be augmented by IL-12, but not IL-18 alone.
addition of IL-12 and/or IL-18 (Fig. 2). Cells polarized under type 1 conditions was not augmented by the addition of IL-12 and/or IL-18 (Fig. 2). Cells could be augmented by the addition of IL-12 and/or IL-18 (Fig. 2) together or type 2 polarizing conditions (left diagrams) or type 2 polarizing conditions (right diagrams) together with IL-12 (10 ng/ml), IL-18 (10 ng/ml), or both IL-12 and IL-18, as indicated. After 5 days, cells were collected, washed, and stimulated for 24 h with PMA and ionomycin. Duplicate culture supernatants were analyzed for IFN-γ/H11022/H11022 and IL-4/H11022/H11022/IL-12 (10 ng/ml), IL-18 (10 ng/ml), or both IL-12 and IL-18, as indicated. After 5 days, cells were collected, washed, and stimulated for 24 h with PMA and ionomycin. Duplicate culture supernatants were analyzed for IFN-γ and IL-4 content by ELISA. Results are expressed as the mean ± SD of four experiments and compared with controls using Student’s t test (*, p < 0.05; **, p < 0.005).

(Fig. 2a, left). However, IL-18 synergized with IL-12 to induce a >10-fold increase in IFN-γ production. IFN-γ secretion by CD8 T cells polarized under type 1 conditions was not augmented by the addition of IL-12 and/or IL-18 (Fig. 2c, left). IL-4 was not detected in supernatants of both CD4 and CD8 T cells polarized under type 1 conditions (Fig. 2, b and d, left). Under type 2 conditions, IL-4 produced by CD4 and CD8 T cells could be inhibited by the addition of IL-12 and/or IL-18 (Fig. 2, b and d, right). Although IFN-γ secretion by type 2 polarized CD4 could not be detected even with the addition of IL-12 and/or IL-18 (Fig. 2a, right), IFN-γ secretion by type 2 polarized CD8 T cells could be augmented by the addition of IL-12 and/or IL-18 (Fig. 2c, right). IL-12 and IL-18 synergized to induce higher levels of IFN-γ from type 2 polarized CD8 T cells. Hence, our results suggest that IL-18, especially with IL-12, has a direct effect on promoting Th1/Tc1 over Th2/Tc2 responses.

FIGURE 2. IL-18 and IL-12 induce IFN-γ and inhibit IL-4 production. CD4 (a and b) and CD8 (c and d) T cells from C57BL/6 mice were cultured at 1 × 10⁶ cells/ml in 24-well plates with plate-bound anti-CD3 and soluble anti-CD28 under type 1 polarizing conditions (left diagrams) or type 2 polarizing conditions (right diagrams) together with IL-12 (10 ng/ml), IL-18 (10 ng/ml), or both IL-12 and IL-18, as indicated. After 5 days, cells were collected, washed, and stimulated for 24 h with PMA and ionomycin. Duplicate culture supernatants were analyzed for IFN-γ and IL-4 content by ELISA. Results are expressed as the mean ± SD of four experiments and compared with controls using Student’s t test (*, p < 0.05; **, p < 0.005).

CD8 T cells enhance the ability of DCs to produce IL-12 and IL-18

To determine whether CD8 T cells can induce DC to produce IL-12, DC were pulsed overnight with either OVA¹²⁵–²⁶⁴ or irrelevant peptide GAD²⁷⁴–²⁸⁶, washed, and subsequently cultured with freshly isolated OT-I CD8 T cells at 5:1 T-DC ratio for 72 and 144 h. Supernatants were harvested and tested for IL-12 p40 (Fig. 3a) and IL-18 (Fig. 3b) by ELISA. CD8 T cells enhanced the secretion of both IL-12 and IL-18 from DCs pulsed with OVA¹²⁵–²⁶⁴, whereas DC pulsed with irrelevant peptide GAD²⁷⁴–²⁸⁶ secreted levels of IL-12 and IL-18 that were similar to unpulsed DC. This suggests that CD8 T cells can enhance IL-12 and IL-18 production by DC during an Ag-specific CD8 T cell-DC interaction.

IgE and IgG1 Ab responses of IL-18⁻/⁻, IL-12⁻/⁻, IFN-γ⁻/⁻, and wild-type mice to OVA/alum immunization

Because IL-18, especially together with IL-12, can act directly on T cells to promote Th1/Tc1 and inhibit Th2/Tc2 differentiation, we ascertained the contribution of IL-18 to the suppression of Th2 responses in vivo by comparing the levels of IgG1 and IgE generated in IL-18 knockout mice that were immunized with OVA/alum i.p. For this, we compared the Ab responses of IL-18⁻/⁻-immunized mice with IL-12⁻/⁻- and IFN-γ⁻/⁻-immunized mice. Immunized C57BL/6 mice served as an indicator of the normal Ab response, and saline-injected C57BL/6 mice as the negative control. Peak IgE responses were obtained at day 14 for all OVA/alum-immunized mice (Fig. 4, a and b). As expected, the peak responses in IL-12⁻/⁻- and IFN-γ⁻/⁻-immunized mice on day 14 were significantly higher than for wild-type immunized C57BL/6 mice. This was also true for IL-18⁻/⁻-immunized mice. Interestingly, early phase IgE responses at day 7 were highest in...
IL-18−/−-immunized mice, even when compared with IgE levels of IL-12−/− and IFN-γ−/−-immunized mice at day 7. OVA-specific IgE levels were ~20-fold greater in IL-18−/− mice than IL-12−/− at this time point. This was also true for anti-OVA IgG1 responses (Fig. 4c). Furthermore, although IgE responses in IL-12−/− and IFN-γ−/−-immunized mice dropped to levels comparable to immunized wild-type C57BL/6 mice by day 21, the levels of IgE in IL-18−/−-immunized mice were sustained at significantly higher (>10-fold at day 21) levels. This indicates that IL-18 plays an important nonredundant role in the regulation of both early and late phases of an IgE response.

**CD8 T cells inhibit OVA-specific IgE responses in wild-type, but not in IL-18−/−, mice**

We previously showed that IgE responses induced by i.p. immunization of OVA/alum could be suppressed by the adoptive transfer of CD8 T cells from day 21 OVA/alum-immunized mice in an Ag-specific and IL-12-dependent manner (19). We used a similar approach to determine whether IL-18 is also involved in this mechanism of immune regulation. Consistent to our previous study, the adoptive transfer of CD8 T cells from nonimmunized C57BL/6 mice had no effect on the levels of IgE induced by OVA/alum immunization (Fig. 5a), whereas adoptive transfer of CD8 T cells from day 21 OVA/alum-immunized mice inhibited the IgE response in recipient wild-type mice (Fig. 5a) (p < 0.005). To determine the contribution of IL-18 to IgE suppression, CD8 T cells from nonimmunized C57BL/6 mice or CD8 T cells from day 21 OVA/alum-immunized C57BL/6 mice were also transferred to IL-18−/− mice (Fig. 5b). No suppression of OVA-specific IgE was observed in IL-18−/−-immunized hosts. This indicates that IgE suppression by CD8 T cells required the host to be competent in producing IL-18.

**CD8 T cell-mediated IgE inhibition in vivo depends on IL-18**

The inability of CD8 T cells from OVA-primed mice to suppress IgE responses in IL-18−/− mice suggested that host animal-derived IL-18 as well as IL-12 was required for CD8 T cell-mediated IgE suppression. Because CD8 T cells could enhance IL-18 production from DC in vitro (Fig. 3a), we speculated that IgE suppression could be reinstated by transferring IL-18-competent cells into IL-18-deficient hosts. This was investigated by the reconstitution of IL-18−/− mice with 10-fold incremental (10²–10⁵ per mouse) numbers of BMDCp from IL-18-competent, wild-type C57BL/6 mice (Fig. 6). CD8 T cells from OVA-immunized C57BL/6 mice were unable to inhibit IgE in IL-18−/− mice when transferred without wild-type BMDCp (Fig. 6, group B), whereas suppression of IgE response was restored if CD8 T cells from day 21 OVA/alum-immunized C57BL/6 mice were cotransferred with IL-18-competent BMDCp. This effect was dependent on the number of BMDCp transferred, with the greatest IgE suppression achieved by cotransfer of 10⁵ DC progenitors. The IgE anti-OVA Ab titer was significantly inhibited by as few as 10² cotransfected DC progenitors (Fig. 6, groups D–F). The inability of IL-18-competent BMDCp from wild-type C57BL/6 mice to suppress IgE responses in the absence of CD8 T cells from OVA-immunized mice (Fig. 6, group A) suggests that the inhibition of IgE responses induced by transfer of both cells is due to CD8 T cell induction of IL-18. These results show that IL-18 produced by BMDCp was an
important element in the suppression of IgE responses by CD8 T cells.

Discussion
In the present study, we established for the first time the role of IL-18 in CD8 T cell-mediated IgE suppression. In vitro, IL-18 synergized with IL-12 to promote Th1/Tc1 development and IFN-γ secretion. CD8 T cells also induced the production of IL-18 and IL-12 by DCs in an Ag-specific manner. In vivo, IgE responses to OVA/alum immunization were dramatically elevated in the absence of IL-18.

Adoptive transfer of CD8 T cells into wild-type mice results in the suppression of IgE responses. To determine whether IL-18 played a role in this process, we used IL-18−/− mice as recipients of CD8 T cells (Fig. 5). OVA-specific IgE responses in IL-18−/− mice that had received CD8 T cells from OVA-immunized mice were significantly higher than those of comparably treated wild-type mice. This suggested that IL-18 was crucial for CD8 T cell-mediated IgE suppression. To confirm this finding, we restored the IL-18−/− mice to IL-18−/− mice by the adoptive transfer of BMDCp from wild-type mice. The transfer of wild-type cells capable of producing IL-18 reinstated the ability of CD8 T cells to suppress IgE responses. This was dependent on the number of coadaptively transferred BMDCp. The possibility of an alternative factor that bypassed the need for IL-18 was introduced by BMDCp in these experiments while possible seems unlikely because these cells were unable to inhibit IgE in the absence of OVA-specific CD8 T cells (Fig. 6, group A). Furthermore, if an alternative factor was involved, it is hard to explain the lack of IgE suppression by CD8 T cells in IL-18−/− mice, whereas it was clearly demonstrated that IL-12 and IL-18 synergized to induce Th1 cells in vitro (Figs. 1 and 2).

Our results differ from a previous study by Pollock et al. (26), who reported no observable difference between the levels of OVA-specific IgE in response to OVA/alum challenge between wild-type and IL-18−/− mice. This difference raises interesting questions. Pollock et al. (26) used IL-18−/− BALB/c mice in which CD8 T cell IgE suppression has not been described. In addition, the route of immunization, timing, and dose of OVA were different in the two studies. Induction of OVA-specific CD8 T cells requires cross-presentation of OVA. At this stage, it is not clear whether IgE suppression is mediated by a specific subpopulation of CD8 T cells. IgE inhibitory CD8 T cells may need to be of a particular activation stage or differentiation status. However, Thomas et al. (19) showed that both Tc1 and Tc2 CD8 T cells could trigger this pathway. It is clear that recognition of Ag by CD8 T cells is important because IgE suppression was not observed in mice that received CD8 T cells from nonimmunized mice or in mice that were immunized with a different Ag (27). Based on pentamer staining of mouse blood, we find that after immunization, percentage of OVA-specific CD8 T cells in blood was increased from 0.2 to 1.0% of total CD8 T cells (data not shown). The proportion in the spleen and lymph nodes is likely to be higher.

Consistent with our in vitro experiments, IL-18 and IL-12 production were enhanced during an Ag-specific interaction between CD8 T cells and DCs (Fig. 3). The factor(s) involved in IL-18 induction is not known, and it would be of particular interest to determine whether IFN-γ secretion by CD8 cells was required, although for regulation of IgE we have clearly shown that CD8 T cell IFN-γ is not required (19). The roles of other cytokines and molecules such as IFN regulatory factor-1 (IRF-1) also remain undefined. IRF-1 is a transcriptional factor that induces Th1 responses (28, 29); by regulating caspase-1 and IL-18-binding protein expression, IRF-1 regulates IL-18 assembly and release (30).

Although IL-18 was first described as an IFN-γ-inducing cytokine (21), it is clear from subsequent studies that IL-18 can influence both Th1 and Th2 immunity (20). In general, IL-18 enhances IL-12-driven Th1-immune responses, whereas IL-18 alone stimulates Th2 responses (20). The present study suggests that IL-18 deficiency results in enhanced Th2 responses. We also show that IL-18 antagonizes Th2 and Th1/cell development in vitro. IgE responses in IL-18−/− mice were significantly higher compared with wild-type mice in response to OVA/alum immunization (Fig. 4). Furthermore, although peak IgE responses were not significantly different between IL-18−/−, IL-12−/−, and IFN-γ−/− mice, the IgE response of IL-18−/− mice occurred earlier and was sustained.

Consistent with our observation that the lack of IL-18 augments Th2 responses, other investigators have shown that IL-18−/− mice lack protective Th1 responses during Candida albicans, Propionibacterium acnes, and Mycobacterium bovis infection (31, 32). Immunization using Helicobacter pylori with cholera toxin adjuvant also failed to elicit protective Th1 responses in IL-18−/− mice (33). In a model of allergic asthma, IL-18−/− mice had increased levels of eosinophilia and lung damage, which could be reverted with administration of IL-18 (34). Collectively, these and other studies support our observation that IL-18 deficiency leads to increased Th2 responses. In contrast, administration or overexpression of IL-18 can also result in enhanced Th2 responses. For instance, stimulation of naive CD4 T cells via TCR and IL-18 is reported to induce the development of IL-4-producing cells in vitro (24). In vivo, the administration or overexpression of IL-18 resulted in increased levels of Th2 cytokines and IgE Abs, and can potentiate IgE production (25, 35–38). Furthermore, histamine was also reported to stimulate IL-18 production without induction of IL-12 (39).

Further studies show that IL-18 can act in ways that defy the Th1/Th2 paradigm. Transgenic mice overexpressing IL-18 produced higher levels of IFN-γ, IL-4, IL-5, and IL-13; thus, aberrant expression of IL-18 in vivo results in increased production of both Th1 and Th2 cytokines (40). Memory Th1 cells, which do not normally induce airway hyperresponsiveness, acquired the ability to induce airway hyperresponsiveness after stimulation with IL-18 (41). In another report, the systemic neutralization of IL-18 resulted in the accumulation of allergen-associated eosinophils,
whereas airway installment of IL-18 increased peribronchial eosinophil accumulation (42). These studies and others demonstrate that depending on conditions, IL-18 can promote either a Th1 or Th2 response. It is evident that the cellular source and locality of IL-18 secretion are crucial to its role in allergic sensitization. For example, the presence of IL-18 in the lung, where IL-12 production may be lacking due to DC immaturity, enhances IgE responses and eosinophil inflammation (36). However, IL-18 produced in conjunction with IL-12 from mature DC after migration to draining lymph nodes would promote Th1 responses and suppression of allergy. Likewise, the down-regulation of IgE production from B cells in lymphoid organs would require IL-18 as shown in this study, whereas prolonged IL-18 secretion from inflammatory cells in the lung could exacerbate allergic asthma, for example, via eotaxin-mediated eosinophil recruitment (40). Our data indicate that the potent role of CD8 T cells in regulating developing immunity needs to be considered to explain the complex effects of IL-18. Specifically, the ability of CD8 cells to simultaneously induce IL-12 and IL-18 from DC may explain the dominance of IL-18-dependent IgE suppression seen in our model. By contrast, the ability of IL-18 to induce IgE responses is dependent on CD4 Th2 responses triggered by early NKT cell secretion of IL-4 (43).

The current study defines a novel role for IL-18 in CD8 T cell-mediated suppression of IgE responses. The effect described in this study may be important for defining the set point at which a Th1 or Th2 dominant immune response ensues. This might allow the prevention of allergic sensitization by inhibition of Th2 responses and consequent IgE production. In fact, a number of novel therapeutic approaches for the treatment of allergy, including the use of oligodeoxynucleotides containing CpG motifs (44), chitin particles composed of N-acetyl-glucosamine polymer (45), and heat-killed Listeria monocytogenes (46), appeared to exert their effect by the regulation of IL-12 and IL-18. DNA vaccination with OVA cDNA fused to IL-18 cDNA inhibited allergic sensitization in an ILN-γ- and CD8-dependent manner (47). Similarly, an anti-CD3/IL-18 fusion protein inhibited Th2 responses to OVA (48, 49). Hence, therapies that target IL-18 should simultaneously target the CD8 T cell, MHC class I-restricted pathway via DC activation to ensure effective suppression of allergy.

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Disclosures

The authors have no financial conflict of interest.

References

11. Uozuma, J. E., K. L. Joyce, and P. Scott. 2004. Low dose Leishmania major promotes a Th2 response in the lung, whereas prolonged IL-18 secretion from inflammatory cells in the lung could exacerbate allergic asthma, for example, via eotaxin-mediated eosinophil recruitment (40). Our data indicate that the potent role of CD8 T cells in regulating developing immunity needs to be considered to explain the complex effects of IL-18. Specifically, the ability of CD8 cells to simultaneously induce IL-12 and IL-18 from DC may explain the dominance of IL-18-dependent IgE suppression seen in our model. By contrast, the ability of IL-18 to induce IgE responses is dependent on CD4 Th2 responses triggered by early NKT cell secretion of IL-4 (43).

The current study defines a novel role for IL-18 in CD8 T cell-mediated suppression of IgE responses. The effect described in this study may be important for defining the set point at which a Th1 or Th2 dominant immune response ensues. This might allow the prevention of allergic sensitization by inhibition of Th2 responses and consequent IgE production. In fact, a number of novel therapeutic approaches for the treatment of allergy, including the use of oligodeoxynucleotides containing CpG motifs (44), chitin particles composed of N-acetyl-glucosamine polymer (45), and heat-killed Listeria monocytogenes (46), appeared to exert their effect by the regulation of IL-12 and IL-18. DNA vaccination with OVA cDNA fused to IL-18 cDNA inhibited allergic sensitization in an IFN-γ- and CD8-dependent manner (47). Similarly, an anti-CD3/IL-18 fusion protein inhibited Th2 responses to OVA (48, 49). Hence, therapies that target IL-18 should simultaneously target the CD8 T cell, MHC class I-restricted pathway via DC activation to ensure effective suppression of allergy.

Acknowledgment

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Disclosures

The authors have no financial conflict of interest.

References


CORRECTIONS


In Results, in the third sentence of the first paragraph under the heading Characteristics of the initial hybridomas and their variable region genes, the first, third, and fourth GenBank accession numbers are incorrect. The corrected sentence is shown below.

Analysis of the nucleotide and amino acid sequences indicated that clones F428 and F429 contained the same H chain V region but different L chain V region, whereas clone F431 had a distinct H chain V region but shared the L chain V region of clone F428 (GenBank accession numbers: AY626661, IGLV-J of mAbs F428 and F431; AY626662, IGLV-J of mAb F429; AY626664, IGHV-D-J of mAbs F428 and F429; and AY626663, IGHV-D-J of mAb F431).


Sentence seven of the Abstract is incorrect. The corrected sentence is shown below.

Nephrotoxic Ab and TLR2 ligation caused a neutrophil influx in both types of chimera above that seen in the sham chimeras totally TLR2 deficient.

In Fig. 3, the statistics presented represent p values from t tests performed on data that should have been transformed, as stated in Materials and Methods. In addition, p values were not given for several comparisons that are relevant. The figure below shows new p values on transformed data. The revised p values do not change the message of the paper, since the same comparisons are still significant when performed on the transformed data. The corrected figure and legend are shown below.

FIGURE 3. A, Glomerular neutrophil influx at 2 h. B, Albuminuria in 24 h following i.v. Pam3CysSK4 and NTS in chimeric and sham chimeric animals. The graphs show results from all statistical analyses.

As a part of other studies, the authors found that the Iγ2a primer used to amplify Iγ2aCμ products in one panel of Fig. 4 was contaminated with a Cγ2a primer. Therefore, the products designated as “Iγ2aCμ” in the second from the top panel of Fig. 4 are actually Iγ2aCγ2a germline transcripts. The authors have prepared new Iγ2a and new Cμ primers and redone the RT-PCR for Iγ2aCμ transcripts. As this required new cDNA, the authors also repeated the RT-PCR for hypoxanthine phosphoribosyltransferase (HPRT) of the same samples.

The original version of the second from the top panel of Fig. 4 is incorrect in that only one authentic RT-PCR product is detected for Iγ2aCμ RNA (see revised Fig. 4); the original Fig. 4 showed the two products that the authors now know derive from the two splice variants of γ2a germline transcripts (Ref. 43 in original article). As expected, revised Fig. 4 demonstrates that the expression of authentic Iγ2aCμ RNA (a postswitch RNA) lags behind that of germline transcripts; the expression of CD40L-induced Iγ2aCμ transcripts cannot be detected at day 2 of induction and does not peak until day 3 (or even later, not tested). Nevertheless, the major point of this figure remains correct. CD40 ligation of B cells results in switch recombination to γ2a. Postswitch Iγ2aCμ transcripts do not pre-exist in the B cell population (they cannot be detected at 5 h or 1 day of culture), but γ2a postswitch transcripts do appear at day 3, after germline transcription. This correction does not alter any major conclusion of the publication. The corrected figure and legend are shown below.

**FIGURE 4.** T-depleted splenic lymphocytes from IFN-γ−/− mice were cultured in LPS, CD40L, or LPS plus IFN-γ for 5 h, 1 d, 2 d, or 3 d. Transcripts, as indicated on the left side of the figure, were detected in RNA from these cells by RT-PCR with incorporation of 32P-dATP. The primers for amplification (35 cycles) of Iγ2aCμ were GCTGAT-GTACCTACCTGAGAG (Iγ2a) and CCAGGTGAAGGAAATG-GAGC (Cμ), and annealing was at 67°C. Other aspects of the RT-PCR reaction, including HPRT amplification, were as described (28). Other RT-PCR products, none of which was the correct size, were inconsistently amplified in the Iγ2aCμ reaction. To verify that the designated 294-bp fragment is indeed an authentic Iγ2aCμ product, we cloned and sequenced it from RNA of both C57BL/6 and BALB/c cells cultured in CD40L plus IFN-γ. DNA sequence verified that the 294-bp product is the authentic Iγ2aCμ product with 169 bp of Iγ2a sequence joined to 125 bp of Cμ product, using the expected splice site (43).

In Table II, the SAP−/− rows were shifted to the left. The corrected Table is shown below.

<table>
<thead>
<tr>
<th>Days Postinfection</th>
<th>Group</th>
<th>No. of Spleen Cells (×10^6/spleen)</th>
<th>No. of B Cells (×10^6/spleen)</th>
<th>No. of Memory B Cells (×10^6/spleen)</th>
</tr>
</thead>
<tbody>
<tr>
<td>90</td>
<td>SAP+/+</td>
<td>80.0</td>
<td>10.5</td>
<td>49.4</td>
</tr>
<tr>
<td></td>
<td>SAP−/−</td>
<td>44.4</td>
<td>6.2</td>
<td>4.6</td>
</tr>
<tr>
<td>150</td>
<td>SAP+/+</td>
<td>104.0</td>
<td>23.2</td>
<td>371.0</td>
</tr>
<tr>
<td></td>
<td>SAP−/−</td>
<td>29.6</td>
<td>3.2</td>
<td>13.3</td>
</tr>
<tr>
<td>210</td>
<td>SAP+/+</td>
<td>69.7</td>
<td>8.2</td>
<td>41.8</td>
</tr>
<tr>
<td></td>
<td>SAP−/−</td>
<td>6.2</td>
<td>1.2</td>
<td>1.2</td>
</tr>
<tr>
<td>420^d</td>
<td>SAP+/+</td>
<td>89.6 ± 34.1^e</td>
<td>37.7 ± 8.9</td>
<td>ND^f</td>
</tr>
<tr>
<td></td>
<td>SAP−/−</td>
<td>52.8 ± 23.1^e</td>
<td>26.1 ± 9.9</td>
<td>ND^f</td>
</tr>
<tr>
<td>540^d</td>
<td>SAP+/+</td>
<td>58.8</td>
<td>20.1</td>
<td>147.0</td>
</tr>
<tr>
<td></td>
<td>SAP−/−</td>
<td>3.1</td>
<td>9.9</td>
<td>18.2</td>
</tr>
</tbody>
</table>

^a Three to eight spleens were pooled and counted after RBC lysis. Total pooled spleen cells were divided by the number of mice pooled to calculate number of cells per spleen. CD19^+ B cells and memory B cells were analyzed and calculated based on percent positive cells by FACS analysis. A representative experiment at each timepoint is shown.

^b CD19^+ cells were gated and number of CD19^+ B cells was calculated based on the frequency of CD19^+ cells by FACS analysis within the total spleen cell number.

^c Memory B cells are isotype switched sIgG^+ CD38^high cells.

^d Spleen cells were dissociated with collagenase D to improve cell yield.

^e Data shown are the mean ± SD of five individual mice per group.

^f ND, Not done.


In Materials and Methods, under the heading Microarray analysis and real-time RT-PCR, the accession number should have been included as the last sentence in the second paragraph. The omitted sentence is shown below.

The microarray data were deposited in the public Gene Expression Omnibus (GEO) database (www.ncbi.nlm.nih.gov/geo/) under accession number GSE7216.

The last name of the fifth author was misspelled. The correct name is Drew A. Roennburg.

In Table I, “off insulin” in footnote b and “ceased insulin” in footnote f should be “off immunsuppression.” The corrected footnotes are shown below.

b Kidney transplant from a deceased donor in 1993 for reflux nephropathy. Off immunosuppression 1995; clinical course described as patient DS elsewhere (25) and in Fig. 3.

f Kidney transplant from a deceased donor in 1986 for chronic glomerulonephritis; off immunosuppression in 2003 due to renal carcinoma in native kidney that was removed in 2004. Current serum creatinine is 1.1 mg/dl.

In Table II, under “Peptide” in the middle column, the labels to the peptides “allo” and “self” are transposed. The corrected table is shown below.

<table>
<thead>
<tr>
<th>HLA-B Ag</th>
<th>Peptide</th>
<th>Amino Acid Sequences</th>
</tr>
</thead>
<tbody>
<tr>
<td>B*1501 p106 (p106–123) allo</td>
<td>NH2-DGRLLRHDQSSAYDGKDY-COOH</td>
<td></td>
</tr>
<tr>
<td>B*1501 p149 (p149–166) allo</td>
<td>NH2-AREAEOEQYALEGLCVE-COOH</td>
<td></td>
</tr>
<tr>
<td>B<em>1501; B</em>5701 p37-MA (p37–54) allo</td>
<td>NH2-DSDAAPPRAIPWIEQ-COOH</td>
<td></td>
</tr>
<tr>
<td>B*0801 p37-TE (p37–54) self</td>
<td>NH2-DSDAAPPRAIPWIEQ-COOH</td>
<td></td>
</tr>
<tr>
<td>B*0801 p61-F (p61–77) allo</td>
<td>NH2-DRNQTIFKNTQTQTDRES-COOH</td>
<td></td>
</tr>
<tr>
<td>B*0801 p61-C (p61–77) self</td>
<td>NH2-DRNQTICKNTQTQTDRES-COOH</td>
<td></td>
</tr>
</tbody>
</table>

* For the peptide defined by the region from aa 37 to aa 54 we designated the donor allopeptide p37-MA where "MA" refers to the polymorphic residues methionine and alanine at positions 44 and 45 (in boldface type), while the corresponding "self" peptide p37-TE has threonine and glutamic acid. The MA polymorphism is found predominantly in only two common Caucasian HLA-B antigens, HLA-B*1501 and HLA-B57, a mismatched HLA-B antigen for patient K2.

b The polymorphic phenylalanine (F) amino acid is present at position 67 (in boldface type) in all HLA-B8 family members, while the cysteine (C) amino acid (boldface type) is present in the HLA-B14 of patient K2.

In Results, there are several errors as follows. In the last sentence of the paragraph under the heading Nonregulated and regulated PBMC samples contain different proportions of IFN-γ vs TGF-β1-inducible T cells, “TGF-β1” is missing from “anti-TGF-β1 Ab.” The corrected sentence is shown below.

DTH responses to the control peptide, p37-TE, were negative with or without anti-TGF-β1 Ab (Fig. 5C).

In the first sentence of the first paragraph under the heading CD4+CD25low T cells show variable CD25 expression but retain allopeptide-specific TGF-β1 responsiveness after flow sorting and short-term culture, “CD25low T cells” should be “CD25low T_{R} cells” and “TGF-β1” is missing. In the last sentence of the same paragraph, the word “retained” should be “remained.” The corrected sentences are shown below.

The adoptive transfer data, coupled with the finding that mainly CD25low T_{R} cells were induced to express surface TGF-β1 by allopeptide stimulation in vitro (Fig. 4C), raised the possibility that adaptive T_{R} cells are among the CD4+TGF-β1+ T cells in the graft that showed variable CD25 expression by immunostaining.

However, in cultures with medium alone or peptides, >85% remained negative for CD25 and there was no induction of surface TGF-β1 expression by p37-MA relative to p37-TE.


The second author’s name is incorrect. The correct name is Kok Loon Wong.