Early IL-4 Induction in Bone Marrow Lymphoid Precursor Cells by Mycobacterial Lipoarabinomannan

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IL-4 is produced promptly in response to certain infections and plays a key role in the Th1/Th2 T cell dichotomy; however, the cellular source remains a matter of debate. Here we describe the induction of IL-4 in bone marrow cells of normal and RAG−/− mice by both Mycobacterium tuberculosis and its major wall glycolipid, lipoarabinomannan. Characterization of the cell type responsible indicated that it was distinct from the NK1+ or CD4+ T cell previously ascribed the function of rapid IL-4 secretion. Cell-sorting experiments identified CD19+/B220+ precursor cells, presumably pre-B cells that produced IL-4 constitutively and whose frequency was rapidly and markedly up-regulated by lipoarabinomannan. Thus, pathogenic mycobacteria and their glycolipids may influence hemopoiesis by rapidly inducing IL-4 secretion in the bone marrow.


Interleukin-4 is an immunoregulatory cytokine with many effects on both hemopoietic and nonhemopoietic cells. Numerous target cells have been reported for IL-4, including fibroblasts, B cells, T cells, macrophages, and hemopoietic precursor cells (reviewed in Refs. 1 and 2). IL-4 has been attributed both stimulatory and inhibitory activities on hemopoietic cell proliferation (3), in that IL-4 enhances granulopoiesis but inhibits monopoiesis from human CD34+ bone marrow precursor cells (4). In different studies it has been shown to induce colony formation of B-lineage cells (5) and to inhibit the growth and maturation of such precursors (6).

Despite its ill-defined role in hemopoietic processes, the contribution of IL-4 to the regulation of the host response to infection is much clearer. Its primary role is to direct the development of a Th2-type immune response while inhibiting the generation of Th1 cells (7). One of the major effector functions of this cytokine is to control Ig heavy chain class switching from IgM to IgG1 and IgE, and it is high levels of these classes of Abs that generally characterize a Th2 reaction, usually occurring in response to pathogens typified by helminth infections (8). Conversely, infection with intracellular bacteria characteristically results in a Th1 immune response with cellular effector mechanisms such as macrophage activation mediated by IFN-γ (9, 10).

To date the requirements for the generation of a Th1 response have been relatively well defined, involving the initial production of IL-12 by macrophages and the subsequent induction of IFN-γ in NK cells (reviewed in Ref. 11). This activation of components of the innate immune system creates the optimal cytokine environment for the differentiation of naïve T cells into Th1 cells (12, 13). In contrast, the source of the initial IL-4 required to drive differentiation toward a Th2-type response has yet to be identified, although many candidates have been proposed, including cells of the mast cell and basophil lineage (14–15), naïve CD4+ T cells themselves (16, 17), some CD8+ cells (18), a subpopulation of CD4+ thymocytes (19), and NK1+ T cells (20). This latter population in the spleen responds to in vivo anti-CD3 mAb treatment by rapidly producing IL-4 within 90 min of injection (21). However, recent evidence suggests that the IL-4 produced by these cells may have a function distinct from Th2 cell induction, as mice lacking this cell population still mount appropriate Th2 responses to a variety of stimuli (22–24).

The intracellular pathogens of the Mycobacterium species, which include the tubercle bacilli, characteristically induce Th1-type responses upon experimental infection of mice (reviewed in Ref. 25). A major cell wall component of these organisms, lipoarabinomannan, is a potent cytokine inducer in cells, especially macrophages, in vitro (26–28). In this report we present the first evidence that this molecule induces rapid and profound IL-4 induction in bone marrow cells in vivo and demonstrate that the cell type responsible for the IL-4 production is B220+ and probably an early B lymphoid precursor cell and thus distinct from the NK1+ T cell. We suggest that microbial glycolipids have important potential not only for inducing cytokines to direct the host immune response, but also in shaping hemopoietic maturation processes by inducing IL-4 secretion.

Materials and Methods

Mice

All mouse strains used in these experiments were purchased from Bornholgard Breeding and Research Center (Ry, Denmark) or bred under specific pathogen-free conditions at the central animal facilities of the Bundesinstitut für gesundheitlichen Verbraucherschutz und Veterinärmedizin (Berlin, Germany). RAG-2−/− mice (backcrossed 11 times onto the B6 background) were provided by Dr. Johannes Barsig (University of Ulm, Ulm, Germany) and Dr. A. Rolink (Basel Institute for Immunology, Basel, Switzerland). In the experiments presented here, female mice were used at 6–10 wk of age.

Induction of IL-4-producing cells

Purified mannose- and arabinose-capped lipoarabinomannan (manLAM and araLAM, respectively) isolated from Mycobacterium tuberculosis (strain H37Rv) were provided by Dr. John Belisle, Colorado State University (Fort Collins, CO). Both LAM preparations contained approximately $7 \times 10^{-3}$ ng of LPS/mg of material as determined by the Limulus

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3 Abbreviations used in this paper: LAM, lipoarabinomannan; araLAM, arabinose-capped LAM; manLAM, mannose-capped LAM; BCG, Calmette-Guérin bacillus; ELISPOT, enzyme-linked immunospot; PE, phycoerythrin.

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amoebocyte assay. Routinely, 100 ng of LAM diluted in 0.1 ml of PBS (Biochrom, Berlin, Germany) were injected i.v., and cells were harvested after 90 min. LPS purified from *Escherichia coli* (Sigma, St. Louis, MO) was diluted in PBS, and 100 ng/mouse was injected i.v.

**Flow cytometric analysis of cells**

The following Abs were used: anti-B220 (RA3-6B2-FTC conjugated, Pharmingen), anti-CD11b (Mac-1 aM-phycocerythrin (PE) conjugated, Pharmingen), anti-CD3 (145-2C11-PE conjugated, Pharmingen), anti-CD4 (191-FTC conjugated), anti-CD8 (169-PE conjugated, Life Technologies, Paisley, U.K.), anti-MHC class I (B22.249-FTC conjugated), anti-y6 TCR (GL3-PE conjugated, Pharmingen), anti-TCR-aβ (H57-597-FTC conjugated), and anti-NK.1.1 (PK136-PE conjugated, Pharmingen). Unless specified otherwise, all Abs were purified from hybridoma culture supernatants as described above and were labeled with biotin or FITC by conventional methods. Routinely, 10^5 cells were incubated for 60 min at 4°C with the appropriate concentrations of Ab according to the manufacturer’s instructions, diluted in PBS, 5% horse serum, 5% goat serum, and 0.01% sodium azide (FACS buffer). Following three washes with FACS buffer, cells were fixed in 2% paraformaldehyde for 30 min. Cells were analyzed using a Becton Dickinson FACStar Plus equipped with the CellQuest software (Becton Dickinson, Mountain View, CA). Routinely, 10^5 uncultured cells were analyzed per sample.

**Intracellular cytokine detection**

Bone marrow cells were allowed to adhere overnight on eight-chamber tissue culture slides (Nunc, Roskilde, Denmark), and the slides were washed to remove nonadherent cells. This procedure was used to enrich the IL-4-producing cell population that resided within the adherent cell population. Following washing, cells were cultured for 6 h in 2.5 µg/ml brefeldin A (Calbiochem, Bad Soden, Germany) and 2 µM monensin (Sigma) to deplete intracellular transport and to promote the retention of the cytokine within the Golgi compartment of the cell (35). Cells were fixed in 4% formaldehyde at 4°C overnight and were incubated for 10 min at room temperature in blocking buffer consisting of PBS and 10% normal goat serum (Life Technologies). Abs against cell surface markers were diluted in block buffer following the manufacturer’s suggestions and added to the cells for 1 h at 4°C. The following mAbs were used: anti-CD14 (rmC5.3, Pharmingen), anti-CD34-biotin (RAM54, Pharmingen), anti-FcyRIII (2.4G2, Pharmingen), and anti-CD74 (P4H5, Pharmingen). For additional mAbs see the section entitled Flow cytometric analysis of cells. Following washing with PBS, the appropriate second Abs were added at a 1/200 dilution in blocking buffer (goat anti-rat IgG-PE (Dianova), streptavidin-PE (Dianova), and mouse anti-hamster IgG-FITC (Dianova)) for 1 h at 4°C in the dark. Following washing, cells were permeabilized for 5 min on ice in PBS containing 0.08% saponin and were further blocked for 10 min in blocking buffer with saponin. Anti-IL-4 FITC-labeled Ab (11B11, Pharmingen) or an FITC-labeled isotope-matched control Ab (rat IgG1, Pharmingen) was diluted in blocking buffer and saponin and added to cells for 1 h at 4°C. The following mAbs were used: anti-CD11c (H57-597-FTC, Pharmingen), anti-CD34-biotin (RAM54, Pharmingen), anti-FcyRIII (2.4G2, Pharmingen), and anti-CD74 (P4H5, Pharmingen). For additional mAbs see the section entitled Flow cytometric analysis of cells. Following washing with PBS, the appropriate second Abs were added at a 1/200 dilution in blocking buffer (goat anti-rat IgG-PE (Dianova), streptavidin-PE (Dianova), and mouse anti-hamster IgG-FITC (Dianova)) for 1 h at 4°C in the dark. Following washing, cells were permeabilized for 5 min on ice in PBS containing 0.08% saponin (Sigma) and were further blocked for 10 min in blocking buffer with saponin. Anti-IL-4 FITC-labeled Ab (11B11, Pharmingen) or an FITC-labeled isotope-matched control Ab (rat IgG1, Pharmingen) was diluted in blocking buffer and saponin and added to cells for 1 h at 4°C in the dark. Following washing, cells were mounted in Prolong Anti-Fade (Molecular Probes, Eugene, OR) and viewed using a fluorescent microscope. In all cases control cells were included that had not been cultured in monensin and brefeldin A, and all appropriate second Ab controls were negative.

**Depletion of B220^-^ cells by magnetic-activated cell sorting**

Bone marrow cells were harvested, and a single cell suspension was prepared as described above. Cells were incubated with anti-B220 mAb conjugated to magnetic beads (Miltenyi Biotech, Bergisch Gladbach, Germany) at the concentrations recommended by the manufacturers (29) for 15 min in PBS/BSA at 4°C and washed three times. Cells were pipetted onto a magnetic column (Miltenyi Biotech) that had been precoated by passing ice-cold PBS/BSA over it three times. The cells were allowed to pass through at a slow flow rate, and the column was washed three times with PBS/BSA. Cells were collected and analyzed by flow cytometry to check the efficiency of depletion.

**Cell sorting**

Bone marrow cells were harvested, and a single cell suspension was prepared as described previously. A portion of the cells was incubated for 30 min on ice with anti-B220-FTC (RA3-6B2, Pharmingen) and anti-CD11c mAbs (H57-597, Pharmingen) washed extensively, and sterile sorted on a FACSVantage cell sorter (Becton Dickinson). The remaining cells were incubated with unlabeled anti-CD19 for 30 min, washed twice in medium, and then incubated for 30 min with goat anti-rat IgG coupled to magnetic beads (Dynal, Great Neck, NY). CD19^-^ cells were then depleted by passing the cells through a magnetic field. The remaining cells were washed and incubated on ice for 30 min with anti-CD4-FTC and anti-NK.1.1-PE and sorted as described above. In all cases, reanalysis of the sorted population confirmed the purity of the sorted cells.
Infection with Mycobacteria species leads to rapid IL-4 production from bone marrow but not spleen cells. C57BL/6 mice were injected i.v. with PBS, $5 \times 10^5$ M. bovis BCG, or $M$. tuberculosis diluted in 0.1 ml of PBS. At the times indicated, spleen and bone marrow cells were harvested and assayed for IL-4 production by ELISPOT as detailed in Materials and Methods. Results are expressed as the mean ± SD number of IL-4-producing cells per $10^6$ total cells from eight replicate wells. T = 0 is represented by naive, untreated mice.

Results

Infection with mycobacteria induces IL-4 production from bone marrow cells

Mice were inoculated i.v. with Mycobacterium bovis BCG, $M$. tuberculosis, or PBS, and at various times after infection cells were harvested from spleens and bone marrow and assayed for IL-4 production by ELISPOT. A low frequency of bone marrow cells was found to constitutively secrete IL-4 (Fig. 1); however, infection with either strain of mycobacteria resulted in a rapid up-regulation of the frequency of IL-4-producing cells in the bone marrow, but not the spleen, of infected animals (Fig. 1). It should be noted that a small, transient increase in IL-4-secreting cell frequency occurred as a result of control saline injection; however, the increase in IL-4 production in response to the bacteria was increased fourfold and was prolonged, not returning to background levels until 48 h, or even longer in M. bovis BCG-infected animals. A similar increase was not observed in the spleens of these animals over the course of the infection. This rapid induction of cytokine production was not an artifact of the ELISPOT assay, as similar results were obtained when the cells were harvested from mice 90 min after treatment and were incubated for 3 h in the ELISPOT assay rather than the 16-h assay routinely used (data not shown).

To determine whether mycobacteria were present in the bone marrow after a short infection period, mice were infected with $5 \times 10^5$ bacteria, and 90 min after infection spleen, liver, and bone marrow were harvested and assayed for the presence of mycobacteria. In all mice tested, a small number of CFU of acid fast bacilli were isolated from the bone marrow (data not shown).

IL-4 is induced by the major mycobacterial cell wall component LAM

To determine whether the major glycolipid cell wall component of mycobacteria also induced IL-4 production in bone marrow cells, we injected 100 ng of LAM into mice, a concentration that had previously been shown to activate macrophages in vitro (26, 27). Ninety minutes later, spleen and bone marrow cells were harvested, and the frequencies of IL-4-producing cells were measured by ELISPOT. For comparison we also included M. bovis BCG-infected and anti-CD3ε mAb-stimulated mice (Fig. 2). LAM administration induced a rapid increase in the numbers of IL-4-producing cells within total bone marrow, even higher than that caused by the bacterial infection itself. However, treatment with the glycolipid did not increase IL-4 production by spleen cells within the same time frame. In contrast, in vivo administration of anti-CD3ε mAb rapidly and significantly increased the number of IL-4-producing spleen cells as previously described (21), and additionally resulted in a higher frequency of IL-4 secretors in bone marrow cells, although this was considerably less than that observed following in vivo stimulation with LAM.

ManLAM, but not araLAM, induces IL-4

It has been previously shown that LAM isolated from fast growing vs slow growing mycobacteria differ in their ability to activate early gene expression in macrophages and induce cytokine and nitric oxide synthase production (26–28). In light of these observations we investigated whether the araLAM differed in its ability to induce bone marrow cells to produce IL-4 compared with that of manLAM isolated from M. tuberculosis. Injection of 100 ng of the araLAM preparation failed to increase IL-4 production in either spleen or bone marrow over the constitutive background of cytokine-producing cells (Fig. 3). In contrast, the manLAM preparation induced a rapid increase in the frequency of IL-4-producing cells within bone marrow but not spleen. Furthermore, these results are taken as an indication that the effects seen with manLAM were not due to contamination with, for example, LPS, as both preparations contained equivalent amounts of LPS. This was further confirmed by injection of mice with 100 ng of LPS, which failed to increase the frequency of IL-4 producers to the same extent as LAM (PBS, 40 ± 16; LPS, 78.3 ± 15; LAM, 498.3 ± 33 IL-4-producing cells/10^6 total bone marrow cells).

We were interested to determine whether LAM was a specific inducer of IL-4 or a general nonspecific activator of different cytokines in bone marrow cells. Because IL-4 is generally recognized as a cytokine that both promotes the development of and is produced by Th2 cells, we examined in parallel the ability of LAM to induce IFN-γ and IL-12, which are recognized as critical cytokines in the establishment of a Th1 response (12, 13). In contrast to the rapid IL-4 induction, no significant IL-12 production was detected from either spleen or bone marrow cells (Fig. 3). The kinetics of IFN-γ secretion is slower than that of the other two cytokines, and for this reason the ELISPOT plates were incubated for 48 h to permit maximum cytokine secretion. However, even after this time we observed no IFN-γ production from either spleen or bone marrow cells in response to LAM injection (Fig. 3).
The IL-4-producing cell type is distinct from the NK1$^+$ T cell
To identify the bone marrow cells that produced IL-4 in response to in vivo stimulation with LAM, cells from control and LAM-stimulated mice were analyzed by flow cytometry. Total, ungated cell populations were analyzed to avoid any bias in the system toward cells of lymphocyte origin. In several experiments we did not see any significant differences in the number of cells marked by a variety of cell surface markers (Table I), suggesting that it was a resident population in the bone marrow that was responsible for the IL-4 production, rather than recruitment of a cell into that location in response to treatment with LAM.

It has been previously described that a rapid burst of IL-4 is produced following in vivo stimulation with anti-CD3 mAb (21), and the IL-4-secreting cell was identified to be among a population of NK1$^+$ T cells. β2m gene knockout (β2m$^{-/-}$) mice are virtually deficient in this cell population and consequently are unable to make IL-4 in response to in vivo challenge with anti-CD3 mAb (31–33). To determine whether the IL-4 secretors in our system stimulated by a natural microbial glycolipid were also NK1$^+$ T cells, we injected β2m knockout mice with LAM. The data shown in Fig. 4 reveal that LAM administration resulted in a dramatic induction of IL-4-producing cells within the bone marrow again within 90 min after injection (Fig. 4), to frequencies comparable to those in wild-type mice. As a control, spleen cells from these mutant mice were unable to produce IL-4 in response to anti-CD3 mAb (C57BL/6(B6): PBS, 16.3 ± 6 13; anti-CD3, 191.3 ± 6 27; β2m$^{-/-}$: PBS, 15.6 ± 11; anti-CD3, 30.3 ± 8 IL-4-producing

### Table I. Flow cytometric analysis of bone marrow cells 90 min after injection with LAM or PBS

<table>
<thead>
<tr>
<th>Surface Marker</th>
<th>Treatment (% positive cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PBS</td>
</tr>
<tr>
<td>B220</td>
<td>67.7</td>
</tr>
<tr>
<td>CD11b</td>
<td>50.1</td>
</tr>
<tr>
<td>CD3</td>
<td>7.9</td>
</tr>
<tr>
<td>CD4</td>
<td>7.5</td>
</tr>
<tr>
<td>CD8</td>
<td>5.3</td>
</tr>
<tr>
<td>MHC I</td>
<td>94.4</td>
</tr>
<tr>
<td>γδ TCR</td>
<td>2.1</td>
</tr>
<tr>
<td>αβ TCR</td>
<td>3.0</td>
</tr>
<tr>
<td>NK1.1</td>
<td>1.4</td>
</tr>
</tbody>
</table>

*Bone marrow cells were harvested and prepared for flow cytometric analysis as described in Materials and Methods. Routinely, 10⁶ ungated cells were analyzed. Results are from one representative experiment of four.
We examined the responses of bone marrow cells from wild-type B6 mice in response to anti-CD3 and to anti-CD3 plus LAM treatment. Additional confirmation that the NK1+ T cell is not the IL-4-producing cell was provided by using SJL mice, which also express this marker. Cells were sorted on the basis of CD19 phenotype. As illustrated in Fig. 5A, the majority of the constitutive IL-4-producing cells of the bone marrow were contained within the CD19+B220− population, and it was this population that was induced to secrete IL-4 following LAM injection. Thus, in RAG−2−/− mice the IL-4-producing cells that are induced in response to LAM are a subset of the lymphocyte population, i.e., before VDJ recombination, or, alternatively, a cell of a separate lineage.

**Phenotypic characterization of the IL-4-producing cell**

We observed that depletion by adherence of bone marrow cells in tissue culture petri dishes dramatically reduced the number of IL-4-producing cells as measured by ELISPOT (PBS: 127 ± 23; LAM: unselected population, 938.3 ± 80; nonadherent cells, 71.6 ± 21 IL-4-producing cells/10⁶ total cells), suggesting that the cytokine-producing cell is contained within the adherent cell population. Consequently, adherence was used to enrich the IL-4-producing cell population. These cells were visualized following intracellular staining with specific mAbs of cells treated with the Golgi transport inhibitors monensin and brefeldin A to prevent cytokine secretion and permit detection by fluorescent Abs (35). Initial experiments ensured that overnight culture in vitro did not reduce the number of IL-4-producing cells (data not shown). When an array of cell surface markers on cells from both B6 and SCID mice were examined for coexpression with IL-4, only B220−/− T cells, yet this is not the population of cells that is induced to make IL-4 in response to LAM. Indeed, the production of IL-4 from bone marrow cells in response to LAM was considerably more pronounced than that observed with anti-CD3 mAb treatment. Additionally, initial experiments were performed using bone marrow cells from SJL and C57BL/6 mice. These mice have a full complement of functional mature T and B lymphocytes. Despite the absence of these cell populations, LAM rapidly up-regulated the frequency of IL-4-producing cells in the bone marrow of these mice (Table II).

Thus, we conclude that the IL-4-producing cell is either a precursor of the lymphocyte population, i.e., before VDJ recombination, or, alternatively, a cell of a separate lineage.

### Materials and Methods

**Comparison of mice strains for induction of IL-4 in bone marrow cells by LAM**

<table>
<thead>
<tr>
<th>Mouse Strain</th>
<th>PBS</th>
<th>LAM</th>
</tr>
</thead>
<tbody>
<tr>
<td>C57BL/6</td>
<td>167.5 ± 31.9</td>
<td>773.8 ± 50.2</td>
</tr>
<tr>
<td>BALB/c</td>
<td>101.6 ± 15</td>
<td>436.6 ± 37</td>
</tr>
<tr>
<td>SCID</td>
<td>227 ± 28</td>
<td>938.3 ± 80</td>
</tr>
<tr>
<td>RAG−2−/−</td>
<td>285 ± 26.6</td>
<td>952 ± 70.7</td>
</tr>
<tr>
<td>αβ TCR−/−</td>
<td>183.3 ± 31</td>
<td>593.3 ± 15</td>
</tr>
<tr>
<td>β,m−/−</td>
<td>36 ± 16</td>
<td>458.3 ± 33</td>
</tr>
<tr>
<td>SJL</td>
<td>478.3 ± 21.9</td>
<td>966 ± 35.4</td>
</tr>
</tbody>
</table>

* Mice were injected i.v. with either PBS or LAM. After 90 min, bone marrow cells were harvested and assayed for IL-4 production by ELISPOT as described in Materials and Methods. For each mouse strain the experiment has been repeated at least twice.

b Mice bred onto B6 background.

c Eleven backcrosses onto B6 background.
mature B cells expressing B220, and thus the use of this marker to select for precursor cells is not recommended. Because the CD19$^+$/c-kit$^+$ cell population of the B6 mouse is equivalent to B220$^+$/CD19$^+$ cells in RAG$^{-/-}$ (36, 37), we sorted B6 bone marrow cells on the basis of c-kit and CD19 expression. Although CD19$^+$/c-kit$^+$ cells did produce IL-4 in response to LAM, these cells did not account for the total number of IL-4-producing cells within the bone marrow (unsorted cells, 1860 ± 126; CD19$^+$/c-kit$^+$ cells, 640 ± 90 IL-4-producing cells/10$^6$ sorted cells). Thus, we suggest that in RAG$^{-/-}$ mice that are devoid of mature T and B lymphocytes, B220$^+$/CD19$^+$ bone marrow cells are responsible for virtually all of the IL-4 produced in response to LAM. In contrast, the bone marrow of immunocompetent mice comprises at least one additional cell population that is probably dependent on VDJ recombination. However, an examination of cells that represent a more mature population along the B cell lineage, namely CD19$^+$/CD25 (TAC)$^+$ small and large cells did not produce IL-4 in response to LAM (data not shown). Thus, the data indicate that microbial contact influences the cytokine milieu within the hematopoietic system.

**Discussion**

In this report we have described a novel cellular source of IL-4, namely the CD19$^+$/B220$^+$ B-lineage precursor cell contained within the bone marrow. To our knowledge this is the first description of a bone marrow precursor cell rapidly secreting IL-4 in response to a microbial component. Infection with *M. tuberculosis* or *M. bovis* BCG is generally considered to be a potent stimulator of IL-12 and IFN-γ production, resulting in a polarization toward Th1 cell development (reviewed in Refs. 10 and 25). Accordingly, Th1 cells are responsible for protection, whereas IL-4-mediated promotion of the development of Th2 cells correlates with susceptibility. It is therefore surprising that infection with *M. tuberculosis* and *M. bovis* BCG induced a rapid increase in the frequency of IL-4-producing cells within the bone marrow.

IL-4 has long been implicated as having a critical role in the development of a Th2-type immune response, as it is both necessary for...
the generation of Th2 cells and produced by these cells (38). However, in contrast to the situation for Th1 priming, the conditions required to generate the appropriate environment for the development of a Th2 response are less well understood. In particular, the cells responsible for the initial production of IL-4 remain to be identified. The most recent interest has revolved around a population of cells expressing both the NK1 molecule and the TCR, NK1\(^{-} T\) cells, which appear to both recognize and require for selection the nonpolymorphic CD1 molecule (23, 39, 40). These cells rapidly produce IL-4 upon in vivo activation with anti-CD3 (21) and in vitro stimulation with CD1 (41), and therefore are prime candidates as initiating cells in the generation of the Th2 response. However, mice deficient in NK1 T cells cannot mount appropriate and functional Th2 responses leading to normal IgE production (22, 24).

Although NK1\(^{-} T\) cells are found within the bone marrow (42), the IL-4-producing cell described herein is present and equally responsive in \(\beta_{m}^{+}\) and SJL mice that are devoid of NK1\(^{+}\) T cells (32, 34). Moreover, from cell-sorting experiments we have characterized this IL-4-secreting cell to be a B220\(^{+}\)/CD19\(^{+}\) early precursor cell of the B-cell lineage (36, 37). Although IL-4 is known to activate mature B cells, these cells do not produce this cytokine themselves but rely on other relevant sources such as T cells. However, little information is available on the cytokine-producing capacities of precursors of the B-cell lineage. Recent evidence studying the regulation of the IL-4 gene has indicated that the IL-4 promoter can function in both Th1 cells and B cells that do not normally produce this cytokine (43). Moreover, a silencer sequence has been identified that appears to be inactive upon binding of the STAT6 transcription factor, which is normally part of the IL-4R signaling pathway. Thus, the possibility exists that this silencer element is inactive in early precursor cells, thereby allowing IL-4 secretion.

To date we believe that this is the first demonstration of IL-4 production from a lymphopoietic precursor cell. Although little is known about the exact role of this cytokine within the bone marrow, IL-4 has been shown to exert effects on the hemopoietic process (1). Thus, it can act in synergy with granulocyte CSF to promote the proliferation and differentiation of precursor cells into mature cells of the myeloid lineage (4) as well as eosinophils and basophils in conjunction with IL-3 (44). IL-4Rs have been shown to be expressed not only on hemopoietic cells but also on bone marrow stromal cells, which suggests that IL-4 may also have a function within the bone marrow environment. Indeed, IL-4 treatment of bone marrow stromal cells reduces cell proliferation via the induction of an as yet unidentified inhibitory factor (45). IL-4-deficient mice have normal B and T cell development, except for a deficiency in IgE and IgG1 Ab responses, but no hemopoietic effects were reported (46). This does not, however, rule out a role of IL-4 in lymphocyte development, because it has recently been shown that IL-13 can substitute for the actions of IL-4 in many biologic processes (47). Experiments are underway to examine the induction of IL-13 by LAM in the bone marrow of both wild-type and IL-4\(^{-/-}\) mice.

Genetically engineered overexpression of IL-4 within the bone marrow resulted in abnormal T cell development, and data suggested that this defect resulted from the expression of IL-4 within a bone marrow cell that was assumed to be a T cell precursor (48). In a different transgenic mouse strain, overexpression of IL-4 led to increases in CD4\(^{+}\), CD8\(^{+}\), and B220\(^{+}\) cells in the periphery as well as overproduction of eosinophils with an increased phagocytic capacity (49). Circumstantial evidence suggests that eosinophils play a role in host defense against \(M.\) \(tuberculosis\) (50, 51); thus, it is tempting to speculate that mycobacterial infection induces a rapid burst of IL-4 within the bone marrow that can, in turn, increase the production of eosinophils, a host cell devoid of the antimycobacterial potency that macrophages possess.

LAMs are plasma membrane-anchored lipopolysaccharides comprising an important component of the mycobacterial cell wall and are thought to influence host responses to this pathogen (28, 52, 53). In this report we have demonstrated the induction of IL-4 in bone marrow cells by manLAM but not araLAM. A converse cytokine activation pattern has been observed previously, in that araLAM is able to induce IL-12 production from macrophages, whereas manLAM cannot (28). Thus, it would seem that the araLAM induces cytokine production, favoring a Th1-type immune response, whereas manLAM induces a Th2-promoting cytokine. These two LAMs also differ in the proposed cellular receptors with which they interact, and this may partly explain their different activation patterns. AraLAM is currently thought to bind to CD14, which also is the receptor for LPS complexed to LPS binding protein (54), whereas manLAM has been shown to interact with the mannose receptor, at least on macrophages (55). Our data suggest that the mannose receptor is in part involved in this process. Furthermore, IL-4 has been demonstrated to up-regulate the expression of the mannose receptor on macrophages (56). Thus, a feedback mechanism within the bone marrow may occur with binding of LAM to the mannose receptor, resulting in IL-4 induction that, in turn, signals receptor up-regulation. Moreover, as the mannose receptor belongs to the family of pattern recognition receptors that can recognize a number of diverse ligands sharing a common molecular pattern (57), such an interaction would have implications for many diverse pathogens.

The data presented here raise the question of whether LAM and/or the mycobacteria themselves directly interact with the precursor cell or whether the induction of IL-4 occurs indirectly. To date we cannot exclude that following i.v. injection mycobacteria or their products induce soluble mediators within the spleen and liver that stimulate IL-4 production in the bone marrow. However, the rapid kinetics of IL-4 production observed favor a more direct interaction between the cell and the microbial components. The isolation of mycobacteria from the bone marrow as rapidly as 90 min after infection is consistent with this theory, and as LAM may not be the only mycobacterially derived molecule that can mediate this effect, experiments are currently in progress to dissect the exact interaction between mycobacteria and their components and the IL-4-producing cell.

The effects of clinical tuberculosis on cells within the bone marrow has not been extensively studied. However, there has been a report of hemologic changes associated with miliary tuberculosis of the bone marrow. These patients all exhibited peripheral lymphopenia, and in 65% of those examined, this condition also extended to the bone marrow (58). These findings of hemopoietic changes are consistent with experimental infection of mice with BCG, in which mice that received a high dose of viable BCG were found to have severe anemia within 14 days of the infection (59). This was found to be a result of an early central change in hemopoiesis resulting in a transient increase in the production of phagocytic cells at the expense of cells of the erythroid lineage (59, 60). Furthermore, polar glycopeptidolipids of \(Mycobacterium chelonae\), which are associated with the mycolic acid layer of the bacterium, have been shown to accelerate the hemopoietic growth and differentiation of murine bone marrow cells (61). Following in vivo administration of these compounds, myelopoiesis was stimulated and resulted in sustained production of granulocytes and monocytes within the peripheral blood. The authors postulate that this effect is mediated via the induction of cytokines by the glycopeptidolipids, which, in turn, stimulates the proliferation
and differentiation of bone marrow progenitors. Thus, in conjunction with these data, we suggest from our findings that constant exposure to mycobacteria may shape hematopoietic processes within the bone marrow microenvironment via the production of cytokines such as IL-4.

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