Table 1. No. of circulating polymorphonuclear neutrophils (PMNs) before or on day 3 of infection with *Candida albicans* blastoconidia (1×10^5 cfu administered intravenously), and recruitment of PMNs 4 h after intraperitoneal injection of heat-killed *C. albicans* blastoconidia (1×10^7 cfu).

Strain	Circulating PMNs, no. $\times 10^9$/L		Intraperitoneal PMN recruitment	
	Before infection	On day 3 of infection	%[a]	No. $\times 10^6$
IL-1$\alpha^{+/+}\beta^{+/+}$	0.89 ± 0.31	0.84 ± 0.71	63 ± 13	5.81 ± 3.26
IL-1$\alpha^{-/-}$	0.42 ± 0.10	0.86 ± 0.30	54 ± 6	5.56 ± 1.62
IL-1$\beta^{-/-}$	1.38 ± 0.47	3.20 ± 0.99	37 ± 12[b]	1.85 ± 0.84[c]
IL-1$\alpha^{-/-}\beta^{-/-}$	0.67 ± 0.35	2.95 ± 0.52	39 ± 8[b]	3.21 ± 0.70

NOTE. Data are the mean ± SD of at least 3 mice/group, were obtained from 1 experiment, and were analyzed using 1-way analysis of variance and the Mann-Whitney *U* test for posttest comparisons. IL, interleukin.

[a] Percentage of the total no. of peritoneal exudate cells

[b] $P < .005$, vs. control or IL-1$\alpha^{-/-}$ mice.

[c] $P < .01$, vs. control or IL-1$\alpha^{-/-}$ mice.

ia, the largest quantity of *C. albicans* was recovered from the kidneys of IL-1$\alpha^{-/-}\beta^{-/-}$ mice (mean ± SD, 5.23 ± 0.37 log cfu), and the difference was significant in comparison with IL-1$\alpha^{+/+}\beta^{+/+}$ mice (mean ± SD, 3.64 ± 0.51 log cfu) ($P < .01$). Outgrowth in the kidneys of IL-1$\alpha^{-/-}$ mice (mean ± SD, 4.27 ± 1.31 log cfu) was similar to that in the kidneys of IL-1$\beta^{-/-}$ mice (mean ± SD, 4.13 ± 0.67 log cfu. (Data are cumulative results of 2 experiments for at least 6 mice/group.)

PMN circulating numbers and recruitment. To study the role of endogenous IL-1α and IL-1β in the recruitment of PMNs to the site of a localized *C. albicans* infection, peritoneal PMNs were collected after intraperitoneal injection of heat-killed *C. albicans* (table 1). Whereas the recruitment of PMNs in IL-1$\alpha^{-/-}$ mice did not differ from that in IL-1$\alpha^{+/+}\beta^{+/+}$ mice, significantly lower numbers of PMNs were recruited to the site of infection in IL-1$\beta^{-/-}$ mice, indicating that IL-1β is most important for PMN recruitment to the site of infection. In contrast, during the early days of infection, increased numbers of circulating PMNs were observed in IL-1$\beta^{-/-}$ mice and IL-1$\alpha^{-/-}\beta^{-/-}$ mice, compared with those in IL-1$\alpha^{-/-}$ and IL-1$\alpha^{+/+}\beta^{+/+}$ mice (table 1), underscoring the importance of IL-1β in the migration of PMNs from the bloodstream to the site of infection. The mean numbers of peripheral blood PMNs were similar for all groups on day 7 of infection (data not shown).

Anticandidal response of PMNs against* C. albicans *blastoconidia and pseudohyphae. To further characterize the influence of endogenous IL-1α and IL-1β on the activity of PMNs against *C. albicans*, the fungicidal capacity of PMNs was determined in vitro. Whereas IL-1$\alpha^{-/-}$ PMNs showed a tendency only toward reduced superoxide production (mean ± SD, $58.3 \times 10^3 \pm 15.4 \times 10^3$ cps), IL-$\beta^{-/-}$ PMNs produced significantly less superoxide than did control PMNs (mean ± SD, $30.6 \times 10^3 \pm 15.9 \times 10^3$ vs. $71.5 \times 10^3 \pm 10.5 \times 10^3$ cps) ($P < .01$). However, this observation was not accompanied by an altered capacity of IL-1–deficient PMNs to phagocytose or kill internalized *C. albicans* blastoconidia; all groups phagocytosed ~25% of the blastoconidia and killed ~75% of internalized blastoconidia.

In contrast, IL-1 modulated the capacity of PMNs to damage pseudohyphae (figure 4). Pseudohyphal damage caused by IL-1$\alpha^{-/-}$ or IL-1$\alpha^{-/-}\beta^{-/-}$ PMNs was significantly less efficient than that caused by IL-1$\alpha^{+/+}\beta^{+/+}$ or IL-1$\beta^{-/-}$ PMNs ($P < .001$) (figure 4). Deficiency of both IL-1 molecules showed a tendency to further impair PMN antipseudohyphal resistance, compared with IL-1α deficiency alone. Deficiency of IL-1β alone did not diminish the capacity of PMNs to damage *C. albicans* pseudohyphae.

Macrophage function and stimulation. Macrophage recruitment was determined by harvesting peritoneal exudate cells 72 h after an intraperitoneal injection of 1×10^7 cfu of heat-killed *C. albicans*. No differences in the mean ± SD numbers of macrophages that were recruited into the peritoneal cavity were observed, compared with those in control mice (IL-1$\alpha^{+/+}\beta^{+/+}$, $1.01 \times 10^9 \pm 0.12 \times 10^9$; IL-1$\alpha^{-/-}$, $1.10 \times 10^9 \pm 0.02 \times 10^9$; IL-1$\beta^{-/-}$, $0.96 \times 10^9 \pm 0.19 \times 10^9$; IL-1$\alpha^{-/-}\beta^{-/-}$, $1.18 \times 10^9 \pm 0.15 \times 10^9$). IL-1 influenced neither the mean ± SD capacity of exudate peritoneal macrophages to phagocytose *C. albicans* blastoconidia (IL-1$\alpha^{+/+}\beta^{+/+}$, 41% ± 5%; IL-1$\alpha^{-/-}$, 50% ± 4%; IL-1$\beta^{-/-}$, 56% ± 3%; IL-1$\alpha^{-/-}\beta^{-/-}$, 41% ± 16%) nor their capacity to damage *C. albicans* pseudohyphae (data not shown). No differences between NO production by macrophages from the mouse strains were observed in response to either heat-killed *C. albicans* (all around the detection limit) or

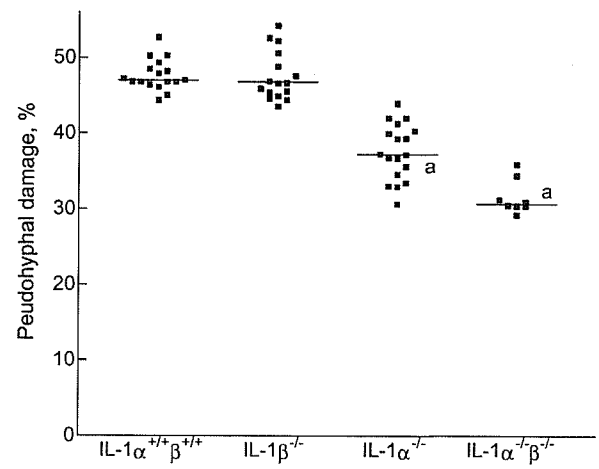

Figure 4. Percentage of exudate polymorphonuclear neutrophil (PMN)–mediated pseudohyphal damage. Pseudohyphae (1×10^5) were incubated in the presence of 8×10^5 PMNs for 2 h. Data are the cumulative results of 2 separate experiments and were analyzed using the Kruskal-Wallis 1-way analysis of variance. For posttest comparisons, the Mann-Whitney *U* test was applied. Horizontal bars indicate medians. [a]$P < .001$, vs. interleukin (IL)–1$\alpha^{+/+}\beta^{+/+}$ or IL-1$\beta^{-/-}$ mice.

LPS (control mice, 1.37 ± 0.54 ng/mL; IL-1$\beta^{-/-}$ mice, 0.78 ± 1.35 ng/mL; IL-$\alpha^{-/-}\beta^{-/-}$ mice, 5.28 ± 0.2 ng/mL; $P > .05$).

IL-1–deficient resident peritoneal macrophages showed impaired production of IL-6 and of the chemokine KC in response to heat-killed *C. albicans* blastoconidia or pseudohyphae. The difference in IL-6 production was significant for IL-1$\alpha^{-/-}$ macrophages and IL-1$\alpha^{-/-}\beta^{-/-}$ macrophages, in comparison with IL-1$\alpha^{+/+}\beta^{+/+}$ macrophages, and the difference in KC production was significant for IL-1$\beta^{-/-}$ macrophages and IL-1$\alpha^{-/-}\beta^{-/-}$ macrophages, in comparison with IL-1$\alpha^{+/+}\beta^{+/+}$ macrophages ($P < .05$) (table 2). No differences between groups were observed in the production of TNF-α, MCP-1, or MIP-2.

Stimulation of splenic lymphocytes. To assess whether IL-1 induces a type 1 or a type 2 immune response, splenocytes were stimulated with heat-killed *C. albicans*. Whereas IL-1$\alpha^{+/+}\beta^{+/+}$ splenocytes produced IFN-γ, neither IL-1$\alpha^{-/-}$ nor IL-1$\beta^{-/-}$ splenocytes did ($P < .05$) (table 3), indicating that both IL-1α and IL-1β are important for protective IFN-γ production. Whereas IL-1$\beta^{-/-}$ and IL-1$\alpha^{-/-}\beta^{-/-}$ splenocytes did not produce any IL-10, both IL-1$\alpha^{+/+}\beta^{+/+}$ splenocytes and IL-1$\alpha^{-/-}$ splenocytes did ($P < .05$) (table 3), indicating that both IL-1α and IL-1β are important for protective IFN-γ production, whereas the residual IL-10 production in IL-1$\alpha^{-/-}$ mice further contributed to a type 2 response.

DISCUSSION

The results of the present study indicate that deficiency of endogenous IL-1α or IL-1β has deleterious effects on the outcome of disseminated candidiasis. Both IL-1$\alpha^{-/-}$ mice and IL-1$\beta^{-/-}$ mice showed increased mortality associated with an increased outgrowth of *C. albicans* in the kidneys. IL-1β proved to be important for PMN recruitment and generation of superoxide production. IL-1α was essential for the capacity of PMNs to damage *C. albicans* pseudohyphae, and both IL-1α and IL-1β were required for the induction of protective Th1 responses.

One of the mechanisms through which IL-1 has been suggested to confer protection is the enhancement of granulopoiesis and influx of PMNs to the site of infection. Although IL-1$\alpha^{-/-}$ mice had 50% fewer circulating granulocytes at the time of infection than control mice, their number in the circulation on day 3 of infection and PMN recruitment to the site of infection did not differ from that of control mice. In IL-1$\beta^{-/-}$ mice, however, a trend toward an increased number of circulating granulocytes during infection was observed, and this coincided with significantly decreased PMN migration to the site of infection in a standardized model of intraperitoneal *C. albicans* challenge. This is in line with earlier evidence that exogenous administration of IL-1α or IL-1β induces peripheral blood granulocytosis [13, 14]. Our data on PMN recruitment in response to heat-killed *C. albicans* also suggest an important role for endogenous IL-1β in the early PMN influx, in line with studies showing that exogenous administration of IL-1 induces PMN accumulation [15, 16]. Moreover, PMN recruitment in IL-1–deficient mice may be further impaired as a result of decreased production of neutrophil chemoattractants. Whereas IL-1$\alpha^{-/-}$ mice showed a tendency toward reduced production of the CXC chemokine KC, KC production by IL-1$\beta^{-/-}$ macrophages was significantly reduced, thus likely contributing to impaired granulocyte recruitment in IL-1$\beta^{-/-}$ mice. Whereas the differences in chemokine production between IL-1$\alpha^{-/-}$ and IL-1$\beta^{-/-}$ mice may not be impressive, the major difference between the mouse strains was the complete absence of IL-1β in the IL-1$\beta^{-/-}$ mice. Since IL-1β has been shown to be an important mediator of

Table 2. In vitro cytokine and chemokine production by resident peritoneal macrophages (1×10^5) stimulated with either heat-killed *Candida albicans* blastoconidia (1×10^7) or heat-killed pseudohyphae (1×10^6).

Stimulation, strain	TNF-α, ng/mL	IL-6, pg/mL	MCP-1, pg/mL	MIP-2, pg/mL	KC, pg/mL
Blastoconidia					
IL-1$\alpha^{+/+}\beta^{+/+}$	0.56 ± 0.14	926 ± 547	87 ± 45	2190 ± 1633	1842 ± 880
IL-1$\alpha^{-/-}$	0.88 ± 0.41	154 ± 9[a]	40 ± 1	2660 ± 1630	380 ± 197
IL-1$\beta^{-/-}$	0.46 ± 0.22	329 ± 235	50 ± 22	2166 ± 1769	308 ± 345[a]
IL-1$\alpha^{-/-}\beta^{-/-}$	0.72 ± 0.41	159 ± 21[a]		1674 ± 753	265 ± 254[a]
Pseudohyphae					
IL-1$\alpha^{+/+}\beta^{+/+}$	1.32 ± 0.82	1378 ± 796	221 ± 135	3350 ± 2699	1920 ± 879
IL-1$\alpha^{-/-}$	0.95 ± 0.42	366 ± 203	152 ± 154	2550 ± 1656	435 ± 196
IL-1$\beta^{-/-}$	0.74 ± 0.29	417 ± 268	98 ± 56	2495 ± 1860	99 ± 87[a]
IL-1$\alpha^{-/-}\beta^{-/-}$	0.98 ± 0.39	233 ± 187[a]		1295 ± 510	303 ± 326[a]

NOTE. Data are the mean ± SD production in 5 mice/group, obtained from 1 experiment, and were analyzed using 1-way analysis of variance and the Mann-Whitney *U* test for posttest comparisons. IL, interleukin; KC, keratinocyte-derived chemokine; MCP, monocyte chemotactic protein; MIP, macrophage inhibitory protein; TNF, tumor necrosis factor

[a] $P < .05$, vs. IL-1$\alpha^{+/+}\beta^{+/+}$ mice.

Table 3. In vitro cytokine production by splenocytes (5×10^6 cells/mL) stimulated with heat-killed *Candida albicans* blastoconidia (10^7/mL).

Strain	IFN-γ, pg/mL	IL-10, pg/mL
IL-1$\alpha^{+/+}\beta^{+/+}$	47.4 ± 30.7	32.3 ± 8.8
IL-1$\alpha^{-/-}$	<15.6[a]	30.8 ± 12.2
IL-1$\beta^{-/-}$	<15.6[a]	<8[b]
IL-1$\alpha^{-/-}\beta^{-/-}$	<15.6[a]	<8[b]

NOTE. Data were obtained from 1 experiment and are expressed as the mean ± SD production in 5 mice/group. IFN, interferon; IL, interleukin.

[a] $P < .05$, vs. IL-1$\alpha^{+/+}\beta^{+/+}$ mice.

[b] $P < .05$, vs. IL-1$\alpha^{+/+}\beta^{+/+}$ and IL-1$\alpha^{-/-}$ mice.

granulocyte recruitment [17, 18], this effect most likely is instrumental in explaining the difference in recruitment between the IL-1$\alpha^{-/-}$ and IL-1$\beta^{-/-}$ mice. However, it has to be taken into account that assessment of PMN recruitment to a peritoneal inflammatory stimulus is, at best, an approximation of early parenchymal PMN recruitment at the various sites of infection.

Transcription of the genes encoding MIP-2 and KC is induced through signals mediated by Toll-like receptor 4 [19], and IL-1 has been shown to selectively stabilize KC mRNA [20]. Hence, it is hypothesized that the normal production of MIP-2—in contrast to the 80% reduced production of KC by IL-1–deficient macrophages—is due to the selective effect of IL-1 on KC mRNA.

The effect of endogenous IL-1 on PMN function has not been investigated previously. The results of the present study indicate that IL-1 affects PMN function and that the modes of action differ for IL-1α and IL-1β. Whereas IL-1$\beta^{-/-}$ PMNs showed impaired superoxide production, IL-1$\alpha^{-/-}$ PMNs showed a decreased capacity to damage *C. albicans* pseudohyphae, indicating that IL-1α is important for PMN degranulation. In addition, production of IL-6, which is induced by IL-1 and known to stimulate PMN function [21–23], was reduced only in IL-1$\beta^{-/-}$ mice, and this may further contribute to impaired PMN function and reduced anticandidal defense. Since the *C. albicans* burden in IL-1$\alpha^{-/-}$ kidneys was significantly higher than that in IL-1$\beta^{-/-}$ kidneys on day 7 of infection, it is suggested that the initially reduced PMN recruitment in IL-1$\beta^{-/-}$ mice is overridden by the effect of the reduced capacity to kill *C. albicans* pseudohyphae in IL-1$\alpha^{-/-}$ mice. However, despite the difference in outgrowth between the 2 mouse strains on day 7, IL-1$\beta^{-/-}$ mice showed susceptibility to infection similar to that of IL-1$\alpha^{-/-}$ mice. Most of the apparent discrepancy is explained by the fact that, on day 14 of infection, the fungal burden in both IL-1$\alpha^{-/-}$ mice and IL-1$\beta^{-/-}$ mice was equally increased. Another possible explanation for this observation is that, in addition to fungal outgrowth, the additional inflammatory damage in the organs of IL-1$\beta^{-/-}$ mice may also have contributed to mortality.

If the beneficial effect of endogenous IL-1 is exerted only through PMNs, the differences in outgrowth between the groups, as observed in nongranulocytopenic mice, should disappear in granulocytopenic mice. However, the observed differences persisted, indicating that the effect of endogenous IL-1 is at least partly mediated through cells or mechanisms other than modulation of PMN recruitment and function, which is in line with previous findings [3, 4]. Nevertheless, because cyclophosphamide also reduces lymphocyte numbers, it cannot be excluded that a minor beneficial effect of IL-1 on *C. albicans* outgrowth may be mediated through PMNs.

Macrophages and lymphocytes are other cells that are likely to mediate the protective effect of IL-1 [24, 25]. Most importantly, IFN-γ production was found to be absent in IL-1–deficient splenocytes. This is in agreement with previous data showing that endogenous IL-1 is important for the production IFN-γ during *C. albicans* stimulation of whole blood [26]. The crucial role of IFN-γ in host defense has become apparent from studies showing that IFN-γ–deficient mice are highly susceptible to disseminated candidiasis and that administration of recombinant IFN-γ reduces outgrowth [12, 27, 28]. Recently, we observed that delayed development of a type 1 response—that is, delayed IFN-γ production—contributed to increased outgrowth in localized *C. albicans* infection [9]. Furthermore, we observed foreign body giant cells (FBGCs) in the kidneys of IL-1–deficient mice. The transition of macrophages to functionally inactive FBGCs is favored in the presence of type 2 cytokines [29]. Therefore, the presence of FBGCs points to an anti-inflammatory type 2 response, which has been shown to be detrimental to anticandidal host defense [30].

In conclusion, the present study has further clarified the mechanisms through which IL-1α and IL-1β enhance host resistance against candidiasis. Interestingly, the absence of either IL-1α or IL-1β had divergent consequences. Although they are products of different genes, IL-1α and IL-1β are highly homologous and have similar tridimensional structures and common receptors. It is unclear at this point what determines the functional differences in IL-1$\alpha^{-/-}$ and IL-1$\beta^{-/-}$ mice—a different distribution of cytokines in the organs, the known dichotomy between cell-bound IL-1α and secreted IL-1β [31], or differential receptor affinity. Although a functional difference between IL-1α and IL-1β was observed in this study, both IL-1α and IL-1β were shown to be essential for anticandidal host defense. The complementary roles of IL-1α and IL-1β in host defense against *C. albicans* infection are further underscored by the even higher susceptibility to disseminated candidiasis of mice lacking both IL-1α and IL-1β. These data constitute an additional body of evidence that IL-1–dependent mechanisms play an important role in innate host defense against *C. albicans*.

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IL-1β, but not IL-1α, is required for antigen-specific T cell activation and the induction of local inflammation in the delayed-type hypersensitivity responses

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Abstract

As IL-1 expression is augmented in delayed-type hypersensitivity (DTH) responses, we analyzed the role of IL-1 in this response. DTH responses against methyl BSA (mBSA) were significantly suppressed in IL-1β-deficient (IL-1β$^{-/-}$) and IL-1α/β$^{-/-}$ mice, but not in IL-1α$^{-/-}$ mice. In contrast, responses in IL-1R antagonist$^{-/-}$ (IL-1Ra$^{-/-}$) mice were exacerbated. Lymph node cells derived from mBSA-sensitized IL-1β$^{-/-}$, IL-1α/β$^{-/-}$ and IL-1R type I (IL-1RI)$^{-/-}$ mice, but not from IL-1α$^{-/-}$ mice, exhibited reduced proliferative responses against mBSA, while these from IL-1Ra$^{-/-}$ mice demonstrated augmented responses. DTH responses in wild-type mice following adoptive transfer of CD4^{+} T cells from mBSA-sensitized IL-1α/β$^{-/-}$ mice were also reduced, while those in mice given cells derived from IL-Ra$^{-/-}$ mice were increased. DTH responses in IL-1RI$^{-/-}$, but not IL-1α/β$^{-/-}$, mice were reduced upon transplantation of mBSA-sensitized CD4^{+} T cells from wild-type mice. The recall response of mBSA-sensitized CD4^{+} T cells against mBSA decreased upon co-culture with dendritic cells (DCs) from IL-1RI$^{-/-}$ mice, while the responses were normal with DCs from IL-1α/β$^{-/-}$ mice. DTH responses in tumor necrosis factor α$^{-/-}$ (TNF$^{-/-}$) mice were also suppressed; the magnitude of the suppression in IL-1α/β$^{-/-}$TNF$^{-/-}$ mice, however, was similar to that observed in IL-1α/β$^{-/-}$ mice. These observations indicate that IL-1 possesses dual functions during the DTH response. IL-1β is necessary for the efficient priming of T cells. In addition, CD4^{+} T cell-derived IL-1 plays an important role in the activation of DCs during the elicitation phase, resulting in the production of TNF, that activate allergen-specific T cells.

Introduction

Delayed-type hypersensitivity (DTH), classified as type IV hypersensitiveness by Coombs and Gell, is an immune response mediated by a variety of inflammatory cells, including neutrophils, macrophages and T cells (1, 2). DTH develops in two phases, a sensitization phase, in which T cells are sensitized and memory T cells are formed, and an elicitation phase, in which T cell recall responses are induced upon secondary challenge with antigens. This second phase results in the induction of inflammation, involving recruitment of inflammatory cells such as neutrophils and macrophages.

Although DTH reactions are classically subdivided into tuberculin-type, Jones–Mote-type and contact hypersensitivity (CHS) reactions, in this study, we analyzed the mechanisms of tuberculin-type reaction as typical of DTH reactions. The DTH response is evoked by T_h1 CD4^{+} T cells, whereas CD8^{+} T cells behave as apparent regulatory cells in this response (3). During the elicitation phase of DTH responses, neutrophils and macrophages infiltrate early into inflammatory sites, followed by T cells. IFN-γ, produced by antigen-specific CD4^{+} T cells, plays an important role in the development of DTH responses by enhancing T_h1 cell development (4), leukocyte recruitment through the induction of chemokines, such as IFN-γ-inducible protein-10 (IP-10) (5), and expression of adhesion molecules, such as intercellular adhesion

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molecule-1, vascular cell adhesion molecule-1, E-selectin and P-selectin by acting on leukocytes, endothelial cells and keratinocytes (6–9). IFN-γ also activates macrophages, resulting in the production of IL-1 and tumor necrosis factor α (TNFα), factors that perpetuate the local inflammation (10).

IL-1 is a pro-inflammatory cytokine that regulates multiple aspects of immune and inflammatory responses (11). The IL-1 family of cytokines includes IL-1α and IL-1β. While these molecules both bind to two cellular receptors, IL-1R type I and type II (IL-1RI and IL-1RII, respectively) (12), the roles for these cytokines differ between types of host defense responses (13). IL-1RI, but not IL-1RII, mediates the functional activities of IL-1α and IL-1β (12). Thus, IL-1RII is considered to be a decoy receptor that negatively regulates IL-1 activity (12). IL-1R antagonist (IL-1Ra), also a negative regulator of IL-1 function, can bind to IL-1RI without generating any signal, competing for IL-1 binding and negatively regulating IL-1α and IL-1β signaling (12).

IL-1 contributes to the development of DTH responses by promoting the maturation of antigen-specific T cells (14) and by augmenting IL-12-dependent IFN-γ production by T_h1 cells (15). The development of DTH responses were reduced in mice lacking the expression of IL-1RI (IL-1RI$^{-/-}$ mice) (16) or treated with peptides derived from IL-1Ra (17). It has also been reported, however, that IL-1β$^{-/-}$ mice exhibit normal DTH responses (18), suggesting that IL-1α, but not IL-1β, contributes to the generation of type IV hypersensitivity reactions.

Recently, we have demonstrated that IL-1α, not IL-1β, potently activates T cells during the sensitization phase of CHS responses (19). The role of IL-1 in DTH responses, however, remains unclear; it is unknown whether IL-1 acts as a T cell-activating factor during the sensitization phase or as a pro-inflammatory cytokine recruiting inflammatory cells during the elicitation phase.

In this study, we have analyzed the activities of IL-1 and the functional differences between IL-1α and IL-1β in the development of DTH responses against methyl BSA (mBSA). We demonstrate that IL-1β, but not IL-1α, functions in the development of DTH responses using IL-1α-, IL-1β-, IL-1α/β-, IL-1RI- and IL-1Ra-deficient mice. We also demonstrate that, in addition to the involvement of IL-1 in T cell sensitization, activated memory T cell-derived IL-1 also induces dendritic cell (DC)-mediated production of TNF, resulting in local inflammation upon secondary stimulation.

Methods

Mice

IL-1α$^{-/-}$, IL-1β$^{-/-}$, IL-1α/β$^{-/-}$ and IL-1Ra$^{-/-}$ mice were generated as described (20). Animals were backcrossed to either C57BL/6J or BALB/cA mice for eight generations. IL-1RI$^{-/-}$ mice, kindly provided by Immunex (21), were backcrossed to C57BL/6J mice for six generations. TNF$^{-/-}$ mice were backcrossed for 10 generations to C57BL/6J mice (22). IL-1α/β$^{-/-}$ TNF$^{-/-}$ mice and IL-1Ra$^{-/-}$TNF$^{-/-}$ mice were obtained by intercrossing IL-1α/β$^{-/-}$ and TNF$^{-/-}$ mice and IL-1Ra$^{-/-}$ and TNF$^{-/-}$ mice, respectively. C57BL/6J and BALB/cA mice were purchased from CLEA-Japan (Shizuoka, Japan). Sex- and age-matched mice, used in all experiments, received simultaneous treatment. All mice were housed under specific pathogen-free conditions in an environmentally controlled clean room (24 ± 2°C, 40–60% moisture, 8:00–20:00 lighting cycle) at the Center for Experimental Medicine, Institute of Medical Science, University of Tokyo. Animals were periodically monitored for infection. Experiments were conducted according to the institutional ethical guidelines for animal experimentation and the safety guidelines for gene manipulation experiments.

DTH response

Evaluation of DTH responses to mBSA (Sigma, St Louis, MO, USA) was performed as described (23–25). Briefly, mice were immunized subcutaneously (s.c.) with 200 μl of 1.25 mg ml^{-1} mBSA emulsified with CFA (Difco, Detoit, MI, USA). Seven days after immunization, mice were challenged s.c. in one footpad with 20 μl of 10 mg ml^{-1} mBSA in PBS. Animals were injected with an equal volume of PBS into another footpad as a control. At the indicated times after the challenge, footpad thickness was measured with a dial caliper (Mitutoyo, Tokyo, Japan). The magnitude of the DTH response was determined as follows: [footpad swelling (mm)] = [footpad thickness of mBSA-injected footpad (mm)] − [footpad thickness of PBS-injected footpad (mm)], [footpad swelling (%)] = ([footpad swelling (mm)]/[footpad thickness of PBS-injected footpad (mm)]) × 100. Footpad swelling (%) was calculated as a percentage of the mean value of the wild-type group.

Histological examination

Twenty-four hours after challenge with either mBSA or PBS, paws were removed and decalcified for 3 days by a standard protocol. After fixation with 10% PFA in PBS, specimens were embedded in paraffin. Paraffin sections were stained with hematoxylin–eosin. Structural changes and the presence of cellular infiltration in sections were determined by light microscopy.

Proliferative responses of lymph node cells

Mice were immunized with 250 μg (when IL-1$^{-/-}$ or IL-1RI$^{-/-}$ mice were immunized) or 100 μg (when IL-1Ra$^{-/-}$ mice were immunized) mBSA/CFA at the base of the tail and two footpads. Seven days after immunization, inguinal and popliteal lymph nodes (LN) were harvested. After preparation of a single cell suspension, LN cells were suspended in RPMI1640 (Sigma) supplemented with 10% FCS (Sigma), 50 U ml^{-1} penicillin (Meiji), 50 μg ml^{-1} streptomycin (Meiji) and 50 mM 2-mercaptoethanol (GIBCO). LN cells (5×10^5) were cultured in the presence or absence of mBSA (50 μg ml^{-1}) in 96-well plates for 72 h, followed by the incorporation of [^{3}H]thymidine ([^{3}H]TdR) (0.25 mCi ml^{-1}) (Amersham, Buckinghamshire, UK) for 9 h. Cells were then harvested using a Micro 96 well harvester (Molecular Devices, Sunnyvale, CA, USA); radioactivity was measured using a Micro Beta counter (Amersham).

Measurement of IFN-γ production

IFN-γ levels in LN cell culture supernatants were determined by sandwich ELISA. IFN-γ Opt EIA kit (BD Biosciences Pharmingen, San Diego, CA, USA) was used for the detection

of IFN-γ. Resulting absolute cytokine levels were determined according to the manufacturer's protocol. The detection limit of the assay for IFN-γ was >5 pg ml^{-1}.

Measurement of serum anti-mBSA IgG

Sera were collected prior to immunization with mBSA/CFA and 4 days after the second immunization with DTH. Anti-mBSA IgG levels were measured by ELISA (25). Briefly, 96-well plates were coated overnight with 100 μl of a 5 μg ml^{-1} mBSA solution at 4°C, then blocked by incubation with 0.1% skim milk (CO-OP, Tokyo, Japan) in PBS for 1 h at room temperature (r.t.). After washing three times in PBS + 0.05% Tween 20, 100 μl of each mouse serum, diluted 316-fold in PBS, was added for 1 h at r.t.. After another round of extensive washing, we added 100 μl of alkaline phosphatase (AP)-conjugated goat anti-mouse IgG (ZYMED, San Francisco, CA, USA). After a 1-h incubation at r.t., plates were washed three times. AP activities were measured on a microplate-reader MP-120 (CORONA, Ibaragi, Japan) using *p*-nitrophenylphosphate SIGMA104® (Sigma) as the substrate.

Adoptive transfer of sensitized T cells

For adoptive T cell transfer experiments, LN cell suspensions were prepared from mBSA/CFA-sensitized mice as described above. To purify $CD4^+$ T cells, LN cells were incubated with anti-mouse B220, CD8, Ter-119 and NK-1.1 microbeads (Miltenyi Biotech, Auburn, CA, USA). $CD4^+$ T cells were isolated following negative selection on a MACS column. We injected the purified $CD4^+$ T cells (2×10^7 cells per mouse) into the tail vain of recipient mice. After 19 h, mice were challenged with mBSA or PBS only into the left or right footpad, respectively. Swelling was monitored over the following days.

mBSA-specific $CD4^+$ T cell proliferation

$CD4^+$ T cells were purified (>90% $CD4^+$ cells) from the draining LNs of immunized mice by positive selection using anti-CD4 magnetic beads (MACS; Miltenyi Biotech). $CD11c^+$ cells (DCs) were purified (>90% $CD11c^+$ cells) from non-immunized splenocytes using anti-CD11c magnetic beads (MACS; Miltenyi Biotech). $CD4^+$ T cells (1×10^5) were cultured for 72 h with 1×10^4 DCs in the presence or absence of 50 μg ml^{-1} mBSA in RPMI1640 (Sigma) complete medium. Proliferation was assessed by measurement of [^{3}H]TdR (0.25 mCi ml^{-1}) (Amersham) incorporation.

Reverse transcription–PCR

Total RNA from $CD4^+$ T cells was isolated by the acid guanidinium thiocyanate-phenol-chloroform extraction (AGPC) method. For reverse transcription (RT), 5 μg of total RNA was transcribed using SuperScript III transcriptase according to the manufacturer's protocol of SuperScript first-strand synthesis system for RT–PCR (Invitrogen, San Diego, CA, USA). The PCR mixture contained 1× PCR buffer, 1.5 mM $MgCl_2$, 0.2 mM deoxynucleoside triphosphate mix, a 5′ and 3′ primer (each at 0.2 μM), 2 units Ex *Taq* DNA polymerase (TaKaRa Bio Inc., Shiga, Japan) and 5 μl (10% of total volume) of the products of RNA reverse transcription in a total volume of 50 μl. After an initial denaturation at 94°C for 2 min, 30 cycles of denaturation (94°C for 20 s), annealing (55°C for 30 s) and extension (72°C for 45 s) were performed using a DNA thermal cycler (icycler, Bio-Rad, Hercules, CA, USA). The primers for PCR amplifying IL-1α, IL-1β, IL-1RI, IL-1Ra and hypoxanthine phosphoribosyltransferase (HPRT) are as follows: forward, 5′-GAGATACAAACTGATGAAGCTC-3′, and reverse, 5′-CAGAAGAAAATGAGGTCGGTC-3′ (IL-1α); forward, 5′-CCTGAACTCAACTGTGAAATGCC-3′, and reverse, 5′-TCATCATCATCCCATGAGTCAC-3′ (IL-1β); forward, 5′-CTGTAAACCTCTGCTTCTTGAC-3′, and reverse, 5′-ACAACACAGATAAACGGATAGCG-3′ (IL-1RI); forward, 5′-GACCCTGCAAGATGCAAGCC-3′, and reverse, 5′-GAGCGGATGAAGGTAAAGCG-3′ (IL-1Ra); forward, 5′-GTTGGATACAGGCCAGACT-3′, and reverse, 5′-CAGGGTAGGCTGGCCTATAGGCT-3′ (HPRT).

Statistical analysis

Each experiment was repeated at least three times. Statistical analysis was performed using the Student's *t*-test. A mean ± SD is shown for all figures. *P*-values <0.05 were considered to be statistically significant.

Results

DTH response in IL-1 KO mice

To elucidate the role of IL-1 in DTH responses, we examined mBSA-induced hypersensitivity in IL-$1^{-/-}$ mice. Footpad swelling in IL-$1\alpha/\beta^{-/-}$ mice on the C57BL/6J background was significantly reduced compared with that seen in wild-type mice (Fig. 1A). A similar reduction was also observed in IL-$1RI^{-/-}$ mice, consistent with the results of Labow *et al.* (16). In contrast, footpad swelling in IL-$1Ra^{-/-}$ mice was markedly enhanced (Fig. 1B). Similar observations were obtained in IL-$1\alpha/\beta^{-/-}$ mice on the BALB/cA background (data not shown).

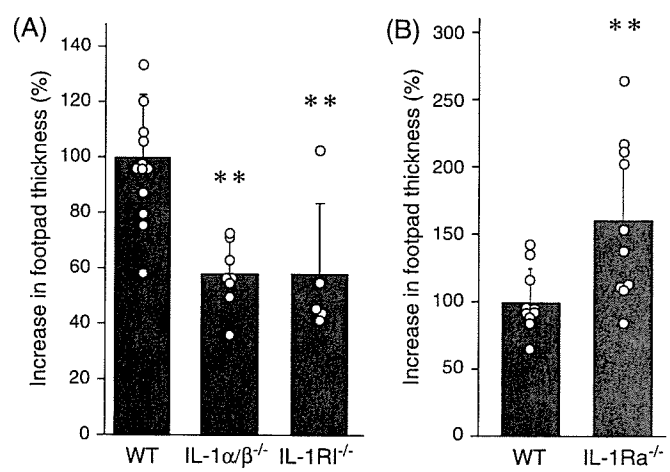

Fig. 1. Induction of DTH responses in IL-$1\alpha/\beta^{-/-}$, IL-$1RI^{-/-}$ and IL-$1Ra^{-/-}$ mice. Mice were sensitized s.c. with mBSA/CFA (250 μg in A, 100 μg in B). One week after sensitization, PBS or mBSA was injected into the footpads of each mouse. Footpad thickness was measured 24 h after the second challenge. Circles indicate the levels for individual mice; a bar shows the mean ± SD for each group. The numbers of the mice used were (A) wild type, $n = 12$; IL-$1\alpha/\beta^{-/-}$, $n = 7$ and IL-$1RI^{-/-}$, $n = 5$; (B) wild type, $n = 8$, and IL-$1Ra^{-/-}$, $n = 4$. **$P < 0.01$ versus wild-type mice.

In the mBSA-injected footpads of wild-type mice, we observed the infiltration of inflammatory cells mainly consisting of neutrophils and lymphocytes in the dermis surrounding small vessels; these infiltrates were not observed in PBS-injected footpads (Fig. 2A–D). Consistent with the reduced footpad thickness (Fig. 1), the numbers of infiltrating cells in the mBSA-challenged footpads of IL-1RI$^{-/-}$ and IL-1$\alpha/\beta^{-/-}$ mice (Fig. 2F and G) were significantly lower than those in wild-type mice, although these levels were increased in comparison with those seen in PBS-injected footpads (Fig. 2B and C). In contrast, a greater number of inflammatory cells were observed in the mBSA-injected footpads of IL-1Ra$^{-/-}$ mice than that seen in wild-type mice (Fig. 2H). The cellular composition infiltrated into the inflammatory sites was similar (data not shown) among IL-1$\alpha/\beta^{-/-}$, IL-1RI$^{-/-}$ and IL-1Ra$^{-/-}$ mice (Fig. 2I–L). These results indicate that IL-1 plays an important role in the development of mBSA-induced DTH responses.

The role for IL-1 in the activation of mBSA-specific T cells

As IL-1 functions as a T cell co-stimulatory molecule in the antigen-specific T cell activation observed during the sensitization phase of immune responses (19, 26), we examined the effect of IL-1 deficiency on antigen-specific T cell responses following mBSA sensitization. Seven days after mBSA sensitization, isolated inguinal LN cells were cultured in the presence or absence of mBSA. LN T cell proliferative responses against mBSA were reduced in both IL-1RI$^{-/-}$ and IL-1$\alpha/\beta^{-/-}$ mice from the levels seen in wild-type mice (Fig. 3A). Under these conditions, IFN-γ levels in the supernatants of LN cell cultures derived from IL-1RI$^{-/-}$ and IL-1$\alpha/\beta^{-/-}$ mice were decreased in comparison with those observed in LN cell cultures from wild-type mice (Fig. 3B). The levels of IL-4 in these LN cell cultures, however, were below the assay limit of the detection among these LN cell cultures (data not shown). The mBSA-specific proliferative responses of LN cells from IL-1Ra$^{-/-}$ mice were increased 2.2-fold from those seen in cultures from wild-type mice (Fig. 3C); the IFN-γ levels in supernatants from IL-1Ra$^{-/-}$ LN cell cultures were also elevated (Fig. 3D). These results indicate that IL-1 is required for optimal T cell activation during the sensitization phase of DTH responses induced by mBSA.

Differential roles of IL-1α and IL-1β in DTH response

To clarify the differential roles of IL-1α and IL-1β in the development of DTH responses, we have examined the expression of IL-1α and IL-1β during DTH reaction. Mice were immunized

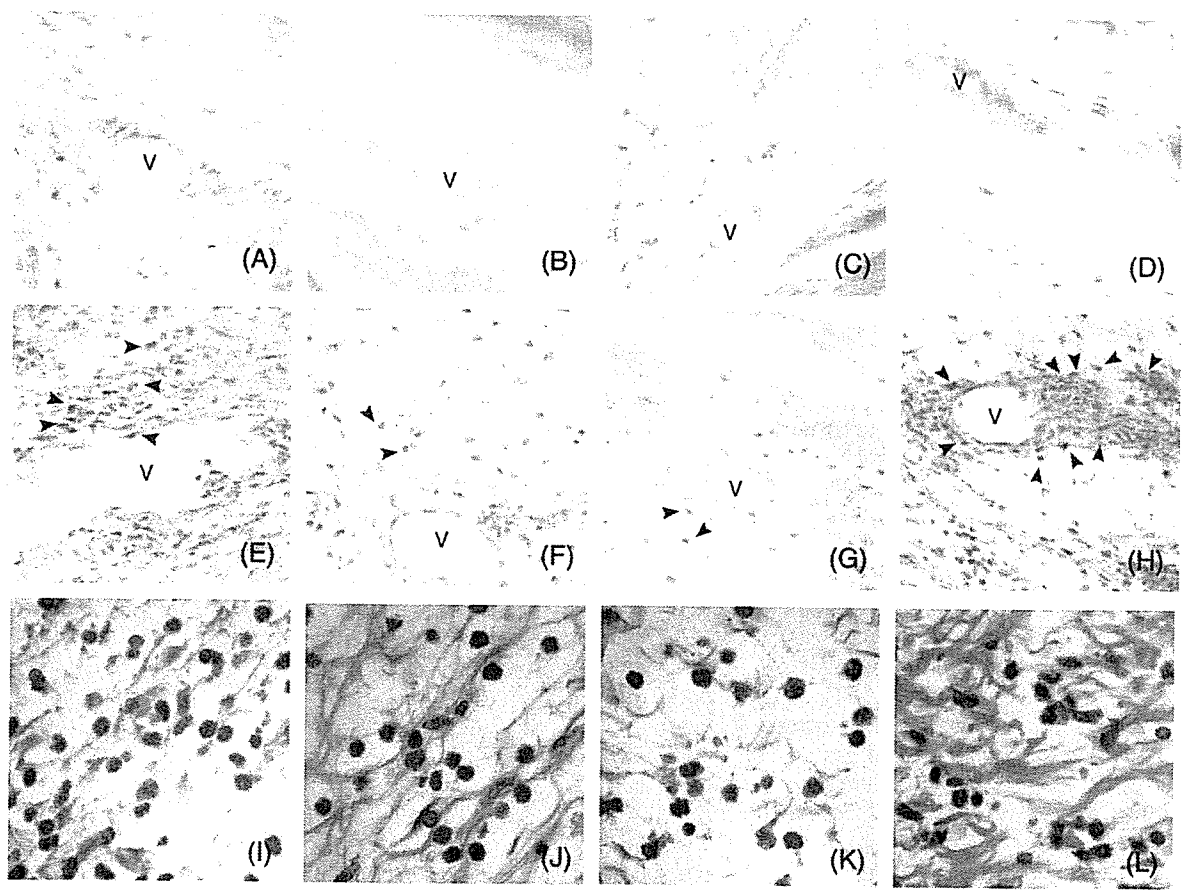

Fig. 2. Histology of footpads from DTH-induced mice. Sections of footpads challenged with PBS (A, B, C and D) or mBSA (E–H) were stained with hematoxylin and eosin. (A) and (E), wild-type mice; (B) and (F), IL-1$\alpha/\beta^{-/-}$ mice; (C) and (G), IL-1RI$^{-/-}$ mice (D) and (H), IL-1Ra$^{-/-}$ mice. "v" points to small vessels. Filled arrow heads in (E–H) indicate infiltration of inflammatory cells in the dermis surrounding small vessels. (A–H): ×400 and (I–L): ×1000

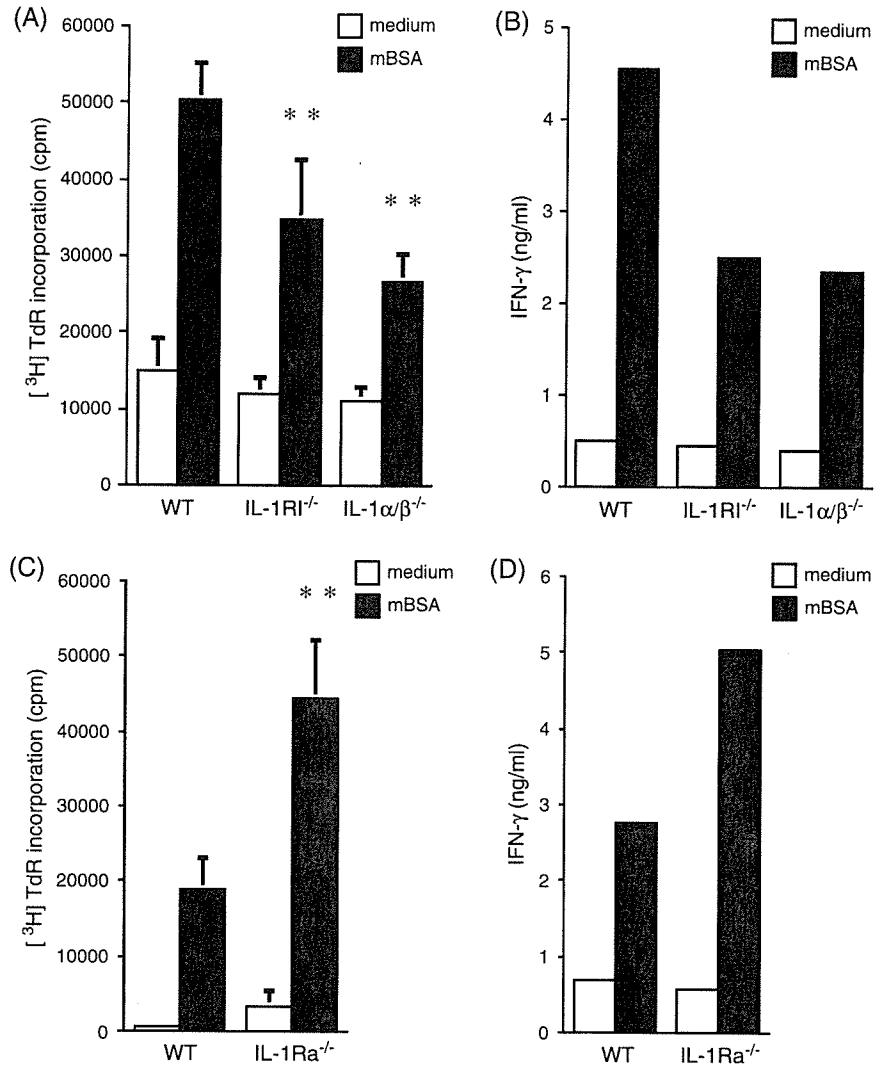

Fig. 3. Proliferative responses and IFN-γ production of LN cells after stimulation with mBSA. Mice were immunized with mBSA/CFA (250 μg in A, B, D; 100 μg in C) as described in Methods. (A), (C) Seven days after immunization, LN cells were harvested. After stimulation with mBSA for 72 h, proliferative responses were measured by [^{3}H]TdR incorporation. A mean ± SD of triplicate measurements is shown. (B), (D) IFN-γ levels in the pooled culture supernatants of triplicate wells from proliferative response assay were determined by ELISA. Similar results were obtained at least in three independent experiments. $^{**}P < 0.01$ versus wild-type mice.

with mBSA/CFA, and after 3 days (for the sensitization phase) or 7 days (for the elicitation phase), the LN cells were harvested. Then, the mRNA levels for IL-1α and IL-1β were determined by real-time PCR. We found that the IL-1β expression level in the LN cells was much higher than that of IL-1α both at the sensitization and elicitation phases (the content of IL-1β mRNA in total IL-1 mRNA: 90% at 3 days after sensitization and 65% at 7 days). The expression level of IL-1β in the inflamed footpads at the elicitation phase was also much higher than that of IL-1α (80% IL-1β). Thus, IL-1β is mainly produced during DTH responses.

Then, we examined mBSA-induced hypersensitivity in IL-1α$^{-/-}$ and IL-1β$^{-/-}$ mice on the C57BL/6J background. Footpad swelling differed markedly among IL-1α$^{-/-}$, IL-1β$^{-/-}$ and wild-type mice; swelling was significantly suppressed in IL-1β$^{-/-}$ and IL-1α/β$^{-/-}$ mice, while it was unaffected in IL-1α$^{-/-}$ mice (Fig. 4A). Microscopic analyses coincided well with these observations (data not shown). Similar results were also observed in mice on the BALB/cA background (data not shown).

The mBSA-specific proliferative responses of LN cells after sensitization with mBSA were significantly reduced in both IL-1β$^{-/-}$ and IL-1α/β$^{-/-}$ mice in comparison with IL-1α$^{-/-}$ and wild-type mice (Fig. 4B). No significant differences were observed between wild-type mice and IL-1α$^{-/-}$ mice. Interestingly, upon stimulation with mBSA, IFN-γ levels in the supernatants of LN cell cultures prepared from both IL-1α$^{-/-}$ and IL-1β$^{-/-}$ mice were decreased from the levels observed in wild-type mice (Fig. 4C). After antigen stimulation, IFN-γ production in IL-1α/β$^{-/-}$ LN cell cultures was significantly reduced in comparison with IL-1β$^{-/-}$ cell cultures. Although IL-1α did not enhance the proliferative responses of LN cells, these findings suggest that both IL-1α and IL-1β are involved in the production of IFN-γ.

We next examined mBSA-specific antibody production in IL-1-deficient mice. At 4 days after the second challenge

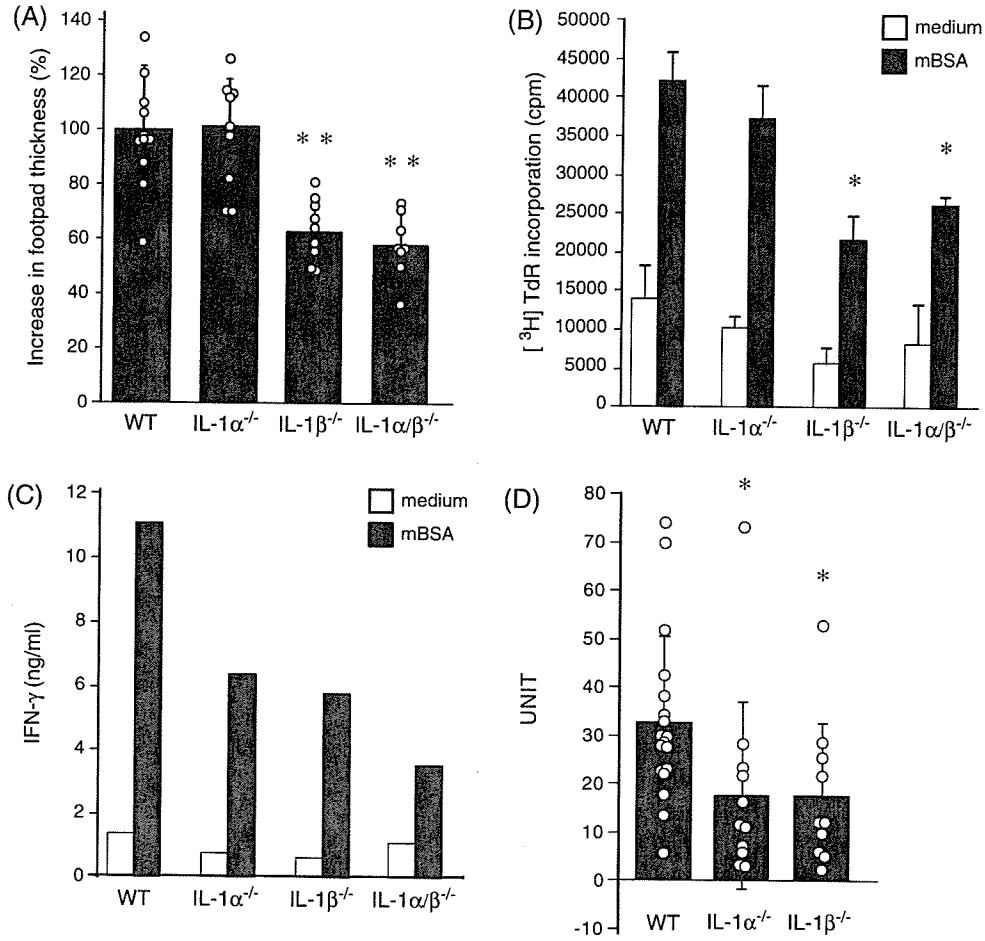

Fig. 4. DTH responses in IL-1$\alpha^{-/-}$ and IL-1$\beta^{-/-}$ mice. mBSA-induced DTH responses in IL-1$\alpha^{-/-}$ and IL-1$\beta^{-/-}$ mice were examined as in Fig. 1. (A) Circles indicate the severity of DTH of individual mice. The mean ± SD of each group is shown. Wild type, $n = 11$; IL-1$\alpha^{-/-}$, $n = 9$; IL-1$\beta^{-/-}$, $n = 9$ and IL-1$\alpha\beta^{-/-}$, $n = 8$. $^{**}P < 0.01$ versus wild-type mice. (B) mBSA-specific LN cell proliferation was assessed by [^{3}H]TdR incorporation. A mean ± SD of triplicate measurements is shown. $^{*}P < 0.05$ versus wild-type mice. (C) IFN-γ levels in pooled culture supernatants of the triplicate wells from the proliferative response assay were determined by ELISA. These results were reproducible in three independent experiments. (D) mBSA-specific IgG levels in sera from DTH-induced mice were determined by ELISA. Circles indicate the levels observed in individual mice. The mean ± SD of each group is also shown. Wild type, $n = 18$; IL-1$\alpha^{-/-}$, $n = 11$ and IL-1$\beta^{-/-}$, $n = 10$. $^{*}P < 0.05$ versus wild-type mice.

with mBSA, mBSA-specific IgG levels in sera were determined by ELISA during the DTH response. mBSA-specific IgG levels were reduced both in IL-1$\alpha^{-/-}$ and IL-1$\beta^{-/-}$ mice in comparison with those observed in wild-type mice (Fig. 4D). The DTH response was only suppressed in IL-1$\beta^{-/-}$ mice. Thus, both IL-1α and IL-1β are involved in mBSA-specific antibody production. These results suggested, however, that these antibodies may not be involved in DTH responses.

An important role for IL-1 in mBSA-specific T cell sensitization in vivo

To discriminate the roles of IL-1 in the sensitization of T cells from its function in the elicitation of inflammation, we performed the adoptive transfer of mBSA-sensitized T cells. Mice were first immunized with mBSA/CFA. One week after immunization, we purified T cells from the draining LNs of these mice. These cells were injected intravenously into naive wild-type mice. Measurement of the development of DTH revealed that footpad swelling was significantly suppressed in mice that received mBSA-sensitized IL-1$\alpha/\beta^{-/-}$ T cells in comparison with animals that were given wild-type T cells (Fig. 5A). In contrast, footpad swelling in mice that received mBSA-sensitized IL-1Ra$^{-/-}$ T cells was significantly increased (Fig. 5B). These results indicate that IL-1 is required for antigen-specific T cell priming during the sensitization phase of mBSA-induced DTH responses.

The role for IL-1 in the elicitation of DTH response

To elucidate the role of IL-1 in the elicitation phase of DTH responses, we adoptively transferred mBSA-sensitized wild-type T cells into wild-type, IL-1$\alpha/\beta^{-/-}$, IL-1RI$^{-/-}$ and IL-1Ra$^{-/-}$ mice. We observed a similar footpad swelling in wild-type and IL-1$\alpha/\beta^{-/-}$ mice, while the resulting footpad swelling in IL-1RI$^{-/-}$ mice was markedly decreased (Fig. 5C). Footpad swelling in IL-1Ra$^{-/-}$ mice receiving mBSA-sensitized T cells was significantly increased in comparison to that seen in wild-type recipient mice (Fig. 5D). These results suggest that donor T cell-produced IL-1 activates recipient leukocytes to induce inflammation. In support for this notion, both IL-1α and IL-1β were transiently expressed in sensitized CD4$^+$ T cells

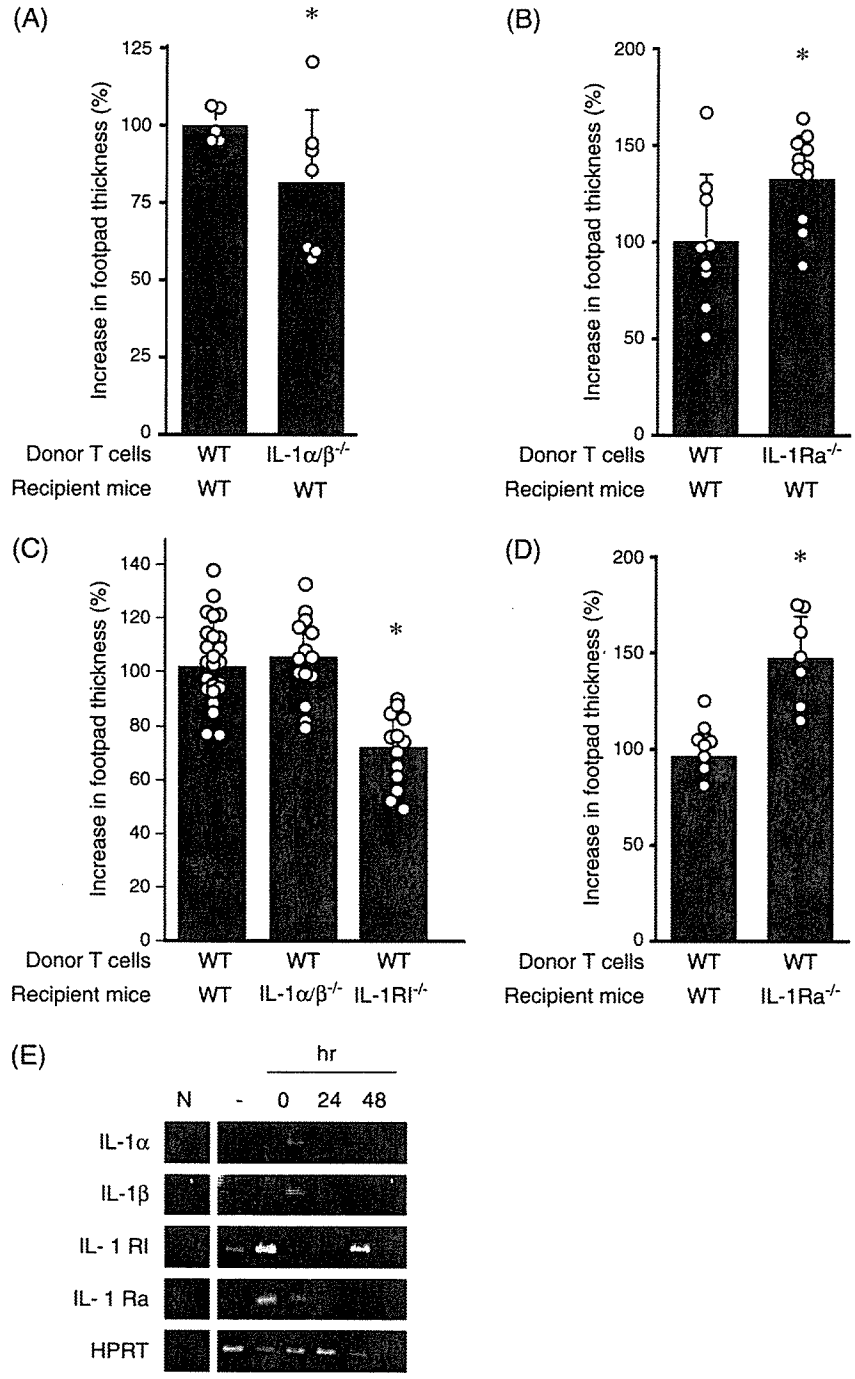

Fig. 5. Analysis of the role of IL-1 in the sensitization and elicitation phases of DTH responses. LN cells (2×10^7 cells per mouse) from mice immunized with mBSA/CFA were transferred into non-sensitized mice intravenously. Nineteen hours after injection, recipient mice were challenged with PBS or mBSA in footpads. Footpad swelling was measured as in Fig. 1. (A) Wild-type mice received wild-type T cells ($n = 5$) or IL-1α/β$^{-/-}$ T cells ($n = 6$), (B) wild-type mice received wild-type T cells ($n = 9$) or IL-1Ra$^{-/-}$ T cells ($n = 12$), (C) wild-type mice received wild-type T cells ($n = 23$), IL-1α/β$^{-/-}$ mice received wild-type T cells ($n = 14$) or IL-1RI$^{-/-}$ mice received wild-type T cells ($n = 13$), (D) wild-type mice received wild-type T cells ($n = 10$) or IL-1Ra$^{-/-}$ mice received wild-type T cells ($n = 10$). Circles indicate the levels for individual mice. The mean ± SD of each group is also shown. *$P < 0.05$ versus wild-type mice. (E) Mice were immunized with mBSA or left untreated. After 7 days, CD4$^+$ T cells were purified and cultured for 0, 24 or 48 h. IL-1α, IL-1β, IL-1RI and IL-1Ra mRNA expression were examined by RT-PCR. "–," non-immunized mice; N, without RT. HPRT mRNA levels served as an internal control.

after a 24-h co-culture with mBSA (Fig. 5E). These observations indicate that IL-1, primarily that derived from T cells, is responsible for the induction of local inflammation during the elicitation phase of DTH responses.

An important role for IL-1 in the activation of DCs during elicitation phase

We next analyzed the effect of T cell-derived IL-1 on the activation of antigen-presenting cells (APCs) during the

elicitation phase. We isolated $CD4^+$ T cells 7 days after the immunization of wild-type mice with mBSA. Purified cells were co-cultured with DCs from naive wild-type, IL-1$\alpha/\beta^{-/-}$, IL-1$RI^{-/-}$ and IL-1$Ra^{-/-}$ mice in the presence or absence of mBSA. mBSA-specific $CD4^+$ T cell proliferative responses were significantly reduced following co-culture with IL-1$RI^{-/-}$ DCs, while co-cultures with IL-1$\alpha/\beta^{-/-}$ DCs generated similar proliferation as that observed in wild-type DCs (Fig. 6A). T cell proliferation in the presence of IL-1$Ra^{-/-}$ DCs, however, was up-regulated (Fig. 6B). These results indicate that T cell-derived IL-1 is important for DC activation, a process that is critical for the subsequent proliferation of antigen-specific $CD4^+$ T cells.

Involvement of TNF downstream of IL-1 in the DTH response

TNF is a potent pro-inflammatory cytokine with similar biological activities to IL-1. As IL-1 deficiency only partially reduced the DTH response induced by mBSA (Fig. 1) and IL-1-deficient DCs could activate memory T cells in recall responses (Fig. 6), we examined the possible involvement of TNF in the development of type IV hypersensitivity. Footpad swelling of $TNF^{-/-}$ mice was significantly, if only slightly, reduced (~80%) from the levels seen in wild-type mice (Fig. 7A), suggesting the involvement of TNF in DTH responses. Footpad swelling in IL-1$\alpha/\beta^{-/-}TNF^{-/-}$ mice, however, was similar to that seen in IL-1$\alpha/\beta^{-/-}$ mice (~60%) (Fig. 7A). No additional effect of TNF deficiency on DTH response could be observed in IL-1$\alpha/\beta^{-/-}$ mice. The footpad swelling of IL-1$Ra^{-/-}TNF^{-/-}$ mice, however, was markedly reduced in comparison with that of IL-1$Ra^{-/-}$ mice (Fig. 7B). These results are compatible with the hypothesis that TNF is

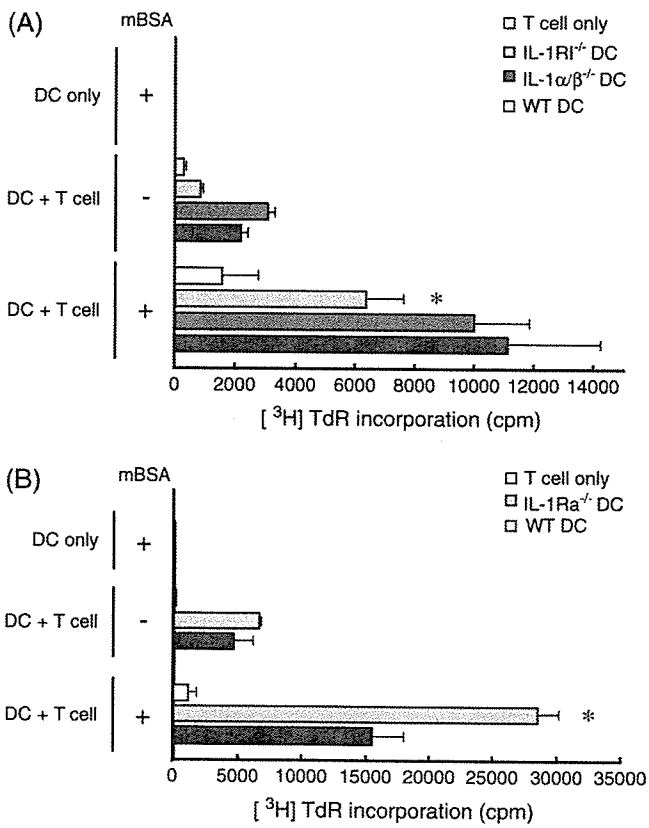

Fig. 6. Effects of DC-derived IL-1 on $CD4^+$ T cell proliferation. $CD4^+$ T cells (1×10^5 per well) from mBSA-immunized wild-type mice were co-cultured with $CD11c^+$ DC cells from a different genotype (1×10^4 per well) in the presence or absence of mBSA. The proliferative responses of T cells were measured by [^{3}H]TdR incorporation. (A) Wild type (WT), IL-1$\alpha/\beta^{-/-}$ and IL-1$RI^{-/-}$ DCs. (B) WT and IL-1$Ra^{-/-}$ DCs. [^{3}H]TdR incorporation was measured in three wells; the mean ± SD of these values is shown. Similar results were obtained in three independent experiments. $^*P < 0.05$ versus wild type.

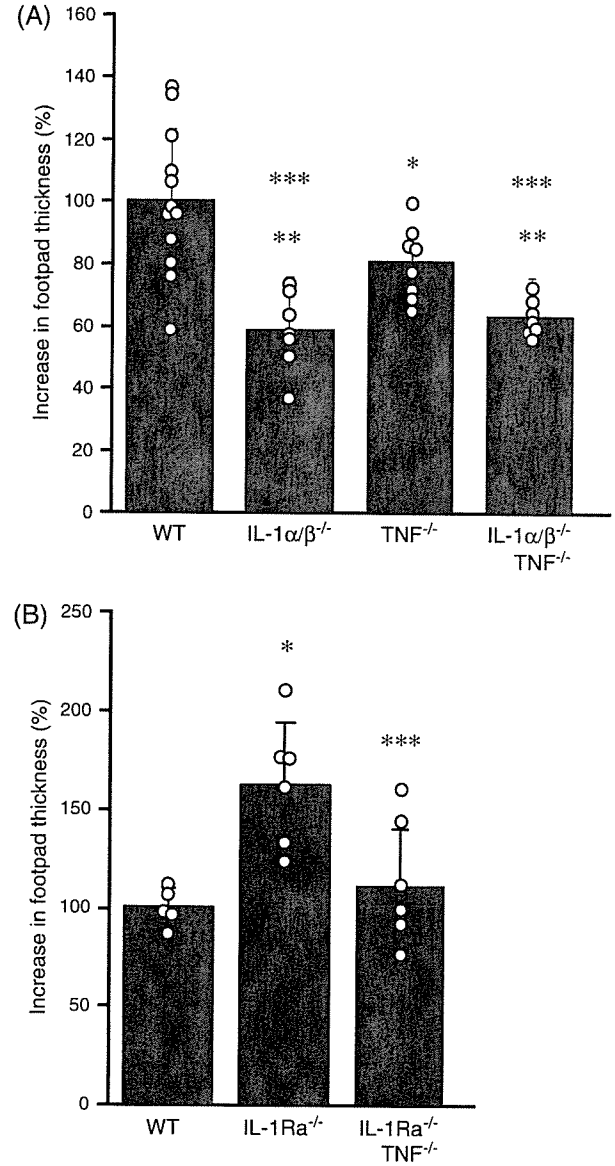

Fig. 7. Roles of IL-1 and TNF in DTH responses. mBSA-induced DTH responses in wild-type, IL-1$\alpha/\beta^{-/-}$, $TNF^{-/-}$ and IL-1$\alpha/\beta^{-/-}TNF^{-/-}$ mice (A) and in wild-type, IL-1$Ra^{-/-}$ and IL-1$Ra^{-/-}TNF^{-/-}$ mice (B) were examined as in Fig. 1. Circles indicate disease severity of individual mice. The mean ± SD of each group is shown. (A) Wild type, $n = 12$; IL-1$\alpha/\beta^{-/-}$, $n = 7$; $TNF^{-/-}$, $n = 8$ and IL-1$\alpha/\beta^{-/-}TNF^{-/-}$, $n = 7$. $^{**}P < 0.01$, $^*P < 0.05$ versus wild-type mice and # $P < 0.05$ versus $TNF^{-/-}$ mice. (B) Wild type, $n = 5$; IL-1$Ra^{-/-}$, $n = 6$ and IL-1$Ra^{-/-}TNF^{-/-}$, $n = 6$. $^*P < 0.05$ versus wild-type mice and $^{***}P < 0.05$ versus IL-1$Ra^{-/-}$ mice.

induced downstream of IL-1 signaling and a portion of the biological activities induced by IL-1 may occur through the induction of TNF.

Discussion

Using IL-1$\alpha^{-/-}$ and IL-1$\beta^{-/-}$ mice, we demonstrated that IL-1β, more so than IL-1α, plays an important role in the development of DTH responses. The proliferative recall responses of T cells derived from both IL-1$\alpha/\beta^{-/-}$ mice and IL-1$\beta^{-/-}$ mice, but not those from IL-1$\alpha^{-/-}$ mice, were significantly reduced, suggesting that IL-1β functions in T cell sensitization. With regard to the function of IL-1 in the sensitization of T cells, we previously showed that IL-1 activate T cells by inducing CD40L and OX40 expression in T cells (26). Recently, it was also shown that IL-1β signaling can activate naive T cells through activation of c-Rel (27).

The involvement of IL-1β in T cell sensitization was also suggested by the responses observed during airway hypersensitivity responses (28, 29). In contrast, IL-1α, but not IL-1β, is crucial for the CHS reaction (19), and both IL-1α and IL-1β are important for the antibody production against mBSA during DTH reaction. As IL-1α and IL-1β bind the same receptors, it seems likely that differential expression of these molecules may explain the differential roles of these molecules among different allergic reactions. In support for this idea, we showed that IL-1β is mainly produced during DTH reactions. The reason why specific IL-1 species are produced during different allergic reactions is not resolved completely. With regard to this, it was reported that different APCs, the major producer of IL-1 during T cell sensitization (30), are required for the antigen presentation during sensitization phase; DCs play a key role in DTH reactions while Langerhans cells (LCs) play an essential role in CHS reactions (3). Human DC1 cells, derived from monocytes, synthesize IL-1α but DC2 cells, derived from plasmacytoid cells, do not (31). $CD11a^{-}CC81^{-}MyD\text{-}1^{+}$ DCs from bovine afferent lymph synthesized IL-1α and stimulated both $CD4^{+}$ and $CD8^{+}$ T cells, while $CD11a^{+}CC81^{+}MyD\text{-}1^{-}$ DCs that did not synthesize IL-1α could not stimulate $CD8^{+}$ T cells (32). On the other hand, both IL-1α and IL-1β are produced in LCs (19). Thus, it is possible that $CD4^{+}$ T cells that are involved in the DTH response as well as in airway hypersensitivity are stimulated by a particular subpopulation of DCs that synthesizes only IL-1β. However, the exact mechanism for the functional discrimination between IL-1α and IL-1β remains to be elucidated.

DTH response has been reported to occur normally in IL-1$\beta^{-/-}$ mice (18). The apparent discrepancy to our results, following two points should be noted. First, the mBSA dosage and the immunization route that they used were different from that we used in the present experiments. It is possible that other cytokines may compensate the deficiency of IL-1β when antigenic stimulation is very strong. In support for this notion, Shornick *et al.* (33) demonstrated that IL-1β deficiency could be overcome either by application of very high doses of sensitizing antigen or by local intra-dermal injection of recombinant IL-1β before antigen application. Second, the genetic backgrounds of the mice used in the experiments were different between these two studies; we used IL-1$\beta^{-/-}$ mice that were backcrossed to either C57BL/6J mice or BALB/cA mice for eight generations, whereas they studied in 129 × B6 IL-1$\beta^{-/-}$ mice. It is possible that a small difference of footpad swelling could not be detected on the mixed background mice.

IFN-γ is a T_h1 cytokine and is critically involved in the development of DTH responses (34, 35). IFN-γ production by $CD4^{+}$ T cells was reduced both in IL-1$\alpha^{-/-}$ and IL-1$\beta^{-/-}$ mice after immunization with mBSA, suggesting that IL-1 induces the production of IFN-γ either by activating T cells or, in the case of IL-1α, by directly stimulating IFN-γ gene transcription as a transcription factor (36). This suppression of IFN-γ production, however, does not explain the differential sensitivity of IL-1$\alpha^{-/-}$ and IL-1$\beta^{-/-}$ mice to the DTH responses, because the IFN-γ production was similar between both mutant mice. Thus, IFN-γ production by IL-1$\alpha^{-/-}$ T cells is sufficient or some other factors compensate for the deficiency of this cytokine to induce a full magnitude DTH response. With this respect, it was reported that DTH reaction is suppressed in IFN-$\gamma^{-/-}$ mice when mice were immunized with antigen alone (37, 38). Since DTH response and pro-inflammatory cytokine expression are much enhanced in the presence of adjuvant, our results suggest that some other cytokines may compensate for the function of IFN-γ. We observed similar compensatory effects of adjuvant for the deficiency of a cytokine in airway hypersensitivity responses (26). In this connection, we examined expression of chemokines in IL-1-deficient mice. The expression levels of RANTES, IP-10 and monocyte chemoattractant protein-1 (MCP-1) were similar between IL-1$\alpha^{-/-}$ and IL-1$\beta^{-/-}$ mice in the elicitation phase. Thus, these chemokine expression levels also could not explain the difference of the DTH responses between IL-1$\alpha^{-/-}$ and IL-1$\beta^{-/-}$ mice, indicating involvement of other factors.

We also discovered that antibody production against mBSA was reduced both in IL-1$\alpha^{-/-}$ and IL-1$\beta^{-/-}$ mice, indicating that both IL-1α and IL-1β are involved in this humoral immune response. However, T cell sensitization was reduced only in IL-1$\beta^{-/-}$ mice. Thus, IL-1 may also function in the process of antibody production other than T cell sensitization. With regard to this, it is known that IL-1 activates DCs and induces maturation of DCs (39–42). IL-1 also stimulates the proliferation of splenic B cells after crossing of their surface Igs (43) and by signals through CD40 and IL-4R (44), and promotes the survival of germinal center B cells (45). IL-1 is strongly expressed in follicular DCs in the germinal center, which play important roles in affinity maturation and isotype switch of Igs through interaction with B cells (46). As another possibility, since IFN-γ plays an important role in the class switching of IgG in B cells (47, 48), the reduction of IFN-γ production in IL-1$\alpha^{-/-}$ and IL-1$\beta^{-/-}$ mice may cause reduction of antibody production against mBSA. These data also indicate that antibody levels against mBSA do not affect greatly the development of DTH, as normal DTH responses were observed in IL-1$\alpha^{-/-}$ mice. The relative independence of DTH on humoral immune responses was also suggested by Lagrange *et al.* (49).

In addition to a role in the sensitization of T cells, IL-1 is also involved in the elicitation of local inflammation during DTH responses. The development of DTH was severely suppressed following the adoptive transfer of mBSA-immunized $CD4^{+}$ T cells into IL-1$RI^{-/-}$ mice, but not IL-1$\alpha/\beta^{-/-}$ mice,

suggesting that activated $CD4^+$ T cell-derived IL-1 plays an important role in the elicitation phase. We also demonstrated that IL-1β derived from activated memory T cells is required for the activation of DCs that activate antigen-specific T cells at inflammatory sites. It is known that IL-1 enhances T-dependent immune responses by amplifying the function of DCs (50). IL-1β acts on APCs to enhance the *in vivo* proliferation of antigen-stimulated naive $CD4^+$ T cells (40), but it does not induce the expression of CD80, CD134 ligand, 4-1BBL or glucocorticoid-induced TNF (40). IL-1β induces functional maturation of DCs by inducing CD40, CD86 and MHC class II (39), although we could not detect any defects in IL-1-deficient DCs (30). In addition, IL-1β induces DCs to secrete IL-12 leading to the activation of cellular immunity (42). Thus, T cell-derived IL-1β may enhance maturation of immature DCs and activate DCs to enhance inflammation during the elicitation phase of the DTH response. These observations suggest dual functions for IL-1 in DTH reactions. During the sensitization phase, IL-1 produced by DCs activates T cells; during the elicitation phase, T cell-derived IL-1 activates DCs to enhance antigen presentation and/or cytokine production.

As IL-1 deficiency only partially reduced the DTH response, it was suggested that other factors contributed to the response. Accordingly, we examined the contribution of TNF, which plays an important role in the development of inflammation during the elicitation phase (26), and found that the elevated DTH response in $IL\text{-}1Ra^{-/-}$ mice was cancelled by TNF deficiency, indicating that the actions of IL-1 are mediated in part by TNF. Other hypersensitivity responses, collagen- and antigen-induced arthritis and experimental autoimmune encephalomyelitis, are also suppressed in $TNF^{-/-}$, $TNFRI^{-/-}$ and $TNFRII^{-/-}$ mice, indicating the importance of this cytokine (51–56). Interestingly, however, the severity of DTH in $IL\text{-}1\alpha/\beta^{-/-}TNF^{-/-}$ triple mutant mice did not differ from $IL\text{-}1^{-/-}$ mice (Fig. 7). With regard to this, we previously demonstrated that TNF production is induced downstream of IL-1 signaling and TNF is not induced in the absence of IL-1 (26). Thus, it seems unlikely that TNFs compensate for the deficiency of IL-1. In this context, several factors are suggested to be involved in the development of DTH responses. Exacerbated inflammation is observed when MCP-1 (57, 58), IL-12 and IL-18 (1, 59) are over-expressed, and on the contrary, DTH responses are suppressed in the absence of these molecules, suggesting that these molecules contribute to the DTH responses through IL-1-independent pathway. Recently, it was also demonstrated that IL-16, a chemoattractant for $CD4^+$ leukocytes (60), is induced in the inflamed footpads and the treatment with anti-IL-16 can inhibit the recruitment of not only $CD4^+$ T cells but also macrophages (24).

In summary, IL-1β, more so than IL-1α, is critical in the development of DTH responses, functioning as both a T cell co-stimulatory molecule and a pro-inflammatory cytokine. T cell-derived IL-1 contributes to the induction of local inflammation by activating DCs. Polymorphism in the genes for both IL-1β and IL-1Ra has been shown to influence both DTH responses against *Mycobacterium tuberculosis* and disease manifestations in human tuberculosis (61). Therefore, our findings may provide a rationale explaining the dependence of the disease on IL-1 gene polymorphisms, which may aid in the future development of novel therapeutics.

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Abbreviations

AP	alkaline phosphatase
APC	antigen-presenting cell
CHS	contact hypersensitivity
DC	dendritic cell
DTH	delayed-type hypersensitivity
HPRT	hypoxanthine phosphoribosyltransferase
IL-1Ra	IL-1R antagonist
IL-1RI	IL-1R type I
IL-1RII	IL-1R type II
LC	Langerhans cells
LN	lymph node
mBSA	methyl BSA
MCP-1	monocyte chemoattractant protein-1
RT	reverse transcription
r.t.	room temperature
s.c.	subcutaneously
TNF	tumor necrosis factor

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mediated by complement bypass systems. Fourth, and probably most important, the type of assay represents the wave of the future in detecting the activity of the complement system in human disease. As these more informative detection systems (like the one used in this report) come into routine clinical use, other examples of these bypass type pathways will likely be uncovered. For human diseases, these more specific and quantitative assay systems will establish which pathway of complement activation is playing a role in disease and elucidate which one to modulate with therapeutic agents.

Finally, a word of caution is in order. These bypass pathways are often not considered by investigators attempting to define the role of the complement system in disease states. For example, C4-deficient animals are widely used to rule out a contribution of the classical pathway and/or lectin pathway in mouse models of human disease. One must be wary of such interpretations in view of bypass cascades that become operative in "deficient" states. Thus the natural maturation of an antibody response to an infectious organism (i.e., to go rapidly into antibody excess) is all that is necessary to trigger these more ancient bypass pathways. Using our current methods, such pathways are not analyzed in clinical medicine or in animal models of human disease.

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The IL-23/IL-17 axis in inflammation

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IL-23 induces the differentiation of naive CD4$^+$ T cells into highly pathogenic helper T cells (Th17/Th$_{IL-17}$) that produce IL-17, IL-17F, IL-6, and TNF-α, but not IFN-γ and IL-4. Two studies in this issue of the *JCI* demonstrate that blocking IL-23 or its downstream factors IL-17 and IL-6, but not the IL-12/IFN-γ pathways, can significantly suppress disease development in animal models of inflammatory bowel disease and MS (see the related articles beginning on pages 1310 and 1317). These studies suggest that the IL-23/IL-17 pathway may be a novel therapeutic target for the treatment of chronic inflammatory diseases.

Th17/Th$_{IL-17}$ is a new CD4$^+$ helper T cell subset that produces IL-17

Upon antigenic stimulation, naive CD4$^+$ T cells differentiate into 2 subsets, Th1 and Th2 cells, characterized by different cytokine production profiles and effector functions (Figure 1). Th1 cells produce large quantities of IFN-γ and mediate cellular immunity while Th2 cells, which are involved in humoral immunity, primarily produce IL-4, IL-5, and IL-13. IL-12, a heterodimer of the p40 and p35 subunits, induces the differentiation of naive CD4$^+$ T cells into IFN-γ–producing Th1 cells through activation of STAT4. IFN-γ signals are transduced by STAT1, which activates a downstream transcription factor, T-bet, that enhances the expression of genes specific to Th1 cells. In contrast, IL-4 induces STAT6 activation, promoting the expression of GATA-3, a transcriptional factor essential for both IL-4 production and Th2 cell differentiation. Recently, it was reported that CD4$^+$ T cells isolated from the inflamed joints of patients with Lyme disease contain a subset of IL-17–producing CD4$^+$ T cells that are distinct from those producing either IL-4 or IFN-γ (Figure 1) (1). These IL-17–producing CD4$^+$ T cells were dubbed Th17 or Th$_{IL-17}$ cells (2–4).

IL-17, a proinflammatory cytokine predominantly produced by activated T cells, enhances T cell priming and stimulates fibroblasts, endothelial cells, macrophages, and epithelial cells to produce multiple proinflammatory mediators, including IL-1, IL-6, TNF-α, NOS-2, metalloproteases, and chemokines, resulting in the induction of inflammation (5, 6). IL-17 expression is increased in patients with a variety of allergic and autoimmune diseases, such as RA, MS, inflamma-

Nonstandard abbreviations used: CIA, collagen-induced arthritis; IBD, inflammatory bowel disease; IL-1Ra, IL-1 receptor antagonist; R, receptor.

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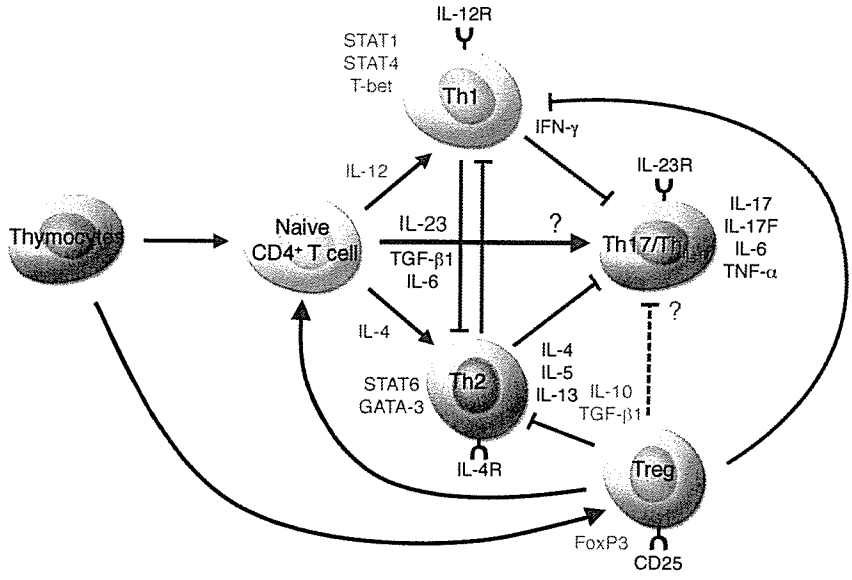

Figure 1

IL-23 promotes the development of an IL-17–producing CD4+ helper T cell subset. IL-23 induces the differentiation of naive CD4+ T cells into IL-17–producing helper T cells (Th17/Th_{IL-17}) via mechanisms that are distinct from the Th1 and Th2 differentiation pathways. The transcriptional factors critical for the development of Th1 (STAT1, STAT4, and T-bet) and Th2 (STAT6) cells are not required for the induction of Th17/Th_{IL-17} cells. The transcriptional factor(s) essential for the development of Th17/Th_{IL-17} cells remain unknown. IFN-γ and IL-4 antagonize each other in the differentiation of Th1 and Th2 cells and the promotion of their function. IFN-γ also suppresses the differentiation of Th17/Th_{IL-17} cells by reducing IL-23R expression on CD4+ T cells. IL-4 also inhibits the development of Th17/Th_{IL-17} cells. It is not known, however, whether Th17/Th_{IL-17} cells inhibit the development of Th1 and Th2 cells. Tregs, an immune-modulating subset of CD4+ T cells, suppress the differentiation and effector function of Th1 and Th2 cells. Recent studies suggest that Treg-derived TGF-β induces the differentiation of Th17/Th_{IL-17} cells from naive CD4+ T cells in the presence of IL-6 in vitro (26). However, the precise effect(s) of Tregs on Th17/Th_{IL-17} cells are as yet unknown.

tory bowel disease (IBD), and asthma, suggesting the contribution of IL-17 to the induction and/or development of such diseases. Supporting this, the involvement of this cytokine in such responses is demonstrated in animal models; autoimmune disorders such as collagen-induced arthritis (CIA) and EAE, animal models for RA and MS, respectively, as well as allergic responses such as contact hypersensitivity, delayed-type hypersensitivity, and airway hypersensitivity were suppressed in IL-17–deficient (IL-$17^{-/-}$) mice (7, 8) (Y. Komiyama et al., University of Tokyo, Tokyo, Japan, unpublished observations). Therefore, Th17/Th_{IL-17} cells are likely to play critical roles in the development of autoimmunity and allergic reactions.

The IL-23/IL-17, but not IL-12/IFN-γ, axis is critical for the development of autoimmune inflammatory diseases

The development of autoimmune diseases, such as RA, MS, and IBD, is thought to be mediated by Th1 cells because high levels of IL-12 and IFN-γ are detected in inflammatory sites (9). In addition, treatment with mAbs against IL-12p40 suppresses such disease development in humans and animal disease models (9, 10). However, mice deficient in IL-12p35, IL-12 receptor β2 (IL-12Rβ2), IFN-γ, IFN-γR, or STAT1, which are critical molecules in IL-12/IFN-γ–mediated responses, exhibit an increased severity of diseases such as EAE and CIA (11–13). These observations are inconsistent with the notion that IL-12 is responsible for such disease development. As IL-23, an IL-12 family cytokine consisting of the p19 and p40 subunits, shares the p40 subunit with IL-12 and anti-p40 mAbs inhibit both cytokines, the involvement of IL-23 is suggested. Current evidence suggests that IL-23 is responsible for the differentiation and expansion of Th17/Th_{IL-17} cells from naive CD4+ T cells (2, 4, 14).

In this issue of the *JCI*, Yen et al. report on their use of IL-23p19$^{-/-}$ and IL-12p35$^{-/-}$ mice to demonstrate that IL-23, but not IL-12, is essential for the development of intestinal inflammation (15). They used IL-$10^{-/-}$ mice as a model of T cell–mediated IBD (16) and showed that the development of colitis was greatly suppressed by IL-23p19 deficiency but not IL-12p35 deficiency. Exogenous IL-23 administration accelerated the onset of colitis in $Rag^{-/-}$ mice engrafted with IL-$10^{-/-}$CD4+ T cells. Notably, IL-17 production was abolished in IL-23p19$^{-/-}$ mice while IFN-γ and IL-4 production were unaffected. IL-17 and IL-6 expression by anti-CD3 mAb–stimulated memory CD4+ T cells were augmented by IL-23, but not by IL-12, indicating that IL-23 can simulate memory CD4+ T cells. This result contrasts with the ability of IL-12 to stimulate naive CD4+ T cells. Moreover, treatment with both anti–IL-6 and anti–IL-17 mAbs significantly ameliorated the severity of the intestinal inflammation induced by IL-23–treated $Rag^{-/-}$ mice engrafted with IL-$10^{-/-}$CD4+CD45RB^{hi} T cells. These observations suggest that IL-17 and IL-6 derived from memory T cells are responsible for the development of intestinal inflammation downstream of IL-23.

Also in this issue of the *JCI*, Chen et al. report on their use of newly developed anti–IL-23p19 mAbs to demonstrate the involvement of IL-23 in EAE (17). The authors previously demonstrated that IL-23p19$^{-/-}$ mice are resistant to EAE and CIA; production of IL-17, but not IFN-γ, is almost completely abolished in these mutant mice (3, 18, 19). In contrast, IL-23p35$^{-/-}$ mice exhibited decreased IFN-γ production and increased IL-17 production, suggesting that IFN-γ may suppress IL-17 production. Furthermore, IL-23–induced but not IL-12–induced proteolipid protein peptide–specific T cells are highly encephalogenic (3). Consistent with these reports, the development of EAE was efficiently suppressed by treatment with anti–IL-23p19 or anti–IL-12/IL-23p40 mAbs by inhibiting infiltration of IL-17–, IFN-γ–, and TNF-α–producing CD4+ T cells in the CNS. Disease severity correlated well with serum IL-17 levels; treatment with anti–IL-17 mAbs ameliorated the clinical disease score. Meanwhile, treatment with anti–IFN-γ mAbs exacerbated disease, consistent with previous observations that IFN-$γ^{-/-}$ and IFN-$γR^{-/-}$ mice are highly susceptible to EAE (12, 13). Thus, it is clearly shown that the IL-23/IL-17 pathway, rather than the IL-12/IFN-γ pathway, is critical for the development of autoimmune diseases.

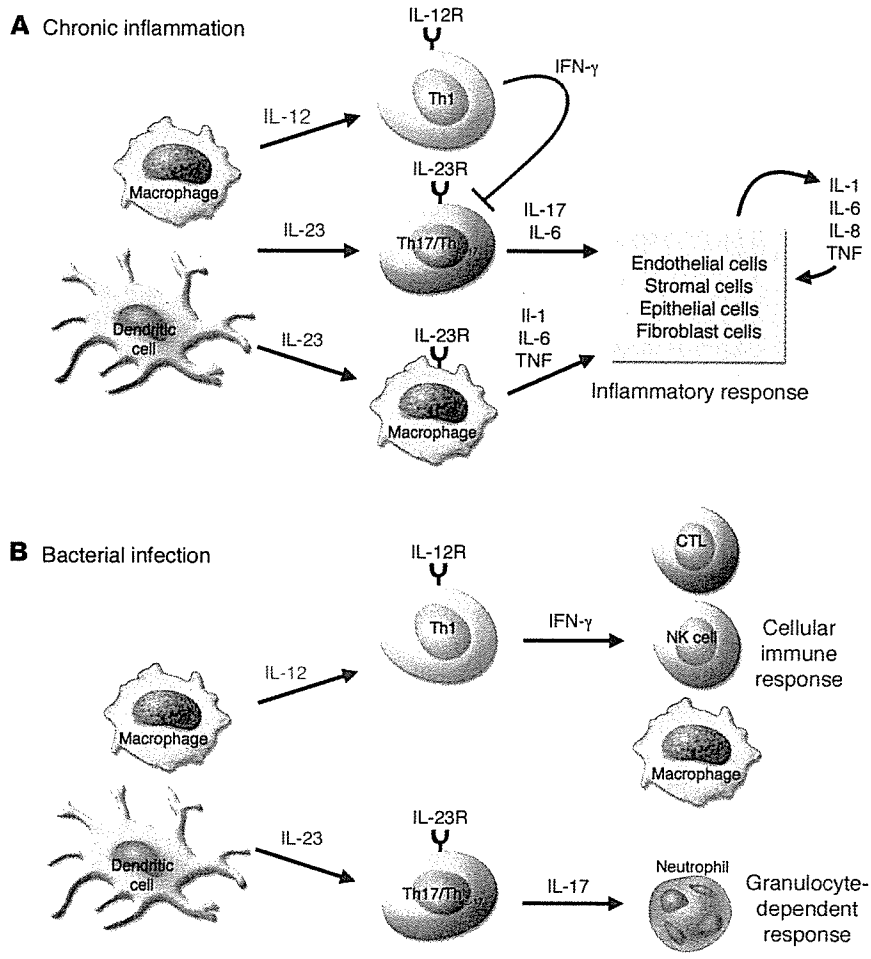

Figure 2
The role of the IL-23/IL-17 axis in inflammation and infection. The IL-23/IL-17 axis plays an important role in the development of chronic inflammation and in host defenses against bacterial infection. (**A**) In chronic inflammation, antigen-stimulated dendritic cells and macrophages produce IL-23, which promotes the development of Th17/Th$_{IL-17}$ cells. Th17/Th$_{IL-17}$ cells produce IL-17, which enhances T cell priming and triggers potent inflammatory responses by inducing the production of a variety of inflammatory mediators. IL-23 also acts on dendritic cells and macrophages in an autocrine/paracrine manner to stimulate the generation of proinflammatory cytokines, such as IL-1, IL-6, and TNF-α. IL-12–stimulated Th1 cells produce IFN-γ and suppress the differentiation of Th17/Th$_{IL-17}$ cells. Th1 cells may play an immunoregulatory, not a pathogenic, role in the development of chronic inflammation. (**B**) Upon bacterial infection, IL-23 is rapidly produced by activated macrophages and dendritic cells at the site of infection. IL-23 then activates local resident Th17/Th$_{IL-17}$ cells and other IL-17–producing cells, such as CD8$^+$ T cells and γδ T cells. Production of IL-17 by these cells induces G-CSF production from stromal cells. The IL-23/IL-17/G-CSF pathway augments neutrophil recruitment to the infection site, contributing to extracellular bacterial clearance. IL-23 also increases the production of IL-1, IL-6, and TNF-α in an autocrine/paracrine manner. In contrast, Th1 cells produce IFN-γ and stimulate CD8$^+$ cytotoxic T lymphocytes, NK cells, and macrophages. IFN-γ enhances antigen presentation by inducing expression of MHC molecules and activates cells to produce cytolytic molecules, including perforin and granzyme, which promote the elimination of intracellular bacteria.

IL-23 causes inflammation through IL-17–dependent and IL-17–independent pathways

Anti-IL-17 treatment did not prevent the onset and relapse of EAE with the same efficiency as anti-IL-23p19 or anti-IL-12/IL-23p40 treatment, suggesting that the effects of IL-23 cannot be explained by the action of IL-17 alone. We also observed that EAE progression is only partially suppressed even in IL-17$^{-/-}$ mice, suggesting the involvement of additional factors (Komiyama et al., unpublished observations). The transcription factor T-bet is responsible for enhancing IFN-γ production and increasing IL-12Rβ2 expression (20). In contrast to IFN-γ$^{-/-}$ mice, T-bet$^{-/-}$ mice are highly resistant to EAE development (11). As Th17/Th$_{IL-17}$ cells were present in T-bet$^{-/-}$ mice, Chen et al. suggested that Th17/Th$_{IL-17}$ cells are not sufficient to induce disease and that additional T-bet–dependent factors and/or cell populations play significant roles in disease pathogenesis (17). With respect to this, T-bet expression on dendritic cells, but not on T cells, is required for IL-1 and chemokine production, contributing to the development of collagen antibody-induced arthritis (21). In the current studies, Chen et al. (17) suggested that IL-23 may directly activate a subset of macrophages and dendritic cells expressing IL-23R, resulting in the production of inflammatory mediators, such as TNF-α and IL-1 (18, 22). However, as progression of diseases such as EAE and CIA is only minimally affected in TNF-α$^{-/-}$ mice and anti-TNF-α treatment of wild-type mice results in more severe MS (23), TNF-α is not critical for the development of this disease. In contrast, IL-6$^{-/-}$ and IL-1$^{-/-}$ mice are significantly resistant, and IL-1 receptor antagonist-deficient (IL-1Ra$^{-/-}$) mice are more susceptible to EAE (24, 25). Thus, IL-6 and IL-1 likely play important roles in the development of this disease. These observations suggest that IL-23 can induce chronic inflammation through 2 independent pathways: (a) activation of Th17/Th$_{IL-17}$ cells; and (b) induction of IL-1 and IL-6 production via myeloid cell activation (Figure 2).

Additional IL-17 family molecules may also play an important role in the development of inflammatory diseases. The IL-17 family currently consists of 6 family members; some of these family members, such as IL-17F, share significant amino acid homology with IL-17 (also known as IL-17A), are induced by IL-23, bind the same receptor as IL-17, and are produced by Th17/Th$_{IL-17}$ cells. Thus, it is possible that these additional IL-17 members may be involved in inflammatory responses. It is important to elucidate the functions of these IL-17 family molecules in normal physiology and in disease pathogenesis.

The Th17/Th$_{IL-17}$ differentiation mechanism is not yet known

Naive CD4$^+$ T cells isolated from mice deficient in STAT1, STAT4, or T-bet

retained the ability to differentiate into Th17/Th_{IL-17} cells in vitro following TCR stimulation in the presence of IL-23 (2). The generation of Th17/Th_{IL-17} cells following immunization with antigen stimulation was also normal in mice deficient for STAT4, STAT6, or T-bet (4). Thus, these Th1- and Th2-specific transcriptional factors are not involved in the differentiation of Th17/Th_{IL-17} cells, indicating that the Th17/Th_{IL-17} lineage is independent from these classical Th cell lineages. In this issue, Chen et al. (17) demonstrate that T-bet$^{-/-}$ lymph node cells produced IL-17 upon stimulation with anti-CD3 mAbs, consistent with previous reports (2, 4). Exogenous IL-23, however, did not further enhance IL-17 production in T-bet$^{-/-}$ lymph node cells, suggesting that T-bet may influence IL-23 responsiveness during early Th17/Th_{IL-17} development (17). Thus, the transcriptional factors involved in Th17/Th_{IL-17} cell development still remain to be elucidated (Figure 1). With regard to this, STAT3 was recently implicated in the IL-23R signaling pathway (22).

As IL-12Rβ1, the common subunit of IL-12R and IL-23R, is constitutively expressed in naive CD4$^+$ T cells, IL-12Rβ2 and IL-23R expression are critical for the responsiveness to IL-12 and IL-23 and development of Th1 and Th17/Th_{IL-17} cell lineages. Only memory and/or activated T cells express IL-23R; naive Th17/Th_{IL-17} progenitor cells are devoid of this molecule (22). However, when naive CD4$^+$ T cells were stimulated with IL-23 in the presence of anti–IL-4 and anti–IFN-γ mAbs, a large IL-17–producing population was observed, indicating that IFN-γ and IL-4 inhibit the differentiation of Th17/Th_{IL-17} cells from naive CD4$^+$ T cells. IFN-γ and STAT1 signaling inhibit the differentiation by downregulating the expression of IL-23R (2). Although IL-4 also inhibits Th17 cell expansion, the mechanism governing this suppression is not known (2, 4). Thus, the identification of the signals that induce IL-23R expression on naive CD4$^+$ T cells is crucial in elucidating the mechanisms of Th17/Th_{IL-17} cell lineage differentiation.

Recently, Veldhoen et al. reported that Treg-derived TGF-β induces the differentiation of Th17/Th_{IL-17} cells from naive CD4$^+$ T cells in the presence of IL-6 in vitro (Figure 1) (26). TGF-β–mediated Th17/Th_{IL-17} cell differentiation is promoted by dendritic cell–derived IL-1β and TNF-α. They showed that IL-23 is not essential for the development of Th17/Th_{IL-17} cells, but required for their survival and expansion through the positive feedback loop that upregulates IL-6, IL-1β, and TNF-α. Thus, current evidence provides us with 2 Th17/Th_{IL-17} cell differentiation pathways; one is IL-23 dependent and the other is IL-23 independent. Further studies are definitely required to address the precise roles of IL-23 and other factors in the development of Th17/Th_{IL-17} cells in vivo.

Concluding remarks

While the importance of IL-12 in host defense against bacteria is widely accepted, the role of IL-23 in host defense is not well understood (Figure 2). Recent studies have revealed that IL-12 and IL-23 have different roles in host defense. Mice deficient in IFN-γ, IFN-γR, or STAT1 are highly susceptible to many different pathogens, including *Leishmania major*, *Listeria monocytogenes*, and *Mycobacterium tuberculosis* (27). IL-23 and IL-17 are also important in host defenses against infection. It should be noted that IL-12/IFN-γ are primarily involved in host defenses against intracellular pathogens while IL-23/IL-17 are important for defenses against extracellular pathogens, including *Klebsiella pneumoniae* (28). This is because IFN-γ stimulates the immune system to kill intracellular bacteria and infected host cells while IL-17 recruits and activates neutrophils. The detailed host defense mechanisms involving IL-23 and IL-17, however, still remain to be elucidated.

IL-17 is produced not only by Th17/Th_{IL-17} cells, but also by activated CD8$^+$ T cells, TCRγδ$^+$ T cells, and neutrophils (5, 29). We have observed that CD4$^-$CD8$^-$TCRγδ$^+$ T cells also produce IL-17 in IL-1Ra$^{-/-}$ mice, which spontaneously develop autoimmune arthritis (Komiyama et al., unpublished observations). Development of arthritis in these mice can be completely suppressed by IL-17 deficiency (6). Thus, IL-17 production by cells distinct from Th17/Th_{IL-17} cells may also be involved in inflammatory responses and host defense mechanisms. It remains unclear, however, which of these producer cells are involved in the different allergic and infectious diseases and how the differentiation pathways of these cell lineages are controlled.

Taken together, accumulating evidence suggests that 3 independent pathways are involved in inflammatory responses: IL-12/IFN-γ, IL-4/IL-5/IL-13, and IL-23/IL-17. These pathways are largely exclusive, although the effect of Th17/Th_{IL-17} cells on Th1 and Th2 cells is not well understood. Identification of the major immune pathways responsible for the development of each disease is important for its treatment because suppression of 1 pathway may accelerate the others. Therapeutic targeting of the newly discovered IL-23/IL-17 immune axis may prove effective for the treatment of autoimmune and allergic inflammatory responses.

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