Increased Bcl-2 and Reduced Bax Expression in Infected Macrophages in Slowly Progressive Primary Murine *Mycobacterium tuberculosis* Infection

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Mycobacterium tuberculosis (MTB) persists in host macrophages (Mϕs) because it has developed mechanisms to escape Mϕ killing. *In vitro* studies have shown that MTB can induce and inhibit apoptosis by causing the expression of Bax and Bcl-2, respectively, suggesting that the infected cells' fate depends on pro- and antiapoptotic signals. In the present study, we investigated the role of Bcl-2 in MTB infection *in situ*. The aim was to study the pattern and distribution of Bcl-2 and Bax in cellular infiltrates of MTB-infected B6D2F1 hybrid mice and correlate the expression with the presence of MTB antigens (MAgs). Using formalin-fixed lung tissues ($n = 45$), our results showed a significant difference in the percentage of Mϕs stained for Bcl-2 or MAgs and Bax ($P < 0.0001$). Bcl-2 expression was increased in a population of Mϕs and corresponded in intensity, colocalization and percentage with that of MAgs on the same cells, while Bax expression was reduced. In lymphocyte aggregates, Bcl-2 and Bax did not show any differences. We conclude that overexpression of Bcl-2 in Mϕs containing MTB may be associated with intracellular survival of the bacilli, thus demonstrating one way by which MTB can escape the host's cellular response and killing.

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INTRODUCTION

Macrophages (Mϕs) provide the first line of defence against infection with *Mycobacterium tuberculosis* (MTB), triggering a cell-mediated immune response in the host [1, 2]. The $CD4^+$ T cells responding to MTB have been shown to secrete cytokines capable of activating Mϕs [3, 4] and enhance their bactericidal capacity [2]. Recent evidence suggests that $CD8^+$ T cells in humans and mice can exert immunoregulatory functions both by secreting cytokines and by cytotoxic cell activity [2, 5]. Activated Mϕs are capable of killing most ingested bacilli. However, MTB has developed mechanisms to resist the hostile Mϕ environment and are thus able to survive within the Mϕs to escape the immune system [1, 6, 7]. Previous studies have shown that MTB can induce increased expression of Fas ligand in infected cells, which may protect these Mϕs from Fas-expressing cells by inducing apoptosis. However, the infected cells are supposed to undergo apoptosis [8]. Apoptosis has been recognized as an important component of protective host responses to virus infection [9]. Apoptosis of the infected cell limits the replication of viruses and reduces the damage caused by the inflammatory process. Viruses have been shown to develop several antiapoptotic genes to evade this defence mechanism [10]. Infection by intracellular bacteria and protozoa will present the host with problems similar to those posed by viruses. Thus, apoptosis might be expected to occur in this setting as well. Bcl-2 is an oncogene known to induce resistance to apoptosis [11, 12] and may also play a role in MTB-induced apoptosis [13]. Previous studies have demonstrated that the mRNA of *Bcl*-2 is downregulated in peripheral blood

monocytes following infection with bacille Calmette–Guérin (BCG) or induction with heat-killed MTB H37Rv [13]. *Bcl*-2 was first located at t (14; 18)(q32; q21) chromosome in follicular cell lymphoma [14] and a founding member of the rapidly expanding family of pro- and antiapoptotic molecules [15]. This protein blocks apoptosis in many cell types [13] and has been shown to contribute to tumourigenesis by prolonging cell survival rather than accelerating the rate of proliferation [16, 17], but the precise mechanism is not clear [11]. *Bax*, another member of the Bcl-2 gene family, promotes apoptosis [17–20] by inhibiting Bcl-2.

A mouse model of slowly progressive primary MTB has been established in our laboratory. In the model, three distinctive phases of infection were described [21]. During the first phase of sub-clinical infection (weeks 0–16), healthy mice had small focal lesions in the lung tissue examined. During the second granulomatous phase starting at week 16, the granulomas increased in size, matured and the Mϕs attained a vacuolated appearance. Mϕs and lymphocytes were organized as separate aggregates, referred to as differentiated granulomas. The mice were moderately sick during this phase, but mortality was low. In the third pneumonic phase (weeks 40–70), the inflammatory cells infiltrated two-thirds of the lung parenchyma, thus losing its focal nature. The number of vacuolated Mϕs was increased, and mortality was very high. Surprisingly, the bacillary numbers were rather stable, indicating a shift in the host's response being responsible for the morbidity and mortality. The main objective of this paper was to examine the pattern of expression and distribution of Bcl-2, Bax and mycobacterial antigens (MAgs) in the lungs of mice during the course of slowly progressive primary MTB and to correlate the expression of Bcl-2 and Bax with MAgs. Our hypothesis is that infection with MTB prevents the infected cells from undergoing apoptosis by inducing antiapoptotic proteins, thereby protecting them.

MATERIALS AND METHODS

B6D2F1 hybrid mice were purchased from Bomholt Gård Breeding and Research Centre Ltd. These were infected intraperitoneally with 1.5×10^6 CFU of H37Rv strain of MTB. The mouse model has been described previously [21]. The number of mice were grouped into phases: 18 in Phase 1 (weeks 1, 2, 4, 8 and 16); 15 in Phase 2 (weeks 20, 24, 29, 33 and 37); and 12 in Phase 3 (weeks 41, 52, 57 and 71). The specimens from the lungs were immediately fixed in 10% buffered formalin and embedded in paraffin as described previously [21]. From each block, 5 μm-thick serial sections were cut and placed on glass slides treated with 0.01% aqueous solution of poly-L-lysine (300,000 molecular weight – Sigma Chemical Co., St. Louis, MO, USA). The first three sections were used for immunohistochemical staining. The fourth section was stained by haematoxylin and eosin for histology. The sections were treated overnight at 37 °C, deparaffinized and prepared for immunohistochemistry as described previously [10, 21–23].

Immunohistochemistry. Briefly, endogenous peroxidase activity was blocked with 0.3% H_2O_2 for 15 min. Antigens were retrieved by treatment with 0.1% protease for 10 min at 37 °C. Nonspecific binding was blocked by incubation with 4% bovine serum albumin in 1.5% normal serum of animal in which the secondary antibody was raised. Endogenous biotin was blocked with avidin and biotin blocking solution (Vector Laboratories, Burlingame, CA, USA). The primary antibodies used were polyclonal rabbit antimouse *Bcl*-2 (PharMingen–Becton Dickinson Company, San Diego, CA, USA) (1 : 1000) and rabbit antimouse Bax (PharMingen–Becton Dickinson Company) (1 : 500). MAgs were detected by anti-BCG (1 : 5000) (Dako A/S, Copenhagen, Denmark). This antibody reacts with multiple antigens of sonicate and culture fluid of BCG Copenhagen (Dako A/S) [23]. The *Mycobacterium bovis*, BCG and MTB are extremely similar in their antigenic content [24]. Antigens detected by anti-BCG are thus referred to as MAgs in our findings. The control sections were treated with phosphate-buffered saline and normal rabbit serum in the same dilution as rabbit anti-*Bcl*-2 and anti-Bax, instead of primary antibodies. Sections were incubated with primary antibodies overnight at 4 °C in a humidified chamber. Secondary antibodies consisted of biotinylated goat antirabbit immunoglobulin G (IgG) (Pharmingen–Becton Dickinson Company) (1 : 200) and biotinylated swine antirabbit IgG (Dako A/S) (1 : 400) and were applied on the sections for 30 min. The sections were washed with respective buffer (×3) between each step. This was followed by incubation at room temperature for 30 min with avidin–biotin complex (ABC – Dako A/S, Glostrup, Denmark). Visualization was achieved with the application of H_2O_2 and 3-amino-9-ethylcarbazol-containing buffer on the sections for 15 min in the dark. The slides were washed with distilled water for 5 min, counterstained with Harris' haematoxylin for 15 s and mounted by an aqueous immunomount medium (Shandon, Pittsburgh, PA, USA).

Cell counting. Cell counts were performed using light microscope (Leitz/Leica, Wetzlar, Germany) using ×40 objective and an ocular fitted with 0.25×0.25 mm graticule. The focal lesions in the lungs consisted of distinct Mϕ and lymphocyte aggregates, classified as Mϕ-predominant aggregates (MPDAs) and lymphocyte-predominant aggregates (LPDAs), respectively, as described earlier [8]. Counting was done by indiscriminate selection of one to five fields in each of the LPDA and MPDA lesions. The positively stained cells and the total number of nucleated cells were counted in each field using Dickinson cell counter. The percentage of all the stained cells was calculated from the overall total of the cells counted. The absolute number of stained cells in the inflammatory lesions in the lung section from each mouse was evaluated as:

$$N_{\text{abs}} = \frac{N_{\text{avg}}}{A_{\text{f}}} \times A_{\text{fl}},$$

where N_{abs} is the absolute number of stained cells in the inflammatory lesions, N_{avg} the average number of stained cells in three to five fields, A_{f} the area of each field and A_{fl} the area of focal lesion in the section. The area of the focal lesion was measured as described previously [21].

Statistical analysis. Cell counts were entered into the SPSS® statistical program. Kruskall–Wallis test was used for the comparison of more than two independent groups, and Mann–Whitney

U-statistical test was used to compare two independent groups. The Wilcoxon signed rank test was used for comparison of dependent samples.

RESULTS

The morphology of the lesions in murine slowly progressive primary tuberculosis has been described previously.

MPDA

In Phase 1, the lesions were not recognized microscopically or were very small, increasing slowly in size from the end of Phase 1 during week 16. With the progress of infection and recruitment of inflammatory cells, the absolute number of stained cells increased similarly. There were only few Mϕs expressing Bcl-2, Bax and MAgs at weeks 1, 2, 4, 8 and 12. At week 16, there were an increased number of positive cells. Figure 1A shows the percentages of Bcl-2-, Bax- and MAg-stained Mϕs during the three phases of infection. The percentages of stained Mϕs were significantly different for Bcl-2 or MAgs and Bax ($P < 0.0001$). Figure 1B shows the absolute number of cells stained with anti-Bax, anti-*Bcl*-2 and anti-BCG. The absolute number of stained Mϕs was significantly different between the phases ($P < 0.0001$), and the number of stained cells correlated with the size of the lesions.

Bax. The percentage of Bax-stained Mϕs (Fig. 1A) remained stable during Phase 1 of infection, except a decrease at week 12. During Phase 2, the percentage of Bax-stained cells increased significantly compared with Phase 1 ($P < 0.0001$). Phase 3 did not demonstrate a significant difference in Bax-stained cells compared with Phase 2 ($P = 0.792$), while it was significantly higher than in Phase 1 ($P < 0.0001$). Bax was strongly expressed on a smaller percentage of activated Mϕs even at high dilution (1 : 8000), at which staining on other cells disappeared (data not shown). The absolute number of Bax-positive cells (Fig. 1B) in Phase 1 corresponded to the size of lesions that either were not recognized microscopically or were very small in size. With the progress of infection and recruitment of inflammatory cells, the size of the lesions increased. The absolute value of

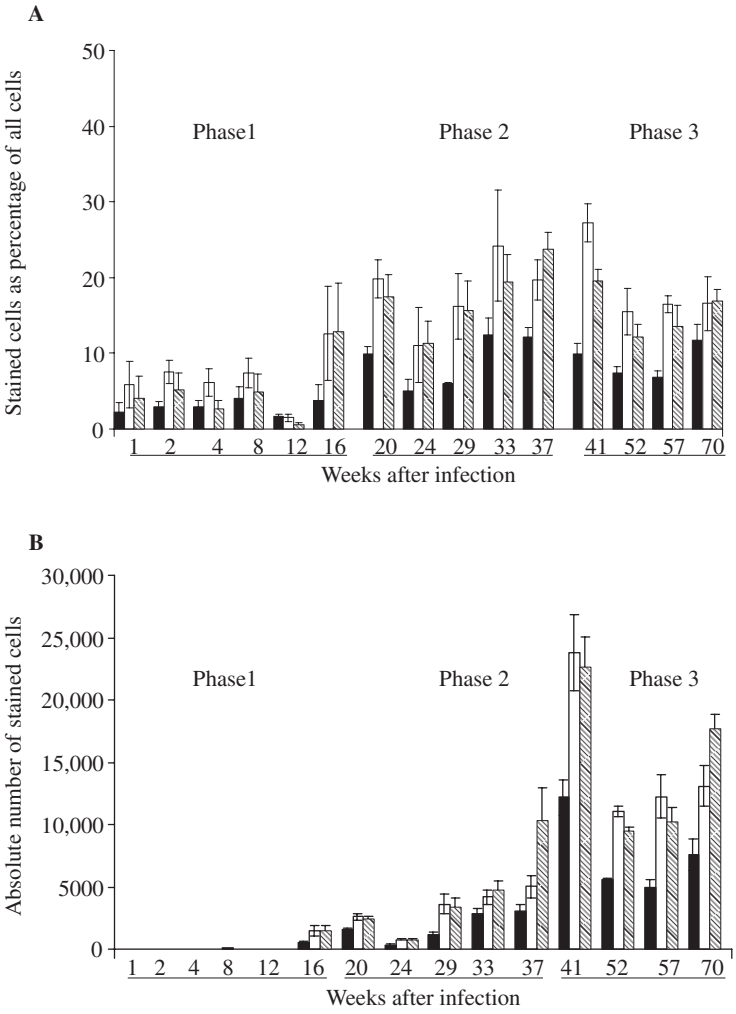

Fig. 1. Distribution of stained cells in macrophage (Mϕ)-predominant aggregate (MPDA) with Bax ■, Bcl-2 □ and *Mycobacterium tuberculosis* (MTB) antigens (MAgs) ▨ through the phases (1–3) of infection with H37Rv MTB in slowly progressive primary disease in mice analysed by using immunohistochemistry. In each group ($n = 3$), the horizontal lines under the weeks represent phases of infection, and bars represent the standard error of the mean (SEM). (A) Stained cells as the percentage of all cells in lesions. The percentage of stained cells expressing Bax and Bcl-2 and MAgs increased with the progress of infection. (B) Distribution of absolute number of stained cells in slowly progressive primary MTB infection in mice showing increase in the number of positively stained cells through the phases of infection.

Bax-stained cells increased significantly with a shift from Phase 1 to 2 ($P < 0.0001$). During Phase 3, the absolute number of Bax-positive cells increased significantly compared with Phase 2 ($P = 0.007$) as well as Phase 1 ($P < 0.0001$). Peak Bax expression was noticed at week 41, declining gradually at weeks 52 and 57 and then slightly increasing at week 70.

Bcl-2. In Phase 2, the percentage of stained cells was significantly higher than in Phase 1 ($P < 0.0001$). There was no difference between the percentage of stained cells in Phase 2 and Phase 3 ($P = 0.648$). Compared with Phase 1, however, the percentage of stained cells in Phase 3 was significantly higher ($P < 0.0001$). The absolute number of Bcl-2-positive cells (Fig. 1B) in Phase 1 corresponded to the size of lesions. During Phase 2, there was an increase in anti-Bcl-2-stained Mϕs at week 20, with a decline in week 24 and then a steady increase until week 37 during Phase 2. The absolute number of stained cells was significantly higher in Phase 2 compared with Phase 1 ($P < 0.0001$). With the progression of infection to Phase 3, the absolute number of cells increased significantly compared with Phase 2 ($P = 0.003$) and Phase 1 ($P < 0.0001$). Interestingly, there was no difference in *Bcl*-2-positive aggregates of Mϕs at week 70 compared with weeks 52 and 57, as noticed during week 41, with corresponding increase in the number of MAg-containing cells during late infection ($P > 0.05$).

MAgs

MAgs were found only in cells of the Mϕ aggregates. The staining pattern corresponded in intensity as well as the percentage of stained cells with that of Bcl-2 on the same cells. There was a significantly higher percentage of stained cells in Phase 2 compared with Phase 1 ($P < 0.0001$). The percentage of MAg-positive cells in Phase 3 was not different compared with Phase 2 ($P = 0.236$) but

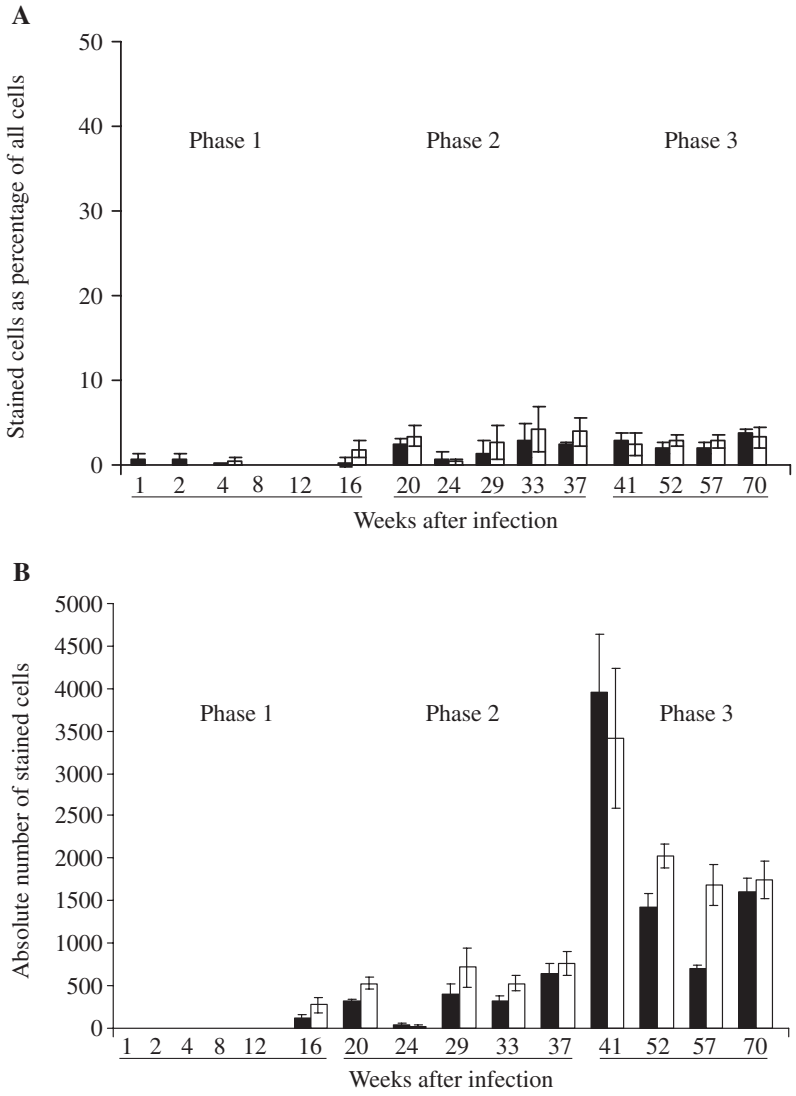

Fig. 2. Distribution of stained cells in lymphocyte-predominant aggregate (LPDA) with Bax- ■ and Bcl-2-positive lymphocyte aggregates □ during the three phases of infection with H37Rv *Mycobacterium tuberculosis* (MTB) in slowly progressive primary disease in mice analysed by using immunohistochemistry. In each group ($n = 3$), the horizontal lines under the weeks represent the phases of infection, and bars represent the standard error of the mean (SEM). (A) Stained lymphocytes as the percentage of all cells in the lesions. The number of stained lymphocytes for both Bax and Bcl-2 increased with the progression of infection. (B) Absolute number of positively stained cells in LPDA. Note that in weeks 1–12, the lesions were small with fewer positive inflammatory cells. From week 16 onwards, the inflammatory foci increased and the absolute number of infiltrating similarly increased. Bars represent the SEM. There was an increase of absolute number of stained cells with the progression of disease from Phase 1 to 2 and 3 for both Bax and Bcl-2.

was higher compared with Phase 1 ($P < 0.0001$). The absolute number of MAg-positive cells (Fig. 1B) in Phase 1 corresponded to the size of lesions. An increase was noticed at week 20, and the absolute number of stained cells was significantly higher during the second phase compared with Phase 1 ($P = 0.005$). The absolute number of cells was lower at weeks 24 and 29. In Phase 3, the number of positive cells was significantly higher than in Phase 2. The number of MAgs increased sharply at week 41 but reduced later in the phase at weeks 52, 57 and 70.

Comparison between Bcl-2-, Bax- and MTB-positive cells

The percentage as well as the absolute number of Mϕs expressing Bcl-2 was significantly higher than Bax expression in Phases 1, 2 and 3 ($P = 0.002$). The percentage of Bcl-2-positive cells was significantly higher than MAg-positive Mϕs during Phase 1 ($P = 0.002$) but was not significantly different during Phases 2 and 3. There was no difference in the absolute values of Bcl-2-positive cells and MAg-positive Mϕs in Phases 1 and 3, but Bcl-2-positive cells were significantly higher than MAg-positive Mϕs during Phase 2 ($P = 0.017$). The percentage of Bax-positive cells was not significantly different from MAg-positive cells in Phase 1, but their percentage was significantly lower in Phase 2 ($P = 0.001$) and Phase 3 ($P = 0.004$). The absolute number of Bax-positive cells was lower than MAg-positive cells in Phase 1 ($P = 0.013$). There was no difference between these stained cells in Phase 2, and in Phase 3, their absolute values were lower than MAg-positive cells ($P = 0.019$).

LPDA

Figure 2A shows the percentage and Fig. 2B the absolute number of lymphocytes stained with Bax and Bcl-2. The lymphocyte aggregate areas did not express any MAg-positive cells. The number of both Bcl-2- and Bax-positive cells was significantly different between the phases ($P < 0.0001$).

Bax. Bax-expressing lymphocytes (Fig. 2A) were present in the lesions except in weeks 8 and 12. With progression to Phase 2 of infection, there was a significantly higher percentage of lymphocytes expressing Bax compared with Phase 1 ($P = 0.007$). In the third phase, there was no significant change in the Bax-expressing lymphocytes compared with Phase 2 but was higher when compared with Phase 1 ($P < 0.0001$).

The absolute number of Bax-expressing cells (Fig. 2B) was higher in Phase 2 compared with Phase 1 ($P = 0.004$). Similarly, it was higher in Phase 3 compared with Phase 2 and Phase 1 ($P < 0.0001$).

Bcl-2. In Phase 1, the percentages of *Bcl*-2-positive lymphocytes were seen to gradually increase in lesions at weeks 4, 8 and 16 but not at weeks 1, 2 and 12 (Fig. 2A). In Phase 2, the percentage of Bcl-2-stained lymphocytes was higher than in Phase 1 ($P = 0.007$). Except for week 24, the percentages of stained cells remained stable during Phase 2. There was no difference in the expression of Bcl-2 on lymphocytes in Phase 3 as compared with Phase 2 ($P = 0.614$), but the percentage was higher in Phase 3 compared with Phase 1 ($P < 0.0001$). There was a significant increase in absolute number of Bcl-2-positive cells between Phases 1 and 2 and 1–3 ($P < 0.0001$), as well as from Phase 2 to 3 ($P = 0.002$).

Cellular colocalization of cells expressing MTB antigens and Bcl-2

The parallel sections at 5–10 μm distance were closely examined for the expression of MAgs, Bcl-2 and Bax in the same cells. The Mϕs, which contained MAgs (Fig. 3B), also showed a strong expression of Bcl-2 (Fig. 3A) at almost all time-points after infection. The expression of Bax was weak on these cells (Fig. 3C). In 45 serially cut sections, colocalization between Bcl-2 and MAgs was found in 61% of the lesions in Phase 1, 93.3% in Phase 2 and 91.7% in Phase 3. When all the phases were combined, positive colocalization between *Bcl*-2 and MAgs on Mϕs was seen in 80% of tissue sections.

Bcl-2, Bax and MTB antigen staining on other structures

Figure 4A and B shows staining of Bax and Bcl-2, respectively, on bronchial epithelium and smooth muscle. During early infection, MAgs were occasionally detected on bronchial epithelial cells, alveolar cells (pneumocytes) as well as some smooth muscle cells. Bcl-2 staining was strongly expressed on smooth muscle and some red blood cells. On bronchial epithelium, the staining was on the luminal side of the epithelium, whereas Bax stained more strongly on both the luminal and basal parts of epithelial cells, red blood cells and smooth muscle cells.

DISCUSSION

These results show that cells expressing high amounts of MAgs in the murine slowly progressive primary MTB lesions colocalize with the cells expressing high amounts of the antiapoptotic molecule Bcl-2, whereas the expression of the apoptosis-inducing Bax molecule was lower on these infected Mϕs. These data suggest that MTB, by upregulating *Bcl*-2 in infected Mϕs, may cause the inhibition of apoptosis of these host cells and use them as a sanctuary to evade the hostile immune response. This result confirms other *in vitro* studies which show that MTB can cause both induction and inhibition of apoptosis, indicating that the cells' fate depends on pro- and antiapoptotic signals from both host and pathogen [6, 7, 25–30]. There is evidence that MTB in its interaction with human alveolar Mϕ can trigger apoptosis of host cells [6]. The apoptotic response to mycobacteria may, however, be restricted to mature Mϕs *in vitro* [27, 30]. Virulent MTBs

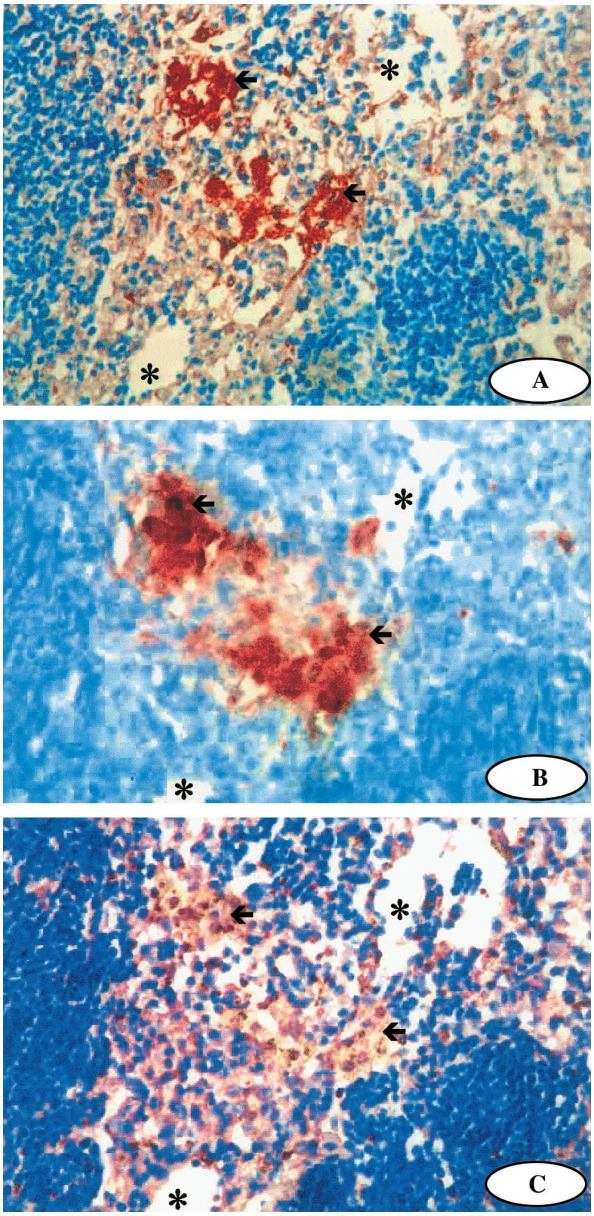

Fig. 3. Expression of Bax, Bcl-2 and *Mycobacterium tuberculosis* (MTB) antigens (MAgs) in the lung tissue granuloma of mice with slowly progressive primary MTB infection at week 16 after infection with H37Rv MTB (×252) detected by using immunohistochemistry. (A) Bcl-2 is expressed strongly in a small population of cells in macrophage (Mϕ)-predominant aggregate (MPDA) (arrows). (B) A parallel section 5 μ apart shows that the same group of cells which express Bcl-2 contains high amount of MAgs (arrows). (C) A parallel section 10–15 μ apart shows that the expression of Bax is weaker on this group of cells (arrows). Asterisks in blood vessels denoting landmarks for lesions A, B, C in the sections for colocalization of stained cells.

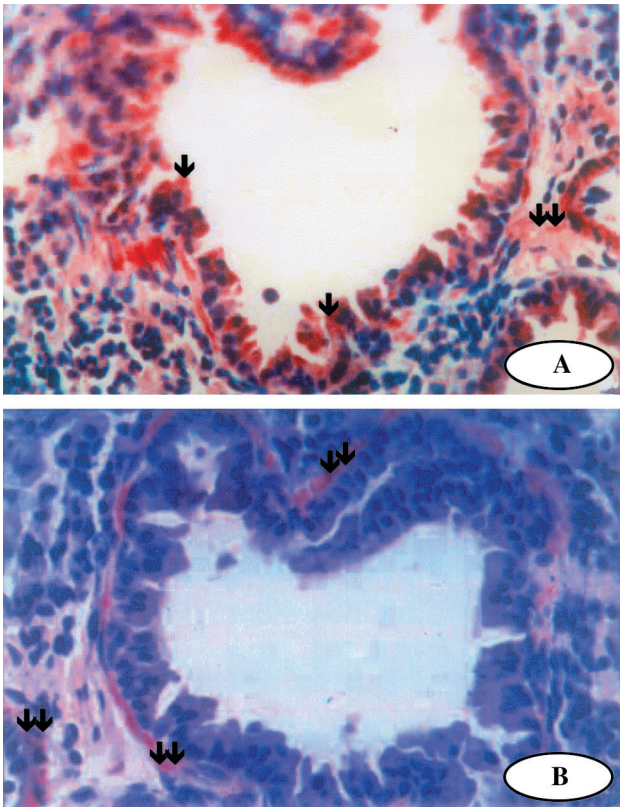

Fig. 4. Expression of Bax and Bcl-2 in the lung tissue (bronchial epithelium) of mice with slowly progressive primary *Mycobacterium tuberculosis* (MTB) infection at week 4 after infection with H37Rv MTB (×252), detected by using immunohistochemistry. There is strong granular expression (arrows) of Bax in bronchial epithelial cells (A) and Bcl-2 expression (double arrows) on bronchial epithelium and smooth muscles (B).

in low numbers have been shown to rescue human monocytes from apoptosis [25, 27]. In contrast, differential Mϕs lose their sensitivity to growth factor withdrawal and are susceptible to activation-induced apoptosis [29]. Thus, the regulation of programmed cell death in terminally differentiated Mϕs is distinct from that of their precursors [31]. These suggestions seem to support our hypothesis that the inhibition of apoptosis could favour MTB by providing an intracellular sanctuary. Support for this hypothesis also comes from other studies which have shown that apoptosis and not lysis of infected Mϕs has a direct antimicrobial effect [27, 32–35]. Furthermore, inverse correlation has been found to occur between virulence and MTB apoptosis [19]. Further support for our results comes from studies which have shown that the infected Mϕs and human HIV-1-infected monocytes also express interleukin-10 (IL-10) and upregulate Bcl-2, thus altering Mϕ accessory function [36, 37]. This cytokine has also been shown to increase the survival of lymphocytes and germinal centre B cells by the induction of Bcl-2 protein [37, 38]. In human alveolar Mϕ, IL-10 can reduce lipopolysaccharide- and interferon-γ-induced apoptosis [39] and similarly prevent apoptosis in murine Mϕs infected with *Salmonella choleraesuis* [40]. Our

laboratory has previously shown that infected Mϕs express high amounts of IL-10 [41]. Taken together, these observations suggest that MTB can induce increased expression of Bcl-2, either through the secretion of cytokines like IL-10 or via other mechanisms, thus preventing the Mϕs containing the bacilli from undergoing apoptosis. This may allow proliferation of the bacilli, explaining the persistence in Mϕs in spite of acquired immunity [39]. The differences found in the expression of MAgs show that some of the Mϕs are probably not able to rid themselves of mycobacteria, suggesting a heterogeneous Mϕ population. The graded overexpression of Bcl-2 in activated Mϕ could lead to reduced expression of Bax. The expression of the latter was found to be lower in the Mϕs containing MAgs, and hence the hyperexpression of Bcl-2 could be a threshold phenomenon depending on the mycobacterial load. The higher expression of Bcl-2 over Bax in the same cells suggests that for Bcl-2 to prevent cell death and for the cells to survive, an early change occurs in the cellular localization and distribution of soluble Bax. This favours the increased expression of Bcl-2.

There is documented evidence that upregulation of Bcl-2 protein may cause systemic autoimmune disease through the accumulation of activated T lymphocytes [42]. Lymphocytes were found to have upregulated the increase of Bcl-2 in multiple sclerosis (MS) plaque [43] oligodendrocytes [44], but lower expression of Bcl-2 was observed in MS plaque intrathecal lymphocytes [45]. This suggests that there is differential upregulation of Bcl-2 protein in different compartments in same or different diseases. In tuberculosis, the infection is characterized the by formation of granulomas and accumulation of T cells; we therefore considered it important to describe the distribution of these proteins in T-cell aggregates as well. We show that most of the lymphocyte aggregates had no detectable Bax or Bcl-2. The detectable Bax and Bcl-2 were not different in the aggregates during infection with MTB. This result confirms previous findings of Shareif and Semra that showed compartmented and significant reduction in Bax/Bcl-2 expression ratio in lymphocyte aggregates in MS [46]. We suggest that the relatively low expression levels of these proteins in lymphocyte aggregates may be important in maintaining homeostasis of the immune system, as lymphocytes adapt to apoptosis signals owing to MAgs.

We have demonstrated the presence of MTB antigens on unique sites in smooth muscle cells and bronchial epithelial cells as well as in antigen-presenting cells. There seems to be other nonantigen-presenting cells, including cells in the blood vessel walls and pneumocytes. The reason for the presence of MAgs in these sites is still unclear, but was first reported by Bermudez [47] and Hernández-Pando *et al*. [48]. These cells may possibly offer protected sites for the persistence of bacilli, as they do not process and present foreign antigens [47, 48].

We conclude from this study that MTB may interfere with the intracellular distribution of Bcl-2 and Bax, causing an increased expression of Bcl-2 and reduced expression of Bax. This may prevent the infected cells from undergoing apoptosis, thereby protecting them. These 'relatively long-lived' infected cells may create an immune privilege site for MTB, thus providing the mycobacteria with an intracellular sanctuary.

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