Contribution of Langerhans Cell-Derived IL-18 to Contact Hypersensitivity

Binghe Wang, Claudio Feliciani, Brandon G. Howell, Irwin Freed, Qinchao Cai, Hideaki Watanabe and Daniel N. Sauder

J Immunol 2002; 168:3303-3308; ;
doi: 10.4049/jimmunol.168.7.3303
http://www.jimmunol.org/content/168/7/3303

The Journal of Immunology is published twice each month by
The American Association of Immunologists, Inc.,
1451 Rockville Pike, Suite 650, Rockville, MD 20852

Print ISSN: 0022-1767 Online ISSN: 1550-6606.

Contribution of Langerhans Cell-Derived IL-18 to Contact Hypersensitivity

Binghe Wang,* Claudio Feliciani,† Brandon G. Howell,* Irwin Freed,* Qinchao Cai,* Hideaki Watanabe,* and Daniel N. Sauder[1]*

The epidermal Langerhans cells (LC), a member of the dendritic cell family, and the LC-derived cytokine IL-12 play a pivotal role in the initiation of contact hypersensitivity (CHS), a Th1 immune response in the skin. Because IL-18, another LC-derived cytokine, shares functional and biological properties with IL-12, we examined a potential role for IL-18 in CHS initiation. Our studies demonstrated that during the induction phase of murine CHS, IL-18 mRNA was significantly up-regulated in the skin-draining lymph nodes (LN). Migratory hapten-modified LC in LN expressed high levels of IL-18 mRNA and secreted functional IL-18 protein. LN cells produced significant amounts of IFN-γ following in vitro IL-12 stimulation, which could be partially blocked by anti-IL-18 Ab, suggesting a synergistic role for endogenous IL-18 in IFN-γ production by LN cells. Because mature IL-18 requires cleavage of immature precursors by caspase-1, we further examined IL-12-induced IFN-γ production in caspase-$1^{-/-}$ LN cells. An impaired IFN-γ production was seen in caspase-$1^{-/-}$ LN cells, which could be restored by addition of exogenous IL-18, supporting a role for caspase-1-cleaved, mature IL-18 in IFN-γ production. Finally, in vivo studies showed that CHS responses were significantly inhibited in mice treated with neutralizing IL-18 Ab as well as in caspase-$1^{-/-}$ mice deficient in mature IL-18, indicating functional relevance for IL-18 in CHS. Taken together, our studies demonstrate that LC-derived IL-18 significantly contributes to CHS initiation. ***The Journal of Immunology*****, 2002, 168: 3303–3308.**

Dendritic cells (DC)[2] constitute a family of APCs that are specialized to prime T lymphocytes (1). DC develop from hemopoietic progenitors and are strategically located at surfaces of the body and in interstitial tissues. The epidermal Langerhans cells (LC), a member of the DC family, represent the major APC in the skin and play an important role in cutaneous immune responses (2).

Contact hypersensitivity (CHS), clinically presenting as allergic contact dermatitis, is one of the most frequent and vexing dermatological problems. The CHS response, traditionally used as a classic model for delayed-type hypersensitivity, is mediated by IFN-γ-producing type 1 T cells (i.e., $CD4^+$ Th1 and/or $CD8^+$ type 1 cytotoxic T (Tc1) cells) (3–5). It has been demonstrated that LC play a central role in CHS. Depending on the allergen, LC can either bind haptens directly to MHC molecules on their surface or process the hapten internally into a complete Ag (6). LC then migrate via afferent lymphatic vessels into skin-draining regional lymph nodes (LN) to position in T cell areas, where they present the haptenated peptide to naive T cells (7–9). During Ag presentation, LC secrete high levels of IL-12 to drive T cell development toward a Th1 type (10, 11). IL-12 promotes Th1 responses by selective up-regulation of IFN-γ in T cells (12). Moreover, IL-12 induces Tc1 cell development and potentiates the cytotoxic activity of CTL (13, 14). In vivo studies have demonstrated that IL-12 serves as a mediator and adjuvant for the induction of CHS (15, 16).

IL-18, initially described as IFN-γ-inducing factor, is a new member of the IL-1 family (17, 18). IL-18 has also been termed IL-1γ due to its structural homology to IL-1β, with both cytokines having a unique, all-β-pleated structure (19, 20). Similar to IL-1β, IL-18 is synthesized as a precursor lacking a typical signal peptide. Both IL-1β and IL-18 require caspase-1 (also known as IL-1β-converting enzyme) for cleavage and release of their mature active molecules from the immature precursors (21–23). Furthermore, the IL-18R system and its signal transduction pathway are analogous to those of the IL-1R (24, 25). Recently, Toll-like receptors have been identified that use signaling pathways shared with IL-1R and IL-18R (26, 27). This has resulted in the defining of a new receptor superfamily, known as the IL-1R/Toll-like receptor superfamily.

Despite the similarities between IL-18 and IL-1, functionally IL-18 shares biological properties with IL-12, such as induction of IFN-γ, enhancement of NK cell cytotoxicity, and promotion of activated T cell proliferation (28, 29). In addition, like IL-12, IL-18 is mainly derived from activated macrophages and DC, including LC (30, 31). IL-18 synergizes with IL-12 to stimulate IFN-γ production by differentiating and committed Th1 cells and is required for significant expression of the Th1 phenotype (32, 33). IL-18 itself does not induce Th1 cell differentiation but serves as a strong costimulatory factor in the activation of Th1 cells stimulated by Ag (34). IL-18 up-regulates IL-12Rβ expression and thereby enhances IL-12-mediated Th1 development (35). Furthermore, IL-18 induces the development of naive $CD8^+$ T cells into Tc1 effector cells (36).

Because LC are a significant source of IL-18, we sought to determine whether LC-derived IL-18 is involved in the initiation of CHS. In this study we examined IL-18 gene expression and protein production by migratory LC in skin-draining LN during the induction phase of CHS. To investigate whether IL-18 plays a

*Department of Dermatology, Johns Hopkins University, Baltimore, MD 21287; and †Department of Dermatology, University G. d'Annunzio, Chieti, Italy

Received for publication October 29, 2001. Accepted for publication January 30, 2002.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] Address correspondence and reprint requests to Dr. Daniel N. Sauder, Department of Dermatology, Johns Hopkins University, Johns Hopkins Outpatient Center, Room 6068, 601 North Caroline Street, Baltimore, MD 21287-0900. E-mail address: dsauder@jhmi.edu

[2] Abbreviations used in this paper: DC, dendritic cell; CHS, contact hypersensitivity; LC, Langerhans cell; hpLC, hapten-modified LC; LN, lymph node; LNC, LN cell; Tc1, type 1 cytotoxic T; WT, wild type.

0022-1767/02/$02.00

synergistic role with IL-12 in IFN-γ production in LN cells (LNC), we performed anti-IL-18 Ab blocking studies. Furthermore, we examined whether caspase-1 deficiency affects the IL-12-induced IFN-γ production in LNC due to the lack of mature IL-18. Finally, to further investigate whether endogenous IL-18 is functionally relevant to the initiation of CHS in vivo, we examined CHS responses in mice treated with neutralizing IL-18 Ab and in caspase-$1^{-/-}$ mice deficient in mature IL-18. Our results suggest that endogenous, caspase-1-dependent IL-18 significantly contributes to CHS initiation.

Materials and Methods

Reagents

Murine rIL-12 was purchased from BD PharMingen (Mississauga, Ontario, Canada), and murine rIL-18 was purchased from PeproTech (Rocky Hill, NJ). Anti-Ia, anti-DEC-205 (NLDC-145), isotype controls, normal rabbit IgG, goat anti-mouse IgM/biotin, goat anti-rat IgG/biotin, streptavidin/FITC, and streptavidin/Texas Red were purchased from Cedarlane Laboratories (Hornby, Ontario, Canada). Oxazolone, collagenase IV, Con A, and metrizamide were purchased from Sigma-Aldrich (St. Louis, MO). DNase I was purchased from Roche (Mannheim, Germany). Anti-IL-18 Ab was a gift from Dr. C. A. Dinarello (University of Colorado Health Science Center, Denver, CO); it was obtained from a New Zealand rabbit immunized by intradermal injection of murine rIL-18, followed by several booster injections (22). This IL-18 antiserum has been used in various mouse models in vivo for specific neutralization of endogenous IL-18 activity (22, 23, 37–41).

Mice

Caspase-1-deficient (capase-$1^{-/-}$) mice were obtained from The Jackson Laboratory (Bar Harbor, ME) (42). C57BL/6 mice were purchased from Charles River Breeding Laboratories (Saint Constant, Quebec, Canada). Six- to 8-wk-old mice were used for the experiments. Each experimental group contained four to six mice. The animal protocol was approved by the institutional animal care and use committee.

XS52 cell line

The XS52 cells, a murine LC-like cell line, were provided by Dr. A. Takashima (University of Texas Southwestern Medical Center, Dallas, TX) (43). The cells were propagated in complete RPMI 1640 medium supplemented with murine rGM-CSF (2 ng/ml) and 10% NS47 cell culture supernatant.

Preparation of LNC

Mice were sensitized with 150 μl 3% oxazolone on the shaved abdomen and four paws, and 24 h later the skin-draining LN (i.e., axillary and inguinal) were collected. To prepare single-cell suspensions, LN were gently disrupted by rubbing between the frosted ends of two microscope slides. The stromal fragments were digested with a collagenase IV/DNase I solution at room temperature for 25 min, and the cells were filtered through nylon mesh (44).

Isolation of migratory hpLC

The migratory hapten-modified LC (hpLC) were isolated from LNC using a metrizamide gradient, as described by Bigby et al. (45). Briefly, skin-draining LN were collected 24 h after oxazolone sensitization. LNC suspensions (5 ml; at 5×10^6 cells/ml) were layered onto 2 ml 14.5% metrizamide and gradient-centrifuged at 1200 rpm for 15 min. Cells at the interface were collected, washed once, and resuspended in RPMI 1640 medium. Consistent with previous reports, immunolabeling studies demonstrated that 50–80% of this fraction was $Ia^+DEC\text{-}205^+$ DCs (5, 45).

RNA isolation and cDNA synthesis

The total RNA from LNC, hpLC, and hpLC-free LNC was extracted by a single-step method using RNA STAT-60 (Tel-Test, Friendswood, TX). Then, 2 μl total RNA, 1 μg oligo$(dT)_{12-18}$ primers, and 500 U Moloney murine leukemia virus reverse transcriptase were used for the synthesis of oligo(dT)-primed cDNA. The RT reaction was stopped by heating at 95°C for 5 min, and 2 μl cDNA was used for each PCR (46).

PCR analysis

The primer sequences were as follows: IL-18: upstream, 5′-ACTGTA CAACCGCAGTAATACGG-3′; downstream, 5′-AGTGAACATTACAG ATTTATCCC-3′ (47); and β-actin: upstream, 5′-GTGGGCCGCTCTAG GCACCAA-3′; downstream, 5′-CTCTTTGATGTCACGCACGATTTC-3′. PCR products were size-fractionated in agarose gel containing ethidium bromide and photographed under UV light. For relative quantitation, amounts of PCR products were determined by scanning photo negatives using a laser densitometer, and then the densitometry value of IL-18 was normalized to that of the housekeeping gene, β-actin.

Quantitation of IL-18 protein in vitro

The hpLC, hpLC-free LNC, and XS52 cells were cultured at 5×10^6 cells/ml in 24-well plates in RPMI 1640 medium for 48 h, and supernatants were collected. IL-18 was quantitated by a sandwich ELISA with a murine IL-18 ELISA kit (R&D Systems, Minneapolis, MN). Each supernatant was analyzed in duplicate.

IL-18 bioassay

IL-18 activity in hpLC culture supernatants was assessed by its ability to induce IFN-γ production in T cells (31). Briefly, the mouse spleen was removed, and cell suspensions were prepared. Spleen cells were resuspended in RPMI 1640 medium supplemented with 10% FBS and cultured at 5×10^6 cells/ml in 24-well plates. Cultures were incubated for 48 h with Con A (0.5 μg/ml) in combination with culture supernatants from hpLC, hpLC-free LNC, and XS52 cells. As a specificity control, supernatants were preincubated with either anti-IL-18 Ab (50 μg/ml) or normal rabbit IgG (50 μg/ml). The amount of IFN-γ produced was measured by a murine IFN-γ ELISA kit (Genzyme, Cambridge, MA).

Assay for IL-12-induced IFN-γ production

Skin-draining LN were obtained from mice 24 h following oxazolone sensitization. LNC were prepared from C57BL/6 mice and cultured at 5×10^6 cells/ml in 24-well plates in RPMI 1640 medium containing murine rIL-12 (10 ng/ml) in the presence of either anti-IL-18 Ab or normal rabbit IgG (50 μg/ml) (23). Caspase-$1^{-/-}$ LNC were prepared from caspase-$1^{-/-}$ mice and cultured in RPMI 1640 medium containing murine rIL-12 (10 ng/ml) in the presence or the absence of murine rIL-18 (30 ng/ml). After 48-h culture, supernatants were collected. IFN-γ was quantitated by ELISA.

Epidermal sheet preparation and immunolabeling

The density of epidermal LC was examined by anti-Ia in situ immunolabeling. Epidermal sheets were obtained from the ears of caspase-$1^{-/-}$ and wild-type (WT) mice using 0.5 M ammonium thiocyanate. Acetone-fixed epidermal sheets were labeled with anti-Ia in a three-step immunolabeling procedure (48). Ia^+ cells (LC) were counted using a micrometer grid.

Induction of CHS

Mice were sensitized with 150 μl 3% oxazolone in alcohol/acetone (3/1) applied to the shaved abdomen and four paws. Five days later mice were challenged by applying 25 μl 1% oxazolone in olive oil/acetone (3/1) on the dorsal and ventral sides of the right ear. As a control, the left ear was painted with an identical amount of vehicle. The CHS response was determined by measuring the degree of ear swelling of the oxazolone-exposed ear compared with that of the vehicle-treated contralateral ear at 24, 48, and 72 h after challenge using a micrometer (49).

In vivo neutralizing IL-18 Ab blocking studies

To study whether IL-18 is involved in the induction phase of CHS, endogenous IL-18 was neutralized by i.p. injection of 100 μl anti-IL-18 antiserum (70 mg/ml) into mice 24 and 2 h before oxazolone sensitization. As a control, equivalent amounts of normal rabbit serum were used.

Statistical analysis

Data are expressed as the mean ± SEM. The statistical significance of differences between the means was determined by applying Student's *t* test. A difference was considered statistically significant at $p < 0.05$.

Results

IL-18 mRNA is up-regulated in skin-draining LN following hapten sensitization

To examine IL-18 gene expression in LN during the induction phase of CHS, RNA was extracted from the skin-draining LN in hapten-sensitized mice and naive mice and subjected to RT-PCR analysis. IL-18 mRNA was constitutively expressed in LN of naive

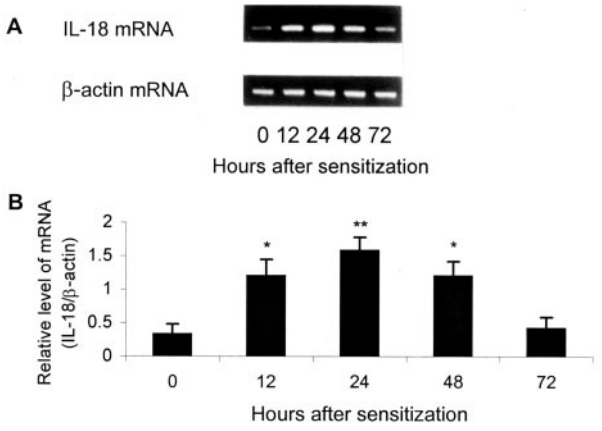

FIGURE 1. Up-regulated expression of IL-18 mRNA in the skin-draining LN of hapten-sensitized mice. Mice were sensitized with oxazolone, and skin-draining LN were collected at various time points. RNA was extracted for RT-PCR analysis. *A*, PCR products were visualized by agarose gel electrophoresis. *B*, Relative amounts of PCR products were determined by densitometer scanning of negative films, and the densitometric values for IL-18 mRNA were normalized to β-actin. The data are the mean $\pm$ SEM of three experiments performed on duplicate samples. *, $p < 0.01$; **, $p < 0.005$.

mice. However, a significantly up-regulated expression of IL-18 mRNA was seen in LN of sensitized mice 12–48 h following epicutaneous hapten application. Maximal expression of IL-18 mRNA was seen at 24 h, and levels returned to baseline by 72 h (Fig. 1).

Migratory hpLC express high levels of IL-18 mRNA and secrete functional IL-18 protein

Because IL-18 is an LC-derived cytokine, we examined whether LC were the main cellular source for the increased IL-18 mRNA expression in skin-draining LN. Migratory hpLC were enriched from LNC and subjected to RT-PCR analysis. Results revealed that the level of IL-18 mRNA was significantly higher in the hpLC compared with hpLC-free LNC and total LNC (Fig. 2). To determine whether LC are able to secrete IL-18 protein, hpLC, hpLC-free LNC, and XS52 cells were cultured for 48 h, and culture supernatants were subjected to ELISA analysis (Fig. 3). A significant amount of IL-18 protein was detected from XS52 cells and hpLC, but not from hpLC-free LNC. To further examine whether LC-derived IL-18 is functionally active, we performed an IL-18 bioassay. IL-18 activity in cell culture supernatants was assayed for its ability to induce IFN-γ production in Con A-stimulated T cells. IL-18-induced IFN-γ production was detected from the supernatants of XS52 cells and hpLC, but not from hpLC-free LNC (Fig. 4). The IFN-γ-inducing activity was significantly blocked (60%) when cells were preincubated with neutralizing anti-IL-18 Ab (data not shown). Importantly, the supernatants of hpLC derived from caspase-$1^{-/-}$ mice showed a significantly lower level of IFN-γ-inducing activity, and addition of anti-IL-18 serum did not block IFN-γ production (data not shown). This confirmed that caspase-$1^{-/-}$ mice do not produce functional IL-18.

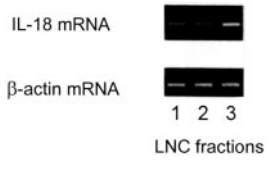

FIGURE 2. High levels of IL-18 mRNA present in migratory hpLC. Migratory hpLC were enriched from LNC using a metrizamide gradient. RNA was extracted from total LNC, the hpLC-enriched fraction, and the hpLC-free fraction, and analyzed by RT-PCR. *Lane 1*, Total LNC; *lane 2*, hpLC-free LNC fraction; *lane 3*, hpLC-enriched LNC fraction.

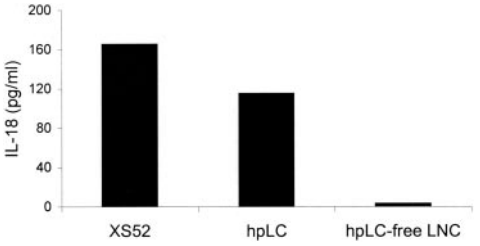

FIGURE 3. Migratory hpLC secrete significant amounts of IL-18 protein. XS52 cells, the hpLC-enriched LNC fraction, and the hpLC-free LNC fraction were cultured for 48 h, and culture supernatants were subjected to ELISA analysis. Significant amounts of IL-18 were detected from the hpLC-enriched LNC fraction as well as XS52 cells, but not from the hpLC-free LNC fraction. The data shown are representative results from one of three individual experiments.

IL-12-induced IFN-γ production by LNC is partially blocked by anti-IL-18 Ab

To induce LNC to produce IFN-γ, LNC were incubated with rIL-12 at 10 ng/ml for 48 h. The quantity of IFN-γ protein in the supernatants was examined by ELISA (Fig. 5). IL-12 induced LNC to produce significant amounts of IFN-γ. To examine whether endogenous IL-18 plays a synergistic role in the IL-12-induced IFN-γ production, LNC were incubated with IL-12 (10 ng/ml) in the presence or the absence of neutralizing anti-IL-18 Ab (50 μg/ml) for 48 h. ELISA analysis on the culture supernatants showed that neutralization of IL-18 significantly reduced IFN-γ production. In contrast, control rabbit IgG did not reduce IL-12-induced IFN-γ production in LNC.

IFN-γ production is decreased in caspase-$1^{-/-}$ LNC but restored by exogenous IL-18

To confirm the synergistic role of IL-18 in the induction of IFN-γ by IL-12, we further examined whether caspase-1 deficiency impairs IL-12-induced IFN-γ production due to the absence of mature IL-18. LNC were obtained from caspase-$1^{-/-}$ and WT mice and incubated with IL-12 for 48 h. Culture supernatants were subjected to ELISA analysis (Fig. 6). The IFN-γ concentration was significantly lower in caspase-$1^{-/-}$ LNC compared with WT LNC. However, the addition of exogenous IL-18 restored IFN-γ production.

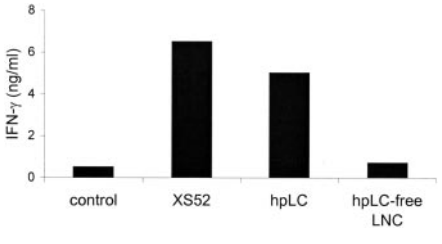

FIGURE 4. Migratory hpLC-derived IL-18 is functionally active. Mouse spleen cells were prepared and cultured for 48 h with Con A (0.5 μg/ml) in combination with culture supernatants of hpLC, hpLC-free LNC, and XS52 cells or as a negative control with Con A alone. The amount of IFN-γ in culture supernatants was measured using a murine IFN-γ ELISA kit. The data shown are representative results from one of three individual experiments.

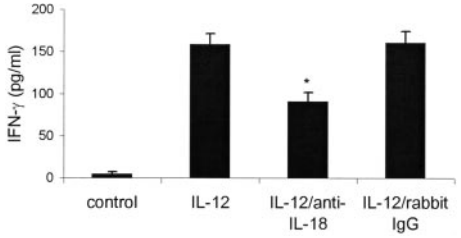

FIGURE 5. Anti-IL-18 Ab inhibits IL-12-induced IFN-γ production by LNC. LNC from oxazolone-sensitized mice were cultured in RPMI 1640 medium containing rIL-12 (10 ng/ml) in the presence of either rabbit anti-mouse IL-18 Ab or normal rabbit IgG (50 μg/ml). After a 48-h culture, supernatants were subjected to ELISA to assess the concentration of IFN-γ. The data are the mean ± SEM of three experiments performed on duplicate samples. *, $p < 0.01$.

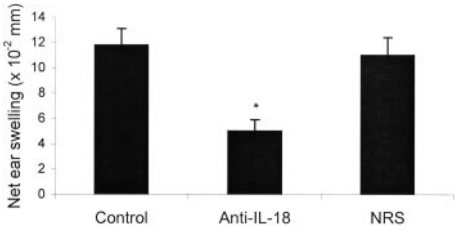

FIGURE 7. Administration of neutralizing anti-IL-18 Ab inhibits CHS. C57BL/6 mice were injected i.p. with 100 μl anti-IL-18 antiserum or normal rabbit serum (NRS) 24 and 2 h before oxazolone sensitization. Mice were sensitized with 3% oxazolone and 5 days later were challenged with 1% oxazolone on the ears. Ear swelling responses were measured 24 h after challenge. The data are the mean ± SEM from groups of five mice. *, $p < 0.005$.

In vivo neutralization of endogenous IL-18 inhibits CHS responses

To investigate whether IL-18 is functionally relevant to the induction of CHS, endogenous IL-18 was neutralized by the administration of anti-IL-18 Ab. C57BL/6 mice were injected i.p. with anti-IL-18 Ab 24 and 2 h before hapten sensitization. Blockage of endogenous IL-18 led to suppression of CHS responses by ~50% (Fig. 7). On the contrary, pretreatment with normal rabbit serum did not significantly alter the ear swelling response.

CHS responses are reduced in caspase-1$^{-/-}$ mice

To further confirm the functional relevance of endogenous IL-18 to CHS, we investigated whether caspase-1 deficiency could impair CHS responses due to the absence of mature IL-18. Before CHS assays, we examined the density of epidermal LC in the epidermal sheets and demonstrated a normal number of LC present in caspase-1$^{-/-}$ mice (data not shown). When caspase-1$^{-/-}$ and WT mice were sensitized to oxazolone, a significantly reduced CHS response was seen in caspase-1$^{-/-}$ mice compared with WT mice (Fig. 8).

Discussion

The balance between two types of specific immune responses (cellular and humoral) is orchestrated by cytokines produced by T cells. Th1 cells synthesize IFN-γ, IL-2, and TNF-α, which promote cell-mediated inflammatory reactions, while Th2 cells synthesize IL-4, IL-5, IL-10, and IL-13, which promote humoral Ab-driven responses. Although populations of T cells may simultaneously produce both cytokine subsets, effective cellular or humoral responses require the selective activation of polarized Th1 or Th2 cells, respectively.

While T cells respond to Ag-derived peptides presented in the context of MHC molecules expressed by APC, the shift of the balance between Th1 and Th2 cytokines is highly determined by APC-associated factors. APC provide T cells not only with an Ag-specific stimulatory signal (signal 1) and a series of costimulatory signals (signal 2), but also with polarizing signals (signal 3, i.e., IL-12) (50).

APC-derived IL-12 is the most potent polarizing cytokine for Th1 cells. IL-12 promotes Th1 cell responses by the selective up-regulation of IFN-γ in T cells. In addition to IL-12, other cytokines, such as IL-1α and TNF-α, are recognized to contribute to Th1 polarization (51). Differential actions of IL-1 on T cell subsets have long been recognized (52). This recognition foreshadowed identification of additional IL-1 family members that can significantly contribute to the biological regulation of Th1 and Th2 cells.

IL-18, a new member of the IL-1 family, has been shown to be a selective activator of IFN-γ in Th1, but not Th2, cells. This is supported by the observation that IL-18 and IL-12 act synergistically on the development of Th1 cells and induction of IFN-γ production by these cells (32). Furthermore, the effects of IL-18 as a costimulator for IFN-γ are selectively targeted toward Th1 cells but not Th2 cells (33). An important role for IL-18 in vivo has been shown by targeted disruption of the IL-18 gene. IL-18-deficient mice were found to have a significant reduction in LPS-induced IFN-γ production and a decreased Th1 response to intracellular bacteria and parasites (53). While many studies indicate that IL-18 cannot induce a Th1 response in the absence of IL-12, other evidence suggests that IL-18 has IL-12-independent effects. This was shown for Th1-directed, delayed-type hypersensitivity-mediated

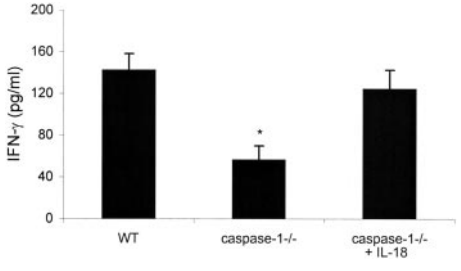

FIGURE 6. Caspase-1-deficient LNC produce lower amounts of IFN-γ. Caspase-1$^{-/-}$ and caspase-1$^{+/+}$ LNC from caspase-1$^{-/-}$ and WT mice were cultured in RPMI 1640 medium containing rIL-12 (10 ng/ml) in the presence or the absence of murine rIL-18 (30 ng/ml). After a 48-h culture, supernatants were collected and subjected to ELISA to assess IFN-γ concentrations. The data are the mean ± SEM of three experiments performed on duplicate samples. *, $p < 0.005$.

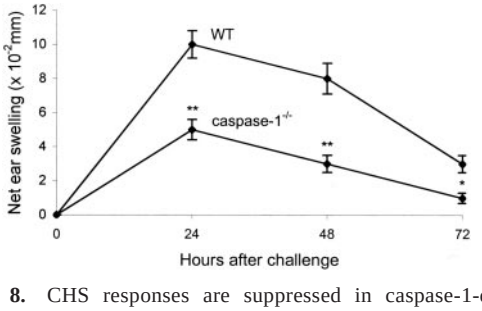

FIGURE 8. CHS responses are suppressed in caspase-1-deficient mice. Caspase-1$^{-/-}$ and WT mice were sensitized with 3% oxazolone and 5 days later were challenged with 1% oxazolone on the ears. Ear swelling responses were determined at various time points. For comparison of caspase-1$^{-/-}$ vs WT mice, p values are as follows: *, $p < 0.05$; **, $p < 0.01$.

crescentic glomerulonephritis and cutaneous delayed-type hypersensitivity in mice (54). Moreover, recent studies reveal that IL-18 can stimulate Th2 immune responses in the absence of IL-12 (55). Further elucidation of the molecular mechanisms of IL-18 on Th1 and Th2 immune responses may provide a novel approach for controlling Th1/Th2 balance at the transcriptional level.

CHS represents a classic Th1 immune response in the skin. Although Th2 cytokines may also mediate CHS in certain cases, it is clear that the Th1 cytokine IFN-γ is the main effector cytokine for CHS (56, 57). The critical role of LC and LC-derived IL-12 in the initiation of CHS has been well documented. For the present studies we investigated whether another LC-derived cytokine, IL-18, plays a role in the induction of CHS. First we demonstrate that during the induction phase of CHS, migratory LC in the skin-draining LN express high levels of IL-18 mRNA and secrete functional IL-18 protein. Examination of IL-18 gene expression in skin-draining LN with RT-PCR showed a significant up-regulation of IL-18 mRNA in LN following hapten sensitization. Further analysis of IL-18 mRNA levels in different fractions of LNC revealed that migratory hapten-modified LC were the main cellular source for the increased IL-18 mRNA expression in skin-draining LN. Moreover, ELISA analysis demonstrated that migratory LC were able to secrete significant amounts of IL-18 protein, which was functionally active, as shown by its ability to induce IFN-γ production in Con A-stimulated T cells.

We also demonstrate a synergistic role for LC-derived IL-18 in IL-12-induced IFN-γ production by LNC. IL-12 induced LNC to produce significant amounts of IFN-γ; however, neutralizing IL-18 Ab could block IFN-γ production by ~50%. IL-12-induced IFN-γ production was also significantly lower in caspase-1$^{-/-}$ LNC compared with WT LNC. This impaired IFN-γ induction appeared to be due to the absence of mature IL-18, because IFN-γ production could be restored by treatment with exogenous IL-18.

Finally, we used in vivo experiments to demonstrate functional relevance for IL-18 production during the induction phase of CHS. To neutralize endogenous IL-18, mice were injected with anti-IL-18 Ab before hapten sensitization. Anti-IL-18 Ab-treated mice showed a reduced CHS response. Additionally, we examined CHS responses in caspase-1$^{-/-}$ mice that could not produce the mature form of IL-18. Caspase-1$^{-/-}$ mice also exhibited reduced CHS responses. Recently, Antonopoulos et al. (58) demonstrated decreased LC migration associated with reduced CHS responses in caspase-1$^{-/-}$ mice. Our observation of a decreased CHS response in caspase-1$^{-/-}$ mice is consistent with their report.

Collectively, the present study demonstrates that during the induction phase of CHS, migratory LC in the LN produce significant amounts of functional IL-18, which acts synergistically with IL-12 to induce IFN-γ production and significantly contributes to CHS initiation. Our results are also consistent with the observations of Wildbaum et al. (59), who recently demonstrated a crucial role for IL-18 in the induction phase of experimental autoimmune encephalomyelitis, a Th1 autoimmune disease of the CNS. They reported that treatment of rats with anti-IL-18 Ab during the induction phase of experimental autoimmune encephalomyelitis resulted in significant inhibition of disease and Ag-specific IFN-γ production (59). However, recent studies also suggest that IL-18 can modulate inflammation at multiple checkpoints, acting not only on initiation of putative autoreactive Th1/Tc1 responses, but also during perpetuation of inflammation. In fact, elevated IL-18 expression has been reported in mouse skin during the elicitation phase of CHS (60). Up-regulated IL-18 expression has also been reported in other Th1 immune/inflammatory responses, such as Crohn's disease, rheumatoid arthritis, psoriasis, and sarcoidosis (61–64). Our studies presented here support a need for better understanding the molecular mechanisms of IL-18 functions. Further defining IL-18-dependent signaling mechanisms in immune/inflammatory responses may provide a rationale for anti-IL-18-based treatment of inflammatory conditions.

References

1. Bell, D., J. W. Young, and J. Banchereau. 1999. Dendritic cells. *Adv. Immunol. 72:255.*
2. Stingl, G., D. Maurer, C. Hauser, and K. Wolff. 1999. The epidermis: an immunologic microenviroment. In *Dermatology in General Medicine*, 5th Ed. I. M. Freedberg, A. Z. Eisen, K. Wolff, K. F. Austin, L. A. Goldsmith, S. I. Katz, and T. B. Fitzpatric, eds. McGraw-Hill, New York, p. 343.
3. Wang, B., H. Fujisawa, L. Zhuang, I. Freed, B. G. Howell, S. Shahid, G. M. Shivji, T. W. Mak, and D. N. Sauder, 2000. CD4$^+$ Th1 and CD8$^+$ type1 cytotoxic T cells both play a crucial role in the full development of contact hypersensitivity. *J. Immunol. 165:6783.*
4. Gocinski, B. L., and R. E. Tigelaar. 1990. Role of CD4$^+$ and CD8$^+$ T cells in murine contact sensitivity by in vivo monoclonal antibody depletion. *J. Immunol. 144:4121.*
5. Xu, H., N. A. Dilulio, and R. L. Fairchild. 1996. T cell populations primed by hapten sensitization in contact sensitivity are distinguished by polarized patterns of cytokine production: interferon γ-producing (Tc1) effector CD8$^+$ T cells and interleukin (IL)-4/IL-10-producing (Th2) negative regulatory CD4$^+$ T cells. *J. Exp. Med. 183:1001.*
6. Belsito, D. V. 1999. Allergic contact dermatitis. In *Dermatology in General Medicine*, 5th Ed. I. M. Freedberg, A. Z. Eisen, K. Wolff, K. F. Austin, L. A. Doldsmith, S. I. Katz, and T. B. Filtzpatric, eds. McGraw-Hill, New York, p. 1447.
7. Mactonia, S. E., S. C. Knight, A. J. Edwards, S. Griffiths, and P. Fryer. 1987. Localization of antigen on lymph node dendritic cells after exposure to the contact sensitizer fluorescein isothiocyanate. *J. Exp. Med. 166:1654.*
8. Kripke, M. L., C. G. Munn, A. Jeevan, J. M. Tang, and C. Bucana. 1990. Evidence that cutaneous APCs migrate to regional lymph nodes during contact sensitization. *J. Immunol. 145:2833.*
9. Wang, B., P. Amerio, and D. N. Sauder. 1999. Role of cytokines in epidermal Langerhans cell migration. *J. Leukocyte Biol. 66:33.*
10. Mactonia, S. E., N. A. Hosken, M. Litton, P. Vieira, C. S. Hsieh, J. A. Culpepper, M. Wysocka, G. Trinchieri, K. M. Murphy, and A. O'Garra. 1995. Dendritic cells produce IL-12 and direct the development of Th1 cells from naive CD4$^+$ T cells. *J. Immunol. 154:5071.*
11. Cella, M., D. Scheidegger, K. Palmer-Lehmann, P. Lane, A. Lanzavecchia, and G. Alber. 1996. Ligation of CD40 on dendritic cells triggers production of high levels of IL-12 and enhances T cell stimulatory capacity: T-T help via APC activation. *J. Exp. Med. 184:747.*
12. O'Garra, A. 1998. Cytokines induce the development of functionally heterogeneous T helper subsets. *Immunity 8:275.*
13. Mehrotra, P. T., D. Wu, J. A. Crim, H. S. Mostowski, and J. P. Siegel. 1993. Effect of IL-12 on the generation of cytotoxic activity in human CD8$^+$ T lymphocytes. *J. Immunol. 151:2444.*
14. Chouaib, S., J. Chehimi, L. Bani, N. Genetet, T. Tursz, F. Gay, G. Trinchieri, and F. M. Chouaib. 1994. Interleukin 12 induces the differentiation of major histocompatibility complex class I-primed cytotoxic T-lymphocyte precursors into allospecific cytotoxic effectors. *Proc. Natl. Acad. Sci. USA 91:12659.*
15. Muller, G., J. Saloga, T. Germann, G. Schuler, J. Knop, and A. H. Enk. 1995. IL-12 as mediator and adjuvant for the induction of contact sensitivity in vivo. *J. Immunol. 155:4661.*
16. Riemann, H., A. Schwarz, S. Grabbe, Y. Aragane, T. A. Luger, M. Wysocka, M. Kubin, G. Trinchieri, and T. Schwarz. 1996. Neutralization of IL-12 in vivo prevents induction of contact hypersensitivity and induces hapten-specific tolerance. *J. Immunol. 156:1799.*
17. Okamura, H., H. Tsutsui, T. Komatsu, M. Yutsudo, A. Hakura, T. Tanimoto, K. Torigoe, T. Okura, Y. Nukada, K. Hattori, et al. 1995. Cloning of a new cytokine that induces IFN-γ production by T cells. *Nature 378:88.*
18. Dinarello, C. A., D. Novick, A. J. Puren, G. Fantuzzi, L. Shapiro, H. Muhl, D.-Y. Yoon, L. L. Reznikov, S.-H. Kim, and M. Rubinstein. 1998. Overview of interleukin-18: more than an interferon-γ inducing factor. *J. Leukocyte Biol. 63:658.*
19. Bazan, J. F., J. C. Timans, and R. A. Kastelein. 1996. A newly defined interleukin-1? *Nature 379:591.*
20. Dinarello, C. A. 1999. IL-18: a Th1-inducing, proinflammatory cytokine and new member of the IL-1 family. *J. Allergy Clin. Immunol. 103:11.*
21. Gu, Y., K. Kuida, H. Tsutsui, G. Ku, K. Hsiao, M. A. Fleming, N. Hayashi, K. Higashino, H. Okamura, K. Nakanishi, et al. 1997. Activation of interferon-γ inducing factor mediated by interleukin-1β converting enzyme. *Science 275:206.*
22. Fantuzzi, G., A. J. Puren, M. W. Harding, D. J. Livingston, and C. A. Dinarello. 1998. Interleukin-18 regulation of interferon-γ production and cell proliferation as shown in interleukin-1β-converting enzyme (caspase-1)-deficient mice. *Blood 91:2118.*
23. Fantuzzi, G., D. A. Reed, and C. A. Dinarello. 1999. IL-12-induced IFN-γ is dependent on caspase-1 processing of the IL-18 precursor. *J. Clin. Invest. 104:761.*
24. Torigoe, K., S. Ushio, T. Okura, S. Kobayashi, M. Taniai, T. Kunikata, T. Murakami, O. Sanou, H. Ikegami, and M. Kurimoto. 1997. Purification and characterization of the human interleukin-18 receptor. *J. Biol. Chem. 272:25737.*
25. Hoshino, K., H. Tsutsui, T. Kawai, K. Takeda, K. Nakanishi, Y. Takeda, and S. Akira. 1999. Generation of IL-18 receptor-deficient mice: evidence for IL-1

receptor-related protein as an essential IL-18 binding receptor. *J. Immunol. 162: 5041.*
26. Adachi, O., T. Kawai, K. Takeda, M. Matsumoto, H. Tsutsui, M. Sakagami, K. Nakanishi, and S. Akira. 1998. Targeted disruption of the MyD88 gene results in loss of IL-1- and IL-18-mediated function. *Immunity 9:143.*
27. O'Neil, L. A., and C. A. Dinarello. 2000. The IL-1 receptor/Toll-like receptor superfamily: crucial receptors for inflammation and host defense. *Immunol. Today 21:206.*
28. Okamura, H., S. Kashiwamura, H. Tsutui, T. Yoshimoto, and K. Nakanishi. 1998. Regulation of IFN-γ production by IL-12 and IL-18. *Curr. Opin. Immunol. 10: 259.*
29. Kohno, K., and M. Kurimoto. 1998. Interleukin 18, a cytokine which resembles IL-1 structurally and IL-12 functionally but exerts its effect independently of both. *Clin. Immunol. Immunopathol. 86:11.*
30. de Saint-Vis, B., I. Fugier-Vivier, C. Massacrier, C. Gaillard, B. Vanbervliet, S. Ait-Yahia, J. Banchereau, Y.-J. Liu, S. Lebecque, and C. Caux. 1998. The cytokine profile expressed by human dendritic cells is dependent on cell subtype and mode of activation. *J. Immunol. 160:1666.*
31. Stoll, S., H. Jonuleit, E. Schmitt, G. Muller, H. Yamauchi, M. Kurimoto, J. Knop, and A. H. Enk. 1998. Production of functional IL-18 by different subtypes of murine and human dendritic cells (DC): DC-derived IL-18 enhances IL-12-dependent Th1 development. *Eur. J. Immunol. 28:3231.*
32. Micallef, M. J., T. Ohtsuki, K. Kohno, F. Tanabe, S. Ushio, M. Namba, T. Tanimoto, K. Torigoe, M. Fujii, M. Ikeda, et al. 1996. Interferon-γ-inducing factor enhances T helper 1 cytokine production by stimulated human T cells: synergism with interleukin-12 for interferon-γ production. *Eur. J. Immunol. 26: 1647.*
33. Kohno, K., J. Kataoka, T. Chtsuki, Y. Suemoto, I. Okamoto, M. Usui, M. Ikeda, and M. Kurimoto. 1997. IFN-γ-inducing factor (IGIF) is a costimulatory factor on the activation of Th1 but not Th2 cells and exerts its effect independently of IL-12. *J. Immunol. 158:1451.*
34. Robinson, D., K. Shibuya, A. Mui, F. Zonin, E. Murphy, T. Sana, S. B. Hartley, S. Menon, R. Kastelein, F. Bazan, et al. 1997. IGIF does not drive Th1 development, but synergizes with IL-12 for IFN-γ production, and activates IRAK and NF-κB. *Immunity 7:571.*
35. Chang, J. T., B. M. Segal, K. Nakanishi, H. Okamura, and E. M. Shevach. 2000. The costimulatory effect of IL-18 on the induction of antigen-specific IFN-γ production by resting T cells is IL-12 dependent and is mediated by up-regulation of the IL-12 receptor β_2 subunit. *Eur. J. Immunol. 30:1113.*
36. Okamoto, I., K. Kohno, T. Tanimoto, K. Ikegami, and M. Kurimoto. 1999. Development of $CD8^+$ effector T cells is differentially regulated by IL-18 and IL-12. *J. Immunol. 162:3202.*
37. Netea, M. G., G. Fantuzzi, B. J. Kullberg, R. J. L. Stuyt, E. J. Pulido, R. C. McIntyre, Jr., L. A. B. Joosten, J. W. M. van der Meer, and C. A. Dinarello. 2000. Neutralization of IL-18 reduces neutrophil tissue accumulation and protects mice against lethal *Escherichia coli* and *Salmonella typhimurium* endotoxemia. *J. Immunol. 164:2644.*
38. Faggioni, R., J. Jones-Carson, D. A. Reed, C. A. Dinarello, K. R. Feingold, C. Grunfeld, and G. Fantuzzi. 2000. Leptin-deficient (*ob/ob*) mice are protected from T cell-mediated hepatotoxicity: role of tumor necrosis factor α and IL-18. *Proc. Natl. Acad. Sci. USA 97:2367.*
39. Joosten, L. A. B., F. A. J. van de Loo, E. Lubberts, M. M. A. Helsen, M. G. Netea, J. W. M. van der Meer, C. A. Dinarello, and W. B. van den Berg. 2000. An IFN-γ-independent proinflammatory role of IL-18 in murine streptococcal cell wall arthritis. *J. Immunol. 165:6553.*
40. Mordue, D. G., F. Monroy, M. L. Regina, C. A. Dinarello, and L. D. Sibley. 2001. Acute toxoplasmosis leads to lethal overproduction of Th1 cytokines. *J. Immunol. 167:4547.*
41. Melnikov, V. Y., T. Ecder, G. Fantuzzi, B. Siegmund, M. S. Lucia, C. A. Dinarello, R. W. Schrier, and C. L. Edelstein. 2001. Impaired IL-18 processing protects caspase-1-deficient mice from ischemic acute renal failure. *J. Clin. Invest. 107:1145.*
42. Kuida, K., A. J. Lippke, G. Ku, M. W. Harding, D. J. Livingston, M. A.-S. Su, and R. A. Flavell. 1995. Altered cytokine export and apoptosis in mice deficient in interleukin-1β converting enzyme. *Science 267:2000.*
43. Xu, S., K. Ariizumi, G. Caceres-Dittmar, D. Edelbaum, K. Hashimoto, P. R. Bergstresser, and A. Takashima. 1995. Successive generation of antigen-presenting, dendritic cell lines from murine epidermis. *J. Immunol. 154:2697.*
44. Wang, B., S. Kondo, G. M. Shivji, H. Fujisawa, T. W. Mak, and D. N. Sauder. 1996. Tumor necrosis factor receptor II (p75) signaling is required for the migration of Langerhans cells. *Immunology 88:284.*
45. Bigby, M., R. Vargas, and M.-S. Sy. 1989. Production of hapten-specific T cell hybridomas and their use to study the effect of ultraviolet B irradiation on the development of contact hypersensitivity. *J. Immunol. 143:3867.*
46. Wang, B., L. Zhuang, H. Fujisawa, G. A. Shinder, C. Feliciani, G. M. Shivji, H. Suzuki, P. Amerio, P. Toto, and D. N. Sauder. 1999. Enhanced epidermal Langerhans cell migration in IL-10 knockout mice. *J. Immunol. 162:277.*
47. Bohn, E., A. Sing, R. Zumbihl, C. Bielfeldt, H. Okamura, M. Kurimoto, J. Heesemann, and I. B. Autenrieth. 1998. IL-18 (IFN-γ-inducing factor) regulates early cytokine production in, and promotes resolution of, bacterial infection in mice. *J. Immunol. 160:299.*
48. Wang, B., A. Rieger, O. Kilgus, K. Ochiai, D. Maurer, D. Fodinger, J. P. Kinet, and G. Stingl. 1992. Epidermal Langerhans cells from human skin bind monomeric IgE via FcϵRI. *J. Exp. Med. 175:1353.*
49. Wang, B., H. Fujisawa, L. Zhuang, S. Kondo, G. M. Shivji, C. S. Kim, T. W. Mak, and D. N. Sauder. 1997. Depressed Langerhans cell migration and reduced contact hypersensitivity response in mice lacking TNF receptor p75. *J. Immunol. 159:6148.*
50. Kapsenberg, M. L., C. M. U. Hilkens, E. A. Wierenga, and P. Kalinski. 1998. The role of antigen-presenting cells in the regulation of allergen-specific T cell responses. *Curr. Opin. Immunol. 10:607.*
51. Shibuya, K., D. Robinson, F. Zonin, S. B. Hartley, S. E. Mactonia, C. Somoza, C. A. Hunter, K. M. Purphy, and A. O'Garra. 1998. IL-1α and TNF-α are required for IL-12-induced development of Th1 cells producing high levels of IFN-γ in BALB/c but not C57BL/6 mice. *J. Immunol. 160:1708.*
52. Lichtman, A. H., J. Chin, J. A. Schmidt, and A. K. Abbas. 1988. Role of interleukin 1 in the activation of T lymphocytes. *Proc. Natl. Acad. Sci. USA 85:9699.*
53. Takeda, K., H. Tsutsui, T. Yoshimoto, O. Adachi, N. Toshida, T. Kishimoto, H. Okamura, K. Nakanishi, and S. Akira. 1998. Defective NK cell activity and Th1 response in IL-18-deficient mice. *Immunity 8:383.*
54. Kitching, A. R., P. G. Tipping, M. Kurimoto, and S. R. Holdsworth. 2000. IL-18 has IL-12-independent effects in delayed-type hypersensitivity: studies in cell-mediated crescentic glomerulonephritis. *J. Immunol. 165:4649.*
55. Nakanishi, K., T. Yoshimoto, H. Tsutsui, and H. Okamura. 2001. Interleukin-18 regulates both Th1 and Th2 responses. *Annu. Rev. Immunol. 19:423.*
56. Wang, B., C. Feliciani, I. Freed, Q. Cai, and D. N. Sauder. 2001. Insights into molecular mechanisms of contact hypersensitivity gained from gene knockout studies. *J. Leukocyte Biol. 70:185.*
57. Dearman, R. J., and I. Kimber. 1992. Divergent immune responses to respiratory and contact chemical allergens: antibody elicited by phthalic anhydride and oxazolone. *Clin. Exp. Allergy 22:241.*
58. Antonopoulos, C., M. Cumberbatch, R. J. Dearman, R. J. Daniel, I. Kimber, and R. W. Groves. 2001. Functional caspase-1 is required for Langerhans cell migration and optimal contact sensitization in mice. *J. Immunol. 166:3672.*
59. Wildbaum, G., S. Youssef, N. Grabie, and N. Karin. 1998. Neutralizing antibodies to IFN-γ-inducing factor prevent experimental autoimmune encephalomyelitis. *J. Immunol. 161:6368.*
60. Xu, B., K. Aoyama, S. Yu, A. Kitani, H. Okamura, M. Kurimoto, T. Matsuyama, and T. Matsushita. 1998. Expression of interleukin-18 in murine contact hypersensitivity. *J. Interferon Cytokine Res. 18:653.*
61. Pizarro, T. T., M. H. Michie, M. Bentz, J. Woraratanadharm, M. F. Smith, Jr., E. Foley, C. A. Moskaluk, S. J. Bickston, and F. Cominelli. 1999. IL-18, a novel immunoregulatory cytokine, is up-regulated in Crohn's disease: expression and localization in intestinal mucosal cells. *J. Immunol. 162:6829.*
62. Gracie, J. A., R. J. Forsey, W. L. Chan, A. Gilmour, B. P. Leung, M. R. Greer, K. Kennedy, R. Carter, X.-Q. Wei, D. Xu, et al. 1999. A proinflammatory role for IL-18 in rheumatoid arthritis. *J. Clin. Invest. 104:1393.*
63. Ohta, Y., Y. Hamada, and K. Katsuoka. 2001. Expression of IL-18 in psoriasis. *Arch. Dermatol. Res. 293:334.*
64. Shigehara, K., N. Shijubo, M. Ohmichi, R. Takahashi, S.-I. Kon, H. Okamura, M. Kurimoto, Y. Hiraga, T. Tatsuno, S. Abe, et al. 2001. IL-12 and IL-18 are increased and stimulate IFN-γ production in sarcoid lungs. *J. Immunol. 166:642.*