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Mice Lacking Bioactive IL-12 Can Generate Protective, Antigen-Specific Cellular Responses to Mycobacterial Infection Only if the IL-12 p40 Subunit Is Present[1]

Andrea M. Cooper,[2]* Andre Kipnis,* Joanne Turner,* Jeanne Magram,[†] Jessica Ferrante,[†] and Ian M. Orme*

Recent evidence suggests that absence of the IL-12p40 subunit is more detrimental to the generation of protective responses than is the absence of the p35 subunit. To determine whether this is the case in tuberculosis, both p35 and p40 knockout mice were infected with *Mycobacterium tuberculosis*. Mice lacking the p40 subunit were highly susceptible to increased bacterial growth, exhibited reduced production of IFN-γ, and had increased mortality. In contrast, mice lacking the p35 subunit exhibited a moderate ability to control bacterial growth, were able to generate Ag-specific IFN-γ responses, and survived infection longer. The superior Ag-specific responses of the p35 gene-disrupted mice, when compared with the p40 gene-disrupted mice, suggest that the p40 subunit may act other than as a component of IL-12. A candidate molecule capable of driving the protective responses in the p35 gene-disrupted mice is the novel cytokine IL-23. This cytokine is composed of the IL-12 p40 subunit and a p19 subunit. In support of a role for this cytokine in protective responses to *M. tuberculosis*, we determined that the p19 subunit is induced in the lungs of infected mice. *The Journal of Immunology*, **2002, 168: 1322–1327.**

Production of IFN-γ is essential in the control of *Mycobacterium tuberculosis*; in its absence, mice are acutely susceptible to both low dose aerogenic infection and high dose i.v. infection (1, 2). In addition, humans lacking the ability to signal via the IFN-γ pathway are acutely susceptible to the most avirulent mycobacteria (3–5). The generation of large amounts of IFN-γ at the site of infection is considered dependent upon Ag-specific lymphocytes. These cells are known to be reliant upon the cytokine IL-12 for their differentiation into an IFN-γ-producing phenotype (6–8).

The importance of IL-12 in generation of the protective response to tuberculosis has been demonstrated indirectly in the aerosol model of murine infection (9) and both indirectly and directly in the high dose i.v. model (10, 11). As expected, in the absence of IL-12, reduced IFN-γ production is observed (10), while the addition of excess IL-12 improves Ag-specific, IFN-γ production (9). It has also been clearly shown that the protective role of IL-12 in mycobacterial infection cannot occur in the absence of IFN-γ (11).

IL-12 is a heterodimeric cytokine made up of two disulfide-linked subunits (p35 and p40 (12)) that acts through a high affinity receptor complex made up of IL-12Rβ1 and β2 (13). Neither subunit is thought to act alone; however, the p40 subunit can form a homodimer with affinity for the IL-12R complex (14, 15). The p40 homodimer is considered an antagonist of the bioactive IL-12 p70 molecule due to its ability to bind the receptor complex without inducing intracellular signals (14, 15). Recent enigmatic data have suggested, however, that the p40 molecule is able to mediate some IL-12-associated effects in the absence of the p35 subunit (16, 17). In particular, mice lacking either the p35 or the p40 subunit differ in their ability to control various infectious diseases (18, 19).

These observed differences suggest that the absence of the IL-12 p40 subunit may result in increased susceptibility to tuberculosis when compared with the risk associated with the absence of the p35 subunit. To test this hypothesis, mice lacking either subunit were infected via the aerosol route with virulent *M. tuberculosis*, and the progression of disease was monitored by enumeration of bacteria, histological analysis, and assessment of Ag-specific responses. Mice lacking the ability to make bioactive IL-12 were more susceptible to pulmonary tuberculosis than were control mice. It was clearly observed, however, that the mice lacking the p40 subunit were more susceptible than those lacking the p35 subunit, and that this correlated with the increased ability of the p35 gene-disrupted (knockout (KO)[3]) mice to generate Ag-specific IFN-γ. Recently, a new cytokine, IL-23, has been identified that is composed of the p40 subunit and a novel, p19 subunit (20). This cytokine may be responsible for the increased survival in the p35 KO mice, and indeed its expression is up-regulated in tuberculosis-affected murine lungs.

Materials and Methods

Mice

IL-12 p40 gene-disrupted mice (p40 KO) and IL-12 p35 gene-disrupted mice were generated as described (21, 22) and were either bred at Hoffmann-LaRoche (Nutley, NJ) or purchased from The Jackson Laboratory (Bar Harbor, ME). C57BL/6 mice were also purchased from The Jackson Laboratory and were used as controls throughout. Female mice between the ages of 6 and 8 wk were used throughout.

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[3] Abbreviations used in this paper: KO, knockout; DTH, delayed-type hypersensitivity; TLR, Toll-like receptor.

Experimental infection

The Erdman (TMCC 107) strain of *M. tuberculosis* was grown in Proskauer Beck medium containing 0.05% Tween 80 to mid-log phase and frozen in 1-ml aliquots at $-70°C$. For aerosol infections, subject animals were infected using a Glas-Col (Terre Haute, IN) airborne infection system, as previously described (23). Mice were euthanized when considered to be failing to thrive. The criteria for "failure to thrive" were contained in a written protocol and were applied equally to all groups.

Bacterial load determination

As previously described (23), infected mice were killed by CO_2 asphyxiation and the lungs were aseptically excised. Each of these organs was individually homogenized in physiological saline, and serial dilutions of the organ homogenate were plated on nutrient 7H11 agar. Bacterial colony formation was counted after 3 wk of incubation at 37°C.

Cell preparation and culture

A single cell suspension was prepared from the spleen, as previously described (24). Briefly, spleens were passed through 70-μm nylon cell strainers and RBCs were lysed using Gey's solution. Cells were counted and were then either cultured with Ag (at 5×10^6/ml) or analyzed by flow cytometry. Lung cell preparations were prepared as described previously (25). Briefly, the lungs were perfused through the heart with cold saline containing heparin. Once lungs appeared white, they were removed and sectioned in ice-cold media using sterile razor blades. Dissected lung tissue was then incubated in Collagenase IX (0.7 mg/ml; Sigma) and DNase (30 μg/ml; Sigma) at 37°C for 30 min. Digested lung tissue was gently disrupted by passage through a 70-μm pore size nylon cell strainer, the resultant single cell suspension was washed twice, and cells were either placed in culture (at 5×10^6/ml) with mycobacterial Ags or analyzed by flow cytometry.

Splenocytes were cultured directly with Ag, whereas lung cells were cultured on bone marrow-derived macrophages generated as previously described (26). Cells were cultured either in the absence of mycobacterial Ag or with *M. tuberculosis*-derived culture filtrate proteins at 25 μg/ml (obtained from J. Belisle, Colorado State University, under National Institutes of Health Contract AI-75320). Cultures were incubated for 72 h at 37°C and 5% CO_2 before cytokine analysis. Supernatants were assayed for the presence of IFN-γ by ELISA using Ab pairs purchased from BD PharMingen (San Diego, CA).

Flow cytometry

Single cell suspensions were prepared from spleens or lungs, as described above. Cells from individual mice were incubated with specific Ab for 30 min at 4°C in the dark. Some cells were incubated with anti-CD3 (clone 145-2C11), anti-CD28 (clone 37.51), and monensin for 4 h before staining. Cells were either washed and analyzed or permeabilized, and the presence of intracellular IFN-γ was probed for with labeled Ab (FITC-labeled clone XMG1.2). Cells were stained with Abs recognizing CD4 (allophycocyanin or PE-labeled clone GK1.5), CD8 (allophycocyanin-labeled clone 53-6.7), CD3 (PerCP-labeled clone 145-2C11), CD44 (FITC-labeled clone IM7), and CD62L (PE-labeled clone MEL-14) (all from BD PharMingen). Control wells containing isotype Abs were also prepared. Cells were then analyzed using CellQuest on a FACSCalibur (BD Biosciences, Mountain View, CA) dual laser flow cytometer with excitation at 488 and 633 nM. Lymphocytes were gated based on their forward and side scatter characteristics, and the number of such lymphocytes per spleen was determined. Twenty thousand CD3- and CD4 (or CD8)-positive lymphocytes were then analyzed for their expression of the activation markers CD44 and CD62L. Coexpression of the CD44 and CD62L cell surface molecules fell into four categories, and gates identifying these categories were applied in an identical manner for all groups and time points, as previously described (27).

Measurement of delayed-type hypersensitivity (DTH)

Mice infected with *M. tuberculosis* were inoculated intradermally with 50 μl saline containing 10 μg purified protein derived from *M. tuberculosis* (Pasteur Mérieux Connaught, Toronto, Ontario, Canada) or sterile saline as a control in the contralateral footpad. Induration was measured at 48 h following inoculation, with dial calipers that can detect swelling in increments of 0.05 mm. Results are expressed as the difference in the mean swelling between the saline- and purified protein derivative-inoculated footpads of individual mice ($n = 4$ in each of two separate experiments).

Real-time PCR analysis of lung tissue

Lung tissue from infected mice was frozen in Ultraspec (Biotexc, Friendswood, TX), and total RNA was extracted following the manufacturer's protocol. RNA was then reverse transcribed and amplified using p19 primers and probe generated using the published sequence of p19 (20) in the Applied Biosystems (Foster City, CA) Primer Express software program. The primer sequences are as follows: forward primer, CAG CAGCTCTCTCGGAAT; reverse primer, ACAACCATCTTCACACTG GATACG; and probe, CATGCTAGCCTGGAACGCACATGC. Briefly, RNA samples ($n = 4$) from each group and each time point were reverse transcribed using Taqman reagents. cDNA was then amplified using Taqman (Applied Biosystems) reagents on the ABI Prism 7700 sequence detection system (Applied Biosystems), and the signal for both p19 and 18S RNA was recorded. The signal for p19 was normalized to the signal for 18S RNA and to the sample with the least p19 signal, following the manufacturer's directions. Samples were also treated with DNase or run in the absence of the reverse-transcription enzyme to confirm that signal was derived from RNA.

Results

Mice lacking bioactive IL-12 are more susceptible to pulmonary tuberculosis

Fig. 1*A* illustrates the fact that neither the p35 KO nor the p40 KO mice were capable of controlling bacterial growth as efficiently as the intact C57BL/6 mice were. It is clear, however, that the mice lacking the p40 subunit of IL-12 were more susceptible to bacterial growth than were the mice lacking the p35 subunit. All three groups allowed bacterial dissemination to, and growth within, the spleen (Fig. 1*B*). The pattern of bacterial growth in this organ did, however, mirror that in the lung, with the control C57BL/6 exhibiting greater control of growth compared with the p35 KO, which in turn was more resistant than the p40 KO.
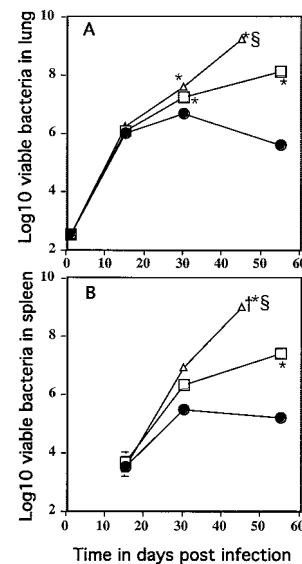

FIGURE 1. Mice lacking subunit components of IL-12 differ in their susceptibility to aerogenically delivered *M. tuberculosis*. Control (●), p35 KO (□), or p40 KO (△) mice were infected by the aerogenic route, and lungs (*A*) or spleens (*B*) were evaluated for bacteria growth over a period of time. Data points represent the mean (±SEM) of values from four mice. Points marked ∗ are significantly different from the data from the C57BL/6 mice ($p \leq 0.05$ by the Student's *t* test). Points marked § are those in which the p40 KO data are significantly higher than that from the p35 KO mice ($p \leq 0.05$ by the Student's *t* test). Points marked † are when mice were moribund and sacrificed. These data are representative of three separate experiments.

Mice lacking p40 are less able to make an Ag-specific DTH response than are mice lacking the p35 subunit

A focused Ag-specific IFN-γ response is required for effective control of *M. tuberculosis*; the ability of the p40 and p35 KO mice to generate such a response was therefore compared. In an initial analysis of the ability of the IL-12-deficient mice to generate Ag-specific cellular responses, the swelling response of the footpad was measured following Ag challenge of infected mice. At 30 and 40 days of infection, both the control and the p35 KO mice exhibited significantly more footpad swelling than the p40 KO mice (Fig. 2).

Mice lacking p40 are less able to make an Ag-specific IFN-γ response than are mice lacking the p35 subunit

To determine whether this cellular recruitment was potentially protective, the ability of the mice to generate Ag-specific IFN-γ-producing cells was determined. Fig. 3 illustrates the ability of cells from the spleen, draining lymph node, and the lung to produce IFN-γ in response to mycobacterial Ag. The peak of response in the control animals is seen in the spleens and draining nodes by day 20 of infection (Fig. 3, *top* and *center panel*); this response then wanes, correlating with the control of bacterial growth (Fig. 1). Cells from the infected lungs were also able to produce IFN-γ in response to Ag when analyzed at day 30 (analysis of lung cell responses was performed at day 30 only) (Fig. 3, *bottom panel*), reflecting the fact that the Ag-specific cells are recruited to the infected organ. Interestingly, the p35 KO mice were also able to generate an Ag-specific IFN-γ response in the spleens, node, and lung (Fig. 3). The response in the p35 KO mice was initially less vigorous in the spleen, waned earlier in the spleen and draining node, and was less vigorous at the principal site of infection, the lung. Although less potent than the response seen in the control animals, the p35 KO response was much more vigorous than that seen in the p40 KO mice. In the p40 KO animals, only a short-lived and minor Ag-specific response could be seen in the draining node, and little to no response was detected in the spleen or lung (Fig. 3).
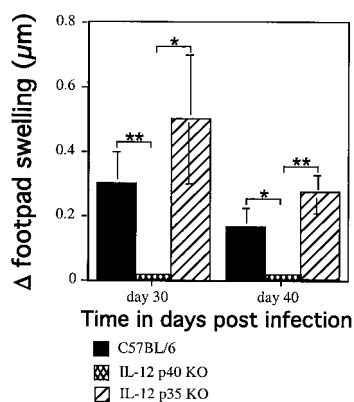

FIGURE 3. Only intact and p35 KO mice were able to generate an Ag-specific, IFN-γ response to mycobacterial Ag in the spleen, draining lymph node, or lung. Control (■), p40 KO (▦), or p35 KO (▨) mice were infected as for Fig. 1, and splenocytes (*top*), mediastinal cells (*center*), and lung cells (*bottom*) were analyzed for their ability to generate IFN-γ in response to mycobacterial Ag. ELISA was used to determine the amount of IFN-γ produced by these cells; each point represents the mean value for four mice. Points marked with an asterisk are significantly higher than the values for nonstimulated culture ($p \leq 0.05$, Student's *t* test). The amount of IFN-γ produced by unstimulated cells was below the level of detection of the assay, while that produced by cells from uninfected mice, in response to mycobacterial Ag, was below 1000 pg/ml. The data shown are representative of two separate experiments. Analysis of lung cell responses was made at day 30 only (ND = not done).

Mice lacking bioactive IL-12 are less able to generate and recruit activated lymphocytes to the site of infection

In a more detailed analysis of the cells being recruited to the lung, lungs were digested and cells were analyzed by flow cytometry for the expression of activation markers and for the presence of intracellular IFN-γ. As might be expected, a high percentage of CD4-positive cells in the infected lung was of the activation phenotype characterized by high expression of CD44 and low expression of CD62L (Fig. 4, *bottom*). The IL-12-deficient mice both had significantly reduced numbers of CD4 cells in the lungs (data not shown), and this resulted in significantly lower numbers of activated CD4 lymphocytes (Fig. 4, *top*). Although total numbers of activated cells were similar for the p35 and p40 KO mice, there was a slight, but significant increased percentage of activated cells in the infected p35 KO lungs compared with the p40 KO lungs (Fig. 4, *bottom*). This pattern of reduced expression of lymphocyte activation markers on CD4 cells was also observed in the spleens

FIGURE 2. Mice lacking the p40 subunit of IL-12 were unable to generate a DTH response to mycobacterial Ag, unlike both intact and p35 KO mice. Control (■), p40 KO (▦), or p35 KO (▨) mice, infected as for Fig. 1, were challenged in the left footpad with saline and the right footpad with mycobacterial Ag. The difference between the swelling in the left and right footpad was measured 48 h after challenge. Data represent a total of four mice and one experiment representative of three. Bars that are significantly higher than the p40 KO bars by the Student's *t* test: *, $p \leq 0.05$; **, $p \leq 0.01$.
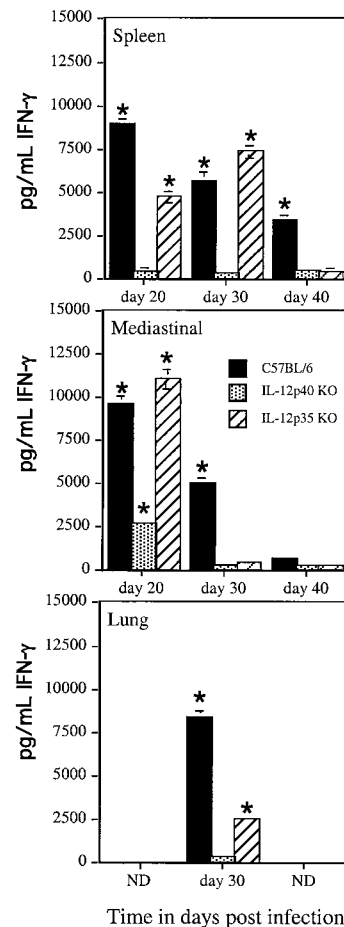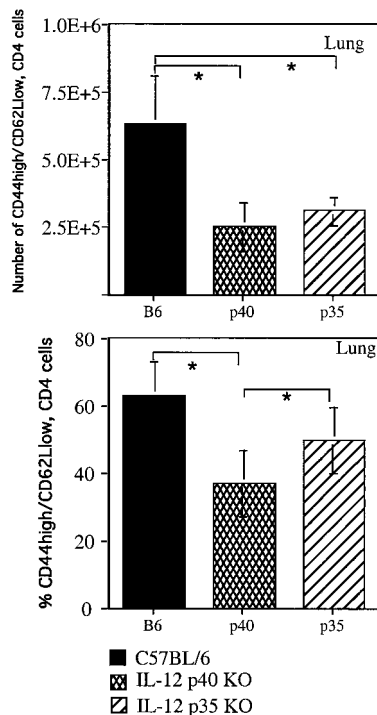
FIGURE 4. Mice lacking IL-12 fail to recruit as many activated lymphocytes to the lung following aerogenic challenge with *M. tuberculosis*. Control (■), p40 KO (▨), or p35 KO (▧) mice were infected for 30 days, as described for Fig. 1, and lung cells were analyzed by flow cytometry for expression of CD4, CD44, and CD62L. Both the percentage (*bottom*) and total number (*top*) of cells, expressing a high level of CD44 and a low level of CD62L, per lung are reported. The bars represent the means of three mice per group, and the graph shows one experiment representative of two. Bars marked with ∗ are significantly different from the bars connected by the line ($p \leq 0.05$, Student's *t* test).
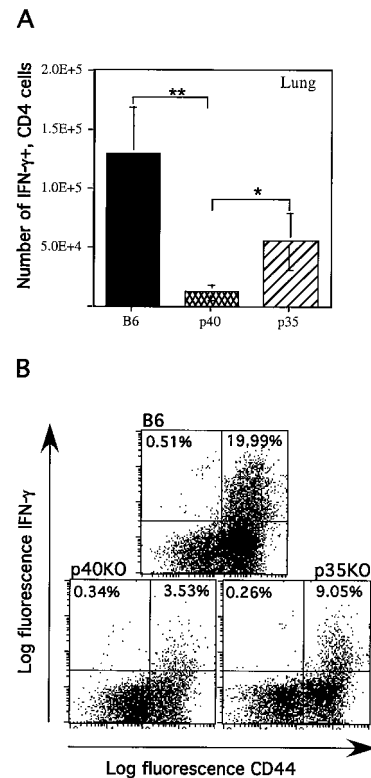
FIGURE 5. A. Mice lacking p40 fail to recruit as many IFN-γ-producing CD4 lymphocytes to the lung compared with both intact and p35 KO mice. Control (■), p40 KO (▨), or p35 KO (▧) mice were infected as for Fig. 1, and lung cells were cultured with anti-CD3, anti-CD28, and monensin and then analyzed for intracellular IFN-γ production. The bars represent the means of three mice per group, and the graph shows one experiment representative of two. Bars marked ∗ are significantly different from the bars connected by the line (∗, $p \leq 0.05$; ∗∗, $p \leq 0.01$, Student's *t* test) (*A*). Cells were also stained for the activation marker CD44, and the *lower panel* (*B*) shows that the expression of IFN-γ is restricted to those cells expressing a high level of CD44. *B*, Shows the results for one animal from each group ($n = 3$); the experiment was repeated twice.

of the p40 and p35 KO mice (data not shown). The number of activated CD8 T cells was between 5- and 10-fold less than the number of activated CD4 T cells in the C57BL/6 mice (data not shown). The low numbers of CD8 cells meant that no statistically significant differences could be detected between the intact and IL-12-deficient mice.

Mice lacking p40 are less able to generate and recruit IFN-γ-producing cells to the lung than are mice lacking the p35 subunit

The significant reduction in activated CD4 cells in the lungs of the IL-12-deficient mice serves to partially explain the increased susceptibility of these mice to tuberculosis. The similarity between the numbers of activated cells in the lungs of the p35 and p40 KO mice does not, however, explain the increased resistance of the p35 compared with the p40 KO. To determine whether the activated cells present in the p35 KO mice were potentially more effective at controlling bacterial growth, their ability to express IFN-γ was assessed. Not surprisingly, a significant number of CD4 cells recruited to the lungs of infected control mice were capable of expressing IFN-γ (Fig. 5*A*). The cytokine-positive cells were also CD44 high (Fig. 5*B*). The percentage of IFN-γ-producing cells in the p35 KO mouse lungs was variable, but generally higher, than was seen in the p40 KO (not shown). This increased percentage translated into significantly higher total numbers of IFN-γ-producing cells in these mice compared with p40 KO mice (Fig. 5*A*). This pattern was also seen in the spleen (data not shown). The number of IFN-γ-producing cells in the p35 KO mice tended to be lower

than for the wild-type mice ($p = 0.057$), and this suboptimal response may explain their eventual failure to control the growth of bacteria.

The number of IFN-γ-producing CD8 T cells was between 5- and 10-fold less than the number of IFN-γ-producing CD4 T cells in the C57BL/6 mice (data not shown). The low numbers of CD8 cells meant that no statistically significant differences could be detected between the intact and IL-12-deficient mice. There was, however, a pattern of reduced numbers of IFN-γ-producing CD8 cells in both of the IL-12-deficient mice; this reduction was most pronounced in the lung (data not shown).

Mice infected with M. tuberculosis *increase the expression of the p19 subunit of IL-23*

The difference in the ability of the p35 and p40 KO mice to generate IFN-γ-producing cells suggested that an additional IFN-γ-inducing agent was active in the p35 KO mice. A candidate cytokine composed of the p40 subunit and a novel p19 subunit has recently been described (20) that acts in a similar, but not identical manner to IL-12. To determine whether this cytokine was available to the p35 KO mice and was therefore potentially responsible for the increased protection seen in these mice, the level of mRNA for the p19 subunit was measured. The p19 message was not detected in uninfected lungs, but was significantly increased by 30 days
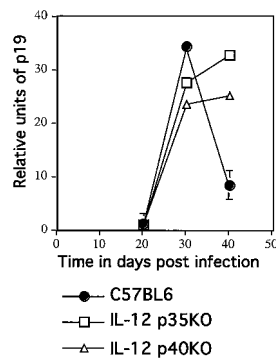
FIGURE 6. Lungs from infected mice expressed mRNA for the p19 subunit of IL-23. Control (●), p35 KO (□), or p40 KO (△) mice were infected as for Fig. 1, and lung tissue was obtained over time. RNA was extracted from the tissue and analyzed using primers and probes specific for the p19 subunit of IL-23. Data were normalized for the amount of RNA added to the reaction using 18S as a control and also normalized to the sample containing the least p19 signal. Data points show the mean (±SEM) value for four mice per group. The data are representative of two independent experiments that both showed the same pattern of p19 induction.

postinfection (Fig. 6). All three groups of infected mice exhibited increased and similar mRNA levels by day 30; however, by day 40, the p19 signal from the p40 and p35 lungs was higher than the p19 signal from the B6 lungs (Fig. 6).

Discussion

We show in this study that the absence of the p40 subunit of IL-12 is more detrimental to the expression of the protective response during tuberculosis than is the absence of the p35 subunit. This suggests that the p40 subunit can act in the absence of p35 to drive cellular responses. In particular, in the absence of p35 and therefore the absence of bioactive IL-12, Ag-specific DTH and cytokine responses to mycobacterial Ags were easily detectable. This was in stark contrast to the almost complete absence of these responses in mice lacking bioactive IL-12, but more specifically lacking the p40 subunit. The recent description of the novel cytokine, IL-23, which contains the p40 subunit of IL-12, prompted us to investigate the availability of this cytokine during the pulmonary response to tuberculosis. We show in this work that the p19 subunit is induced in the lung during the peak of antimycobacterial responses and that, following control of bacterial growth, expression decreases. This expression and the ability of the p35 KO mice to exhibit limited control of bacterial growth implicate the IL-23 cytokine as a possible mediator of protective responses to tuberculosis.

The initial similarity in bacterial numbers in all three groups of mice suggests that any early protective mechanisms in the lung are not dependent upon IL-12 subunits p35 or p40. This is not surprising in view of the fact that even the absence of IFN-γ does not result in a strong susceptible phenotype early in aerosol infection (27). It is therefore likely that the protective effect of both IL-12 subunits is mediated through the differentiation of IFN-γ-producing, Ag-specific lymphocytes. Indeed, the reduction of both DTH and Ag-specific, IFN-γ responses were the most significant differences observed between the control and IL-12-deficient mice. The data also suggest that the IFN-γ response and the DTH response are not necessarily synonymous, as DTH is clearly seen in the p35 KO mice at day 40 when the IFN-γ response in the spleen has waned. We have also reported that DTH is clearly seen in the *M. tuberculosis* IFN-γ gene-deleted mouse (27), further demonstrating that while both responses are required for protection, they are not inseparably linked.

The induction of the IFN-γ gene in lymphocytes can be achieved in the presence or absence of Ag; however, both mechanisms require IL-12 (28, 29). Bioactive IL-12p70 induces IFN-γ gene expression in CD4 T cells via the IL-12R and STAT4 activation (30, 31). IL-23 also binds the IL-12Rβ1 chain of the IL-12R, activates STAT4, and can drive IFN-γ production (20). The p40 homodimer can also bind to the IL-12R, but acts as an antagonist (14, 32, 33). In intact mice, both IL-12p70 and IL-23 are potentially available to drive the protective response; in addition, the p40 homodimer may provide control of the response. In the p40-deficient mice, none of the three IL-12-related molecules can be made, whereas in the p35-deficient mice, both IL-23 and the p40 homodimer are potentially available. The drastically reduced responses in the p40-deficient mouse suggest that Ag-specific DTH and IFN-γ production depend upon the p40 subunit. The presence of these responses in the p35-deficient mouse suggests that IL-12p70 is not essential in initiating these responses, particularly in tuberculosis. Of the alternative molecules available to the p35-deficient mouse, IL-23 is the most likely to drive these protective responses. Although the p40 homodimer has been shown to drive IFN-γ production in CD8 T cells in vitro (17), the p35 KO mice failed to exhibit an increase in IFN-γ-producing CD8 T cells, negating a role for this mechanism in this model.

IL-23 does not drive the proliferation of naive CD4 cells in in vitro models; however, IL-12p70 is not particularly potent in this regard either (20). The expansion of CD4 lymphocytes with an activated phenotype is, however, compromised in both the p35 and p40 KO mice, implicating IL-12p70 as a principal inducer of maximal cellular proliferation in response to mycobacterial infection. Mice lacking the p35 subunit were, however, clearly capable of generating IFN-γ-producing CD4 T cells expressing a high level of CD44. Unfortunately, these cells were not capable of fully protecting the mice, even against the low dose challenge used in this study. There are two possible reasons for this. The first is that the response in the absence of p40 is not optimal and bacterial numbers simply overwhelm the response. This mechanism is supported by the data, as the total number of protective cells in the p35 KO mice was less than that produced by the intact mice. A second explanation, which is not mutually exclusive of the first, is that IL-12p70 is required to keep differentiated cells alive. Again, the data support this explanation, as the Ag-specific IFN-γ response fades more rapidly in the p35 KO mice even in the face of increasing bacterial numbers. IL-12p70 has also been specifically implicated as a mediator of increased Th1 cell survival. In particular, in leishmaniasis and toxoplasmosis, IL-12p70-treated p40 KO mice can only control disease as long as external IL-12p70 is provided (34, 35).

How then do the p40, p35, and p19 subunits interact to protect mice from tuberculosis? Once bacteria enter the lung, they will be phagocytosed by macrophages or immature dendritic cells (36); the dendritic cells then transport the bacteria to the spleen and draining lymph node. At these sites, the newly matured dendritic cells drive Ag-specific T cell activation. Optimum expansion of activated lymphocytes requires the presence of the p40 subunit probably in the form of IL-12p70. In the absence of IL-12p70, however, both activation and differentiation to an IFN-γ-producing cell can occur, and this is most likely mediated through the action of IL-23. The relative roles of either IL-12p70 or IL-23 in driving the IFN-γ response have not been addressed in this work and await the availability of the p19 gene-deleted mouse and/or neutralizing anti-p19 Ab. The presence of p19 in the lungs of infected mice suggests that IL-23 may be available at the site of infection and may serve to augment the response locally. It is likely that the p19 in the lung is derived from activated macrophages and dendritic

cells (20) present in the developing granuloma. It may be induced via Toll-like receptor 2 (TLR2), as dendritic cells produce the p19 subunit following ligation of the TLR2 (37) and a 19-kDa lipoprotein of *M. tuberculosis* is a stimulatory ligand for TLR2 (38).

The identification of the mechanisms involved in the induction of the protective response to tuberculosis will help in the rational design of vaccines. The observation that protective mechanisms can be expressed in the absence of IL-12p70 supports the development of vaccines that target not only the induction of IL-12p70, but also other potentially protective cytokines such as IL-23.

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