Endogenous IL-12 synthesis is not required to prevent hyperexpression of type 2 cytokine and antibody responses

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Endogenous IL-12 production is hypothesized to play an essential role preventing spontaneous expression of type 2 responses, acting as a natural inhibitor limiting development of immediate hypersensitivity. Here, IL-12-deficient p35−/− and p40−/− mice were used to examine the role of endogenous IL-12 and p40 homodimer during in vivo development of exogenous antigen-driven responses. In the absence of deliberate immunization, IL-12-deficient mice exhibited greatly reduced serum IgG2a but IgG1/IgE levels no higher than controls. Immunization to elicit polarized ovalbumin-specific type 1 or type 2 dominant responses, or using Trichinella spiralis extract in the absence of adjuvants, led to IFN-γ production of approximately 10% of C57BL/6 controls yet the kinetics and intensity of primary and secondary type 2 cytokine (IL-4, IL-5, IL-13) and antibody (IgG1, IgE) responses, as well as functional IL-12 receptor expression, were consistently unaltered. Thus, while IL-12 provides an important positive signal for Th1 development, antigen exposure in its absence does not lead to generalized enhancement of type 2 cytokine or antibody responses. The data argue that endogenous IL-12 production is not required as a constitutive negative regulator limiting induction or expression of type 2 effector responses.

Key words: Th1/Th2 activation / Allergy / Cytokine

1 Introduction

The nature of the CD4 T cell response that is elicited upon Ag exposure plays a key role in shaping the type of adaptive immune response that develops. While many factors affect initial T cell differentiation and commitment, studies over the last several years have focussed attention on the central roles that IL-4 and IL-12 play in this process [1–3]. Given the activity of IL-4 as a promoter of Th2-like commitment and the negative consequences that result from excessive production of type 2 cytokines, the induction and expression of this cytokine is very tightly controlled. The discovery that IL-4 production by CD4 T cells becomes independent of extracellular cytokines following commitment to the Th2-like phenotype [4], a situation quite distinct from IFN-γ synthesis [5], underlines the necessity of restricting initial T cell commitment to type 2 patterns.

The capacity of exogenous IL-12 to promote cytokine and effector responses characteristic of type 1 immunity is well established (for review see [6]). Endogenous IL-12 is thought to play a similar role in regulating the type 1: type 2 cytokine and effector balance by promoting commitment of naive T cells to Th1-like patterns of differentiation and, indirectly, by inhibiting Th2 development. This capacity of IL-12 to inhibit undesirable Th2 development may function both indirectly via induction of NK and Th1 cell-derived IFN-γ production and directly by inhibitory activities on Th2 cells [7–11].

Immediate hypersensitivity is the most common human immunological disease [12]. Individuals with atopic dermatitis [13, 14], asthma [15, 16] or allergic rhinitis [16, 17], the most common form of atopy, are reported to exhibit substantially reduced IL-12 synthesis and/or responsiveness relative to non-atopic controls, a finding consistent with a potential role for such defects in the pathogenesis of allergic disease. In vivo studies in mice using anti-IL-12 Ab or with strains genetically deficient in
the capacity to produce IL-12 clearly indicate a critical role for endogenous IL-12 in promoting induction of Th1 responses [6]. These data have reasonably been taken to suggest that endogenous IL-12 synthesis is also responsible for preventing development of type 2 responses [6, 11, 18–20] and, by extension, acts as a natural inhibitor of immediate hypersensitivity. While some data lend support to this hypothesis, in the majority of studies where IL-12 production or function was interrupted, IL-4 (± IL-10) was used as the sole representative of type 2 cytokine synthesis or type 2 cytokine synthesis was not examined at all [21–29]. Indeed, experimental conditions that yield only very low to undetectable IL-4 levels in wild-type controls have frequently been used [18, 21, 24, 27, 28, 30–35].

Here, we examine the impact of p35 and p40 deficiency on the development and maintenance of cytokine and Ab responses generated in C57BL/6 (B6) mice following exogenous Ag-mediated activation under conditions leading to type 1- vs. type 2-dominated responses. The data indicate that p35−/− mice, deficient in the capacity to produce p70, and p40−/− mice, lacking both p70 and p40 homodimers, exhibit reduced type 1 cytokine and Ab responses, but that neither the kinetics nor the intensity of type 2 responses are increased. Taken together, the data argue that endogenous IL-12 synthesis is not required to prevent unregulated development and amplification of type 2 responses in vivo in exogenous Ag-driven responses, nor is it absolutely required for the development of type 1 Ab responses.

2 Results

2.1 Role of endogenous IL-12 synthesis on induction of type 1 and type 2 response in vivo

To evaluate the impact of IL-12 deficiency on the initiation of exogenous Ag-specific responses in vivo, mice were immunized with OVA under conditions that preferentially lead to type 1 (100 μg OVA in CFA)- or type 2-dominated responses (2 μg OVA in alum) [36]. As shown in Fig. 1, primary culture of spleen cells from both p35−/− and p40−/− mice revealed >90% reductions in OVA-specific IFN-γ responses relative to those seen in identically immunized wild-type B6 controls. A small but reproducible IL-12-independent response was consistently seen in both strains of IL-12-deficient mice (means of 10 and 12 U/ml in p35−/− and p40−/−, respectively, over five independent experiments; IFN-γ assay detection limit was ∼0.3 U/ml). Cultures carried out in the presence of OVA and anti-CD4 mAb resulted in abrogation of IFN-γ responses in all three strains of mice. The same pattern of strikingly reduced, but not absent, IFN-γ responses was evident in OVA/CFA-immunized mice (data not shown).

Strikingly, the intensity of Ag-driven IL-4 responses in primary culture was consistently indistinguishable between B6, p35−/− and p40−/− mice (Fig. 2, top panels, ANOVA p > 0.05). As for IFN-γ, the addition of anti-CD4 mAb during culture abrogated ≥80% of cytokine synthesis in all three strains (data not shown).

Previous reports of marked enhancement in Th2-like activity are often based solely on alterations in IL-4 synthesis, often in models where IL-4 production in wild-type controls was extremely low to undetectable. We therefore examined a panel of type 2 cytokines under immunization conditions that preferentially elicit type 1 (Fig. 2, right panels) or type 2 (left panels) dominated immunity. As shown in Fig. 2, OVA (alum) immunization resulted in substantially stronger IL-4, IL-5, IL-10 and IL-13 responses than did immunization using larger amounts of Ag (100 μg) in the presence of CFA. However, regardless of whether type 2 cytokine responses were examined following dominant type 1 or type 2 activation, IL-5, IL-10 or IL-13 responses were not enhanced in either p35−/− or p40−/− mice relative to wild-type controls (p > 0.05 in all cases). In cultures without OVA, type 2 cytokine levels were undetectable in normal B6 and both of the IL-12-deficient strains, further arguing against a pronounced global type 2 bias in the absence of endogenous IL-12 synthesis (data not shown).

To evaluate cytokine responses to an unrelated Ag, mice were immunized with *Trichinella spiralis* sonicates in the absence of adjuvant and killed for culture at various times.
**Fig. 2.** Type 2 cytokine responses in IL-12-deficient mice are equivalent to those in wild-type controls. (B6 (□), p35−/− (◼) and p40−/− (▲) mice were immunized with either 2 µg OVA in alum or 100 µg OVA in CFA. Spleen cells from mice killed 5 days later were cultured with and without OVA (not shown) as described for Fig. 1 and cytokines were analyzed as described in Sect. 4.3. Data represent means ± SEM (n = 24 mice from six independent experiments, except IL-5 and IL-13, which used 20 mice in five experiments). No significant differences from B6 responses were observed (ANOVA p > 0.05 in all cases).

2.2 p35−/− and p40−/− mice do not exhibit increased total or Ag-driven type 2 Ab responses in vivo

Given that isotope switch is a very sensitive biological marker of cytokine synthesis, total serum IgG2a, IgE and IgG1 were examined in naive B6, p35−/− and p40−/− mice as a complementary way of examining the role that endogenous IL-12 production plays in regulating generation of type 2 responses. As indicated in Figs. 4 and 5 (days 0 time point), prior to immunization the mean total serum IgG2a in the 41 mice examined for each strain was approximately 90% lower in p40−/− (78 vs. 8.2 µg/ml) and approximately 50% lower in p35−/− mice (78 vs. 40 µg/ml in B6 vs. p35−/−). Most importantly, total serum IgE levels in resting naive mice (at 4 to 30 weeks of age) were not enhanced in either of the IL-12-deficient groups (49.8, 46.8 and 10.2 ng/ml for B6, p35−/− and p40−/− respectively; ANOVA p > 0.05). Similar results were found for total serum IgG1 levels (171, 183 and 115 µg/ml, p > 0.05). This is in marked contrast to the situation observed in IL-4-deficient mice where both basal and Ag-driven IgE responses were markedly reduced in the absence of IL-4 [37–39] and IgG2a was reported increased [38–40].

We next examined the impact that the absence of IL-12 p70 or p40 homodimer had on development and expression of specific Ab responses stimulated without adjuvant (Fig. 4) or following immunization with OVA under conditions that lead to type 1 (Fig. 5)- and type 2 (Fig. 6)-dominated responses. Mice immunized with *T. spiralis* extract in the absence of adjuvant developed serum IgE and IgG1 responses that were indistinguishable between times thereafter (Fig. 3). Examination of type 1 and type 2 cytokine responses revealed markedly reduced IFN-γ synthesis in IL-12-deficient mice. However, as was found following OVA immunization, IL-12-deficient B6 mice demonstrated *T. spiralis*-driven IL-4, IL-5 and IL-13 responses equivalent to wild-type controls. Mean IL-10 production was slightly elevated (p = 0.03).
Fig. 4. The absence of endogenous IL-12 markedly reduces IgG2a but does not alter IgE synthesis in mice injected with parasite Ag. B6 (●) and p35−/− (■) mice were injected with 50 μg T. spiralis extract on day 0 and boosted with the same amount on day 21. Mice were bled on days 10, 14, 28 and 35. Means ± SEM (n = 12 mice) are shown. Significant differences from B6 are indicated (p < 0.05*, 0.005**).

B6 and p35−/− mice in terms of the kinetics, duration and intensity of both primary and secondary responses. As anticipated in light of the markedly reduced IFN-γ responses seen above, IgG2a levels were significantly, and equally, reduced (~50 %) relative to B6 controls in both IL-12-deficient strains.

Following immunization under type 1-promoting conditions (Fig. 5), total and secondary OVA-specific IgG2a responses were reduced by 50–80 %. Strikingly, total and OVA-specific IgE responses in both primary and secondary responses were similar or, in the case of secondary responses, slightly lower than those of B6 controls. IgG1 production was essentially identical in all groups.

In independent experiments, mice were immunized under conditions favoring OVA-specific type 2 Ab responses. Thus, OVA (alum)-immunized with exhibited total and specific IgE responses markedly higher (~ten-fold) than those following immunization using CFA, while specific IgG2a responses were 80- to 100-fold weaker.

However, as seen above, the intensity of IgE and IgG1 responses did not differ between the three strains of mice examined, again arguing against a generalized enhancement of type 2 activation in the absence of endogenous IL-12 synthesis. These data also indicate that such deficiencies in IL-12 production have equivalent impact on the development of exogenous Ag-specific Ab responses under type 1- and type 2-dominant conditions.
In vivo activation of IL-12–/– and B6 spleen cells with OVA and exogenous IL-12 stimulates intense, equivalent IFN-γ responses

IL-4 and IFN-γ [41], as well as IL-12 itself [42], alters IL-12 responsiveness of T cells by modulating expression of the IL-12R β2 subunit. Given that IL-12–/– mice exhibit > 90% reductions in IFN-γ and IL-12 production upon stimulation with antigenic or polyclonal stimuli, and that cytokine function is frequently regulated in an autocrine manner, we examined IL-12 responsiveness by these mice. As shown in Fig. 7, mice were immunized with OVA (alum), killed 5 days later and their spleen cells cultured in the presence and absence of rIL-12 with and without OVA. In the absence of rIL-12, the mean OVA-dependent IFN-γ response in B6 mice was sevenfold that of either IL-12-deficient strain. Addition of rIL-12 (± rIL-2) resulted in increased IFN-γ responses of similar intensity in B6 and IL-12-deficient strains. Moreover, no difference was detectable in the capacity of IL-12 to activate OVA-driven IFN-γ synthesis in mice lacking p40 homodimer, arguing against a regulatory role for (p40)2 under these conditions. Finally, the intensity of the Ag-independent IFN-γ responses observed, primarily attributable to NK cell activation ([6, 43] and data not shown), were also indistinguishable in B6 and both strains of IL-12-deficient mice.

3 Discussion

Administration of exogenous IL-12 potently inhibits the development of primary IgE responses in vivo (for review see [6]). In light of observations that atopic humans may exhibit substantially reduced IL-12 responses [13–17] and reports of anti-IL-12-treated or IL-12 knockout mice exhibiting enhanced IL-4 synthesis [6, 18, 20, 31, 44], we hypothesized that endogenous IL-12 plays an essential role in preventing spontaneous and unrestricted development of type 2 responses in vivo. However, while we confirm that endogenous IL-12 synthesis is important for promotion of normal type 1 responses, we find that its absence does not lead to general activation of either type 2 cytokine or Ab responses in vivo, either in resting
mice or following immunization with exogenous Ag under a variety of conditions.

Notwithstanding the frequently cited perception that endogenous IL-12 production acts as a constitutive negative regulator of Th2 induction, examination of the primary data obtained following disruption of IL-12 production or receptor expression reveals a complex picture. In many studies, IL-4 and/or IgE production was not examined or was not shown. In others, the immunization methods selected were such that IL-4 responses in wild-type controls were at or below the detection limits of the assays used. Comparison of IL-4 production under such conditions can indicate several fold increases as a result of extremely small denominators rather than due to substantive IL-4 production. It is well established that the optimal conditions for Ag-driven activation of IL-4 and IgE production in vivo differ from those that stimulate Th0 or Th1-like responses [36]. Indeed, rIL-12 has demonstrated a capacity to potentiate, rather than inhibit, human or murine IL-4 responses ([45] and Rempel, Wang and HayGlass, manuscript submitted).

Given the evidence that IL-4 is a potent negative regulator of IL-12Rβ2 expression and that IL-12R expression is a key point of regulation governing the stability or reversibility of T cells during primary activation [2, 3], the observation that IL-12-deficient mice exhibit the same level of responsiveness to rIL-12 stimulation (Fig. 7) further supports the assertion that in vivo responses in these mice are not characterized by hyperexpression of type 2 immunity. This observation of maintained IL-12 responsiveness despite the lack of endogenous IL-12 synthesis parallels a similar finding in alloantigen-driven responses where in vitro addition of exogenous IL-12 led to equivalent enhancement of IFN-γ responses by B6, p35−/− and p40−/− mice [11].

Our conclusion that the failure of B6 mice to mount endogenous IL-12 responses upon environmental Ag exposure (i.e., analysis of cytokine and basal Ig levels without deliberate immunization) or following exogenous Ag challenge (OVA or T. spiralis extract) does not lead to generalized hyperexpression of type 2 cytokine or Ab responses is supported by a few studies in unrelated systems. Anguita et al. [30] found that administration of anti-IL-12 Ab to C3H mice over the course of lyme borreliosis infection greatly reduced type 1 activation but failed to enhance either IL-4 or serum IgE. Similarly, Engwerda et al. [46] observed no increase in IL-4 following anti-IL-12 treatment of Leishmania donovani-infected BALB/c mice. While one might argue that these represent exceptions, reflecting incomplete neutralization of endogenous IL-12 synthesis in vivo, two recent studies examining the impact of mycobacterial infection in IL-12−/− models found that IL-4 responses were not enhanced in the absence of endogenous IL-12 production [32, 35]. Other representative type 2 cytokines, or IgE vs. IgG2a effector responses, were not examined in these studies. Schijns et al. [34] report that there was minimal change in IL-4 responses (IL-5, -10, -13 not examined) following infection of IL-12-deficient mice with a hepatotropic coronavirus (an RNA virus) or a neurotropic pseudorabies virus (DNA virus). Taken together, the data suggest that a wide variety of Ag, capable of stimulating type 1- or type 2-dominated responses by endogenous or exogenous pathways, do not elicit hyperexpression of type 2 responses in the absence of IL-12 production.

At the same time, in contrast to our findings, Keane-Myers et al. [44] observed that anti-IL-12 administration at the time of Ag challenge resulted in 35–50% reductions in IFN-γ synthesis that were associated with twofold increases in IL-4 and an approximately 75% increase in total IgE responses in C3H mice (p < 0.05 in both instances). Anti-IL-12-treated A/J mice did not exhibit changes in either of these parameters. The reasons underlying the differences between C3H and A/J mice, or the B6 mice used in this report remain unclear. Given the variety of strains that have been studied, including those that are “inherently” biased towards development of type 1 (i.e., B6) or type 2 (i.e. BALB/c) responses, the reasons underlying the differences are unlikely to be attributable solely to strain differences.

Both p40- and p35-deficient mice were utilized in this study. The rationale was that while both are deficient in p70 production, p35-deficient mice continue to express substantial levels of serum p40 and (p40), [31]. Functionally, while p40-deficient mice are highly susceptible to Listeria or Cryptococcus neoformans infection, p35-deficient mice can respond to infection by sterile elimination of the bacteria despite their inability to produce p70 [6]. Moreover, in a model of alloreactive Th1 development, p35−/− mice exhibited substantially stronger IFN-γ synthesis than did p40−/− mice as a result of (p40)− dependent promotion of CD8 type 1 commitment [11, 24]. However, we found that OVA-driven responses were consistently indistinguishable between mice lacking p35 (hence p70) or p40 (thus both p70 and p40 homodimer) when comparing either the levels of IFN-γ synthesis or the intensity of type 2 (IL-4, IL-5, IL-10, IL-13) responses under both type 1- and type 2-dominant conditions. The similarity of p35−/− and p40−/− mice was also apparent in serum Ab responses. Thus, in contrast to allostimulation or infectious disease systems, we saw no evidence of a role for endogenous p40/(p40)2 synthesis in shaping development or expression of exogenous Ag-specific responses.
It should be noted that in both p35- and p40-deficient mice, residual antigen-dependent IFN-γ responses that were approximately 10% of those seen in normal controls were observed, consistent with most prior reports. Similarly, while reductions in total and specific IgG2a levels were clear in IL-12-deficient mice, the residual responses were significant. These mice clearly mount substantial Ag-specific primary and secondary IgG2a responses (Figs. 5, 6). Collectively, these data suggest that IL-12 plays an important but nonessential role in development of type 1 cytokine and Ab responses. While the role of IL-12-independent pathways in inducing type 1 immune responses remains to be fully determined, we note that Takeda et al. [47] demonstrate more intense reductions in type 1 responses in IL-12−/−IL18−/− mice than are seen in mice deficient in IL-12 or IL-18 alone. While IL-18 does not itself directly drive Th1 development, instead synergizing with IL-12 in acting on primed Th1-like cells, the finding that functional deletion of IL-18 from IL-12-deficient mice had additional effects on type 1 commitment suggests that the Th1 response can be initiated by IL-18 independently of IL-12. No type 2 cytokine or Ab responses to Ag were evaluated [47].

Inhibition of IL-4 production is the key step in preventing excessive commitment to type 2 cytokine synthesis, Ab responses and the resulting clinical consequences. While it is widely recognized that IL-4 gene expression is tightly controlled, and the data above indicate that IL-12 does not play an essential role in this process, the means by which hyperexpression of type 2 cytokine and Ab responses is prevented following exposure to environmental Ag remains to be determined. In light of the fact that IL-12 production is absent and IFN-γ reduced by approximately 90% in this model, it suggests that the main checkpoints for control of IL-4 and type 2 responses may be primarily intrinsic (i.e., based on up-regulation of IFN-4 signaling that secondarily induces extinction of IL-12 signaling [48]) rather than extrinsic ones that are dependent upon constitutive, inhibitory type 1 (IL-12 ± IFN-γ) cytokine synthesis. At the same time, it cannot be excluded that such knockout mice may have generated alternative compensatory mechanisms for regulation of the type 2 response as a result of the chronic absence of IL-12.

4 Materials and methods

4.1 Mice and immunization

C57BL/6 mice were bred at the University of Manitoba breeding facility (Winnipeg, MB, Canada) or purchased from Charles River Canada. p35−/− and p40−/− mice on a B6 background were purchased from Jackson Laboratories and bred at the University of Manitoba, also under SPF conditions. All mice were used in strict accordance with guidelines issued by the Canadian Council on Animal Care. Mice were immunized on days 0 and 21 by i.p. injection of 2.0 μg OVA absorbed onto 2 mg Al(OH)3, adjuvant (alum) or 100 μg OVA in CFA. In subsequent experiments, mice were challenged with 50 μg T. spiralis whole body extract in the absence of adjuvant, also on days 0 and 21. This extract was prepared by mechanical disruption of T. spiralis L1 muscle larvae in 1% sucrose, Tris-HCl followed by centrifugation at 100,000 × g for 60 min, with the supernatant used as the soluble Ag fraction [49].

4.2 Determination of serum Ab responses

Mice were bled from the tail 10 and 14 days after primary immunization and 7 and 14 days after Ag booster to determine total and Ag-specific serum Ig levels. OVA-specific IgE was determined by 48 h passive cutaneous anaphylaxis, with IgG1 and IgG2a quantified by ELISA as previously described [50]. Total IgE was determined by ELISA using rat anti-mouse IgE (Southern Biotechnology Associates Inc., Birmingham, AL) followed by biotinylated ε-specific rat anti-mouse IgE heavy chain mAb (SeroTec, GB) and streptavidin-alkaline phosphatase. IgE levels were calibrated against purified anti-DNP mouse IgE prepared from hybridoma 26.82 (a gift of Dr. A. Froese, University of Manitoba). Total murine IgG1 and IgG2a were determined using sheep anti-mouse IgG (Jackson ImmunoResearch, Mississauga, Canada) for capture followed by biotinylated goat anti-mouse IgG or IgG2a (Southern Biotechnology Associates Inc.) Internal mAB standards of mouse IgG1 (MAB2) and IgG2a (PK136) culture supernatants were calibrated against purified IgG1 mAb generated by Dr. G. Lang (University of Manitoba, Winnipeg, Canada) and IgG2a (UPC 10, Sigma-Aldrich). The sensitivity of these assays was typically 0.5 ng/ml for IgE, 0.5 ng/ml for IgG1 and 0.1 ng/ml for IgG2a.

4.3 Cell culture

Spleen cells from OVA-immunized mice were cultured at 7.5 × 10^5/ml (2 ml/well) alone or with OVA (0.3, 1.0 mg/ml). Spleen cells from mice injected with T. spiralis sonicate were cultured in the presence of medium, 20 or 100 μg/ml Ag. In some experiments, immobilized anti-CD3 (2C11) or Con A (2 μg/ml) were used as stimuli. Purified anti-CD4 mAb from YTS 191.1 tissue culture supernatant or normal rat IgG, rIL-12 (100 μg/ml) and/or IL-2 (10 U/ml) were added to some cultures (see results). In most experiments mice were killed 5 days after immunization, the time of peak Ag-driven cytokine synthesis. Culture supernatants were harvested for analysis of IL-4 (24 h), IFN-γ (48 h), IL-5, IL-13 (72 h) and IL-10 (96 h), times corresponding to maximum cytokine levels. For each experiment, two to four mice/group/time point were killed, independently cultured in duplicate and analyzed.
4.4 Cytokine analyses

IL-4 levels were determined in an assay using CT.4S cells as previously described [51] and, in most instances, were confirmed in ELISA using 11B11 capture and biotinylated BV66-24G2 as development mAb (PharMingen), IL-5 (PharMingen) and IL-13 (R&D Systems) were similarly assessed using cytokine-specific mAb. IFN-γ and IL-10 were determined in ELISA as previously described [51]. Detection limits for these assays were 0.15 U/ml for IFN-γ, 0.2 U/ml for IL-4, 10 pg/ml for IL-5, 0.3 U/ml for IL-10 and 5 pg/ml for IL-13. All samples were evaluated in at least two assays, with the concentration of each sample calculated from a minimum of three points that fell on the linear portion of titration curves established with recombinant cytokine standards serially diluted on each plate. Inter-assay standard errors ranged from 5% to 10% in most cases.

4.5 Statistical analysis

Ag-specific IgE levels, determined as passive cutaneous anaphylaxis titers, were log2 transformed and are presented as geometric means. Data for all other parameters are expressed as mean ± SEM. Statistical significance was determined using the unpaired two-tailed Student’s t-test or, where appropriate, ANOVA followed by Tukey’s multiple comparison test.

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References

1 Constant, S. L. and Bottomly, K., Induction of Th1 and Th2 CD4+ T cell responses: the alternative approaches. Annu. Rev. Immunol. 1997. 15: 297–322.
2 Murphy, K. M., T lymphocyte differentiation in the periphery. Curr. Opin. Immunol. 1998. 10: 226–232.
3 O’Garra, A., Cytokines induce the development of functionally heterogeneous T helper cell subsets. Immunity 1999. B: 275–283.
4 Huang, H., Hu Li, J., Chen, H., Ben-Sasson, S. Z. and Paul, W. E., IL-4 and IL-13 production in differentiated T helper type 2 cells is not IL-4 dependent. J. Immunol. 1997. 158: 3731–3738.
5 Yang, X. and HayGlass, K. T., Interferon gamma, but not IL-4, synthesis by antigen primed murine T cells is IL-2 dependent. Differential requirements in de novo and mature responses. J. Immunol. 1993. 150: 4354–4363.
6 Gatley, M. K., Renzetti, L. M., Magram, J., Stern, A. S., Adorini, L., Gubler, U. and Presky, D. H., The IL-12/IL-12-Receptor System: role in normal and pathologic immune responses. Annu. Rev. Immunol. 1998. 16: 495–521.
7 Fitch, F. W., McKiscic, M. D., Ancaki, D. W. and Gajewski, T. F., Differential regulation of murine T lymphocyte subsets. Annu. Rev. Immunol. 1993. 11: 29–48.
8 Seder, R. A., Gazzinelli, R., Sher, A. and Paul, W. E., Interleukin 12 acts directly on CD4+ T cells to enhance priming for IFNγ production and diminished IL4 inhibition of such priming. Proc. Natl. Acad. Sci. USA 1993. 90: 10188–10192.
9 Marshall, J. D., Secrist, H., DeKruijff, R. H., Wolf, S. F. and Umemtsu, D. T., IL-12 inhibits the production of IL-4 and IL-10 in allergen-specific human CD4+ T lymphocytes. J. Immunol. 1995. 155: 111–117.
10 DeKruijff, R. H., Fang, Y., Wolf, S. F. and Umemtsu, D. T., IL-12 inhibits IL-4 synthesis in keyhole limpet hemocyanin-primed CD4+ T cells through an effect on antigen-presenting cells. J. Immunol. 1995. 154: 2578–2587.
11 Piccotti, J. R., Li, K., Chan, S. Y., Ferrante, J., Magram, J., Eichwald, E. J. and Bishop, D. K., Allotiotigen-reactive Th1 development in IL-12-deficient mice. J. Immunol. 1998. 160: 1132–1138.
12 HayGlass, K. T., Immunotherapy of Allergic Diseases. In Delves, P. J. and Roitt, I. (Eds.) Encyclopedia of Immunology. Academic Press, London 1999. pp 1353–1356.
13 Lester, M. R., Hofer, M. F., Gately, M., Trumble, A. and Leung, D. Y., Down-regulating effects of IL-4 and IL-10 on the IFNγ response in atopic dermatitis. J. Immunol. 1995. 154: 6174–6181.
14 Konig, B., Neuber, K. and Konig, W., Responsiveness of peripheral blood mononuclear cells from normal and atopic donors to microbial superantigens. Int. Arch. Allergy Immunol. 1995. 106: 124–133.
15 Naseer, T., Minshall, E. M., Leung, D. Y., Laberge, S., Ernst, P., Martin, R. J. and Hamid, O., Expression of IL-12 and IL-13 mRNA in asthma and their modulation in response to steroid therapy. Am. J. Respir. Crit. Care Med. 1997. 155: 845–851.
16 van der Pouw-Kraan, T. C., Boeije, L. C., de-Groot, E. R., Stapel, S. O., Snijders, A., Kapsenberg, M. L., v.d. Zee, J. S. and Aarden, L. A., Reduced production of IL-12 and IL-12-dependent IFNγ release in patients with allergic asthma. J. Immunol. 1997. 158: 5560–5565.
17 HayGlass, K. T., Li, Y., Rempel, J. D., Wang, M. and Simons, F. E., Exogenous IL-12 and directed induction of human and murine Th1-associated responses: IL-12 and atopic immune responses. Int. Arch. Allergy Immunol. 1997. 113: 281–283.
18 Heinzel, F. P., Rerko, R. M., Ahmed, F. and Pearlman, E., Endogenous IL-12 is required for control of Th2 cytokine responses capable of exacerbating leishmaniasis in normally resistant mice. J. Immunol. 1998. 160: 730–739.
19 Hofstra, C. L., Van Ark, I., Hofman, G., Kool, M., Nijkamp, F. P. and Van Oosterhout, A. J., Prevention of Th2-like cell responses by coadministration of IL-12 and IL-18 is associated with inhibition of antigen-induced airway hyper-responsiveness, eosinophilia, and serum IgE levels. J. Immunol. 1998. 161: 5054–5060.
20 Magram, J., Connaughton, S. E., Warrier, R. R., Carvajal, D. M., Wu, C. Y., Ferrante, J., Steart, C., Sarmiento, U., Faherty, D. A. and Gately, M. K., IL-12-deficient mice are defective in IFNγ production and Type 1 cytokine responses. Immunity 1996. 4: 471–481.
21 Gazzinelli, R. T., Wysocka, M., Hayashi, S., Denkers, E. Y., Hieny, S., Caspar, P., Trinchieri, G. and Sher, A., Parasite-induced IL-12 stimulates early IFNγ synthesis and resistance during acute infection with Toxoplasma gondii. J. Immunol. 1994. 153: 2533–2543.
22 Heinzl, F. P., Hujer, A. M., Ahmed, F. N. and Rerko, R. M., In vivo production and function of IL-12 p40 homodimers. J. Immunol. 1997. 158: 4381–4388.
23 Neurath, M. F., Fuss, I., Kelsall, B. L., Stüber, E. and Strober, W., Antibodies to Interleukin 12 abrogate established experimental colitis in mice. J. Exp. Med. 1995. 182: 1281–1290.
24 Piccotti, J. R., Chan, S. Y., Li, K., Eichwald, E. J. and Bishop, D. K., Differential effects of IL-12 receptor blockade with IL-12.
p40 homodimer on the induction of CD4+ and CD8+ IFNγ-producing cells. J. Immunol. 1997. 158: 643–648.

25. Rao, K. L., Varalakshmi, C., Ali, A. M. and Khar, A., Administration of anti-IL-12 antibody in vivo inhibits rejection of a rat histiocytoma and suppresses cytokine response in a tumour-bearing host. J. Immunol. 1997. 92: 381–387.

26. Brewer, J. M., Tetley, L., Richmond, J., Liew, F. Y. and Alexander, J., Lipid vesicle size determines the Th1 or Th2 response to en triggered antengen. J. Immunol. 1998. 161: 4000–4007.

27. Wu, C., Ferrante, J., Gately, M. K. and Magram, J., Characterization of IL-12 receptor beta1 chain (IL-12Rbeta1)-deficient mice: IL-12Rbeta1 is an essential component of the functional mouse IL-12 receptor. J. Immunol. 1997. 159: 1658–1665.

28. Altare, F., Durandy, A., Lamas, D., Emile, J. F., Lamhamedi, S., Le Deist, F., Drysdale, P., Jouanguy, E., Doffinger, R., Bernaudin, F., Jeppsson, O., Gollob, J., Meinl, E., Segal, A. W., Fischer, A., Kumararatne, D. and Casanova, J. L., Impairment of mycobacterial immunity in human interleukin-12 receptor deficiency. Science 1998. 280: 1432–1435.

29. Noben-Trauth, N., Kropf, P. and Müller, I., Effect of anti-interleukin 12 treatment on murine lyme borreliosis. J. Exp. Med. 1998. 182: 1593–1597.

30. Fischer, A., Kumararatne, D. and Casanova, J. L., and Ottenhoff, T. H., Resistance to antigen-induced airway hyperresponsiveness requires endogenous production of IL-12. J. Immunol. 1998. 161: 919–926.

31. Yang, X. and HayGlass, K. T., M., Resistance to antigen-induced airway hyperresponsiveness requires endogenous production of IL-12. J. Immunol. 1998. 161: 1411–1415.

32. Anguita, J., Persing, D. H., Rincón, M., Barthold, S. W. and Fligik, E., Effect of anti-interleukin 12 treatment on murine typhoid. J. Clin. Invest. 1998. 97: 1028–1034.

33. Mattner, F., Magram, J., Ferrante, J., Launois, P., Di Padova, K., Gehin, R., Gately, M. K., Louise, J. A. and Alber, G., Genetically resistant mice lacking interleukin-12 receptor-2 are susceptible to infection with Leishmania major and mount a polarized Th2 cell response. Eur. J. Immunol. 1998. 28: 1553–1559.

34. Cooper, A. M., Magram, J., Ferrante, J. and Orme, I. M., Interleukin-12 (IL-12) is crucial to the development of protective immunity in mice intravenously infected with Mycobacterium tuberculosis. J. Exp. Med. 1997. 186: 39–45.

35. Yoshimoto, T., Wang, C. R., Yoneo, T., Waki, S., Sunaga, S., Komagata, Y., Mitsuyama, M., Miqazaki, J. and Nariuchi, H., Reduced T helper 1 responses in IL-12 p40 transgenic mice. J. Immunol. 1998. 160: 588–594.

36. Schijns, V. E. C. J., Haagmans, B. L., Wierda, C. M., Kruithof, B., Heijnjen, I. A., Alber, G. and Horzinek, M. C., Macrophage modified antigen preferentially elicits induction of Th1-like cytokine synthesis patterns in vivo. J. Immunol. 1998. 160: 6101–6111.

37. Yang, X. and HayGlass, K. T., Allergen dependent induction of interleukin-4 synthesis in vivo. Immunology 1993. 78: 74–79.

38. Kuhn, R., Rajewsky, K. and Muller, W., Generation and analysis of interleukin-4 deficient mice. Science 1991. 254: 707–710.

39. Noben-Trauth, N., Kropf, P. and Müller, I., Susceptibility to Leishmania major infection in interleukin-4-deficient mice. Science 1996. 271: 982–990.

40. Kropf, P., Etges, R., Schopf, L., Chung, C., Sypek, J. and Müller, I., Characterization of T cell-mediated responses in non-healing and healing Leishmania major infections in the absence of endogenous IL-4. J. Immunol. 1997. 159: 3434–3443.

41. Schuler, T., Qin, Z., Ibe, S., Noben-Trauth, N. and Blankenstein, T., Helper cell type1-associated and cytokotoxic T lymphocyte-mediated tumor immunity is impaired in interleukin-4-deficient mice. J. Exp. Med. 1999. 189: 603–810.

42. Szabo, S. J., Jacobson, N. G., Dighe, A. S., Gubler, U. and Murphy, K. M., Developmental commitment to the Th2 lineage by extinction of IL-12 signaling. Immunity 1995. 2: 665–675.

43. Chang, J. T., Shevach, E. M. and Segal, B. M., Regulation of interleukin(IL)-12 receptor β2 subunit expression by endogenous IL-12: A critical step in the differentiation of pathogenic autoreactive T cells. J. Exp. Med. 1999. 189: 969–978.

44. Rempel, J. D., Wang, M. D. and HayGlass, K. T., In vivo IL-12 administration induces profound but transient commitment to T helper type 1-associated patterns of cytokine and antibody production. J. Immunol. 1997. 159: 1490–1496.

45. Keane-Myers, A., Wysocka, M., Trinchieri, G. and Wills-Karp, M., Resistance to antigen-induced airway hyperresponsiveness requires endogenous production of IL-12. J. Immunol. 1998. 161: 919–926.

46. In vitro maturation of human neonatal CD4 T lymphocytes. I. Induction of IL-4 producing cells after long-term culture in the presence of IL-4 plus either IL-2 or IL-7. J. Immunol. 1994. 152: 1141–1153.

47. Anguita, J., Persing, D. H., Rincón, M., Barthold, S. W. and Fligik, E., Effect of anti-interleukin 12 treatment on murine typhoid. J. Clin. Invest. 1998. 97: 1028–1034.

48. Takeda, K., Tsutsui, H., Yoshimoto, T., Adachi, O., Yoshida, N., Kishimoto, T., Okamura, H., Nakanishi, K. and Akira, S., Defective NK cell activity and Th1 response in IL-18-deficient mice. Immunity 1998. 8: 383–390.

49. Wu, C. Y., Demeure, C. E., Gately, M., Podlaski, F., Yssel, H., Kiniwa, M. and Delespesse, G., H. Histoplasma capsulatum infection with Trichinella spiralis. Exp. Parasitol. 1993. 76: 303–411.

50. HayGlass, K. T. and Stefura, W. P., Anti-IFNγ treatment blocks the ability of glutaraldehyde polymerized allergens to inhibit specific IgE responses. J. Exp. Med. 1991. 173: 279–285.

51. Yang, X., Gieni, R., Mosmann, T. R. and HayGlass, K. T., Chemically modified antigen preferentially elicits induction of Th1-like cytokine synthesis patterns in vivo. J. Exp. Med. 1993. 178: 349–453.

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