Induction of cell cycle arrest and apoptosis by BCG infection in cultured human bronchial airway epithelial cells

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Lai Y-M, Mohammed KA, Nasreen N, Baumuratov A, Bellew BF, Antony VB. Induction of cell cycle arrest and apoptosis by BCG infection in cultured human bronchial airway epithelial cells. Am J Physiol Lung Cell Mol Physiol 293: L393–L401, 2007. First published May 25, 2007; doi:10.1152/ajplung.00392.2006.—Bronchial airway epithelial cells (BAEpC) are among the first cells to encounter M. tuberculosis following airborne infection. However, the response of BAEpC to M. tuberculosis infection has been little studied. This study investigates the response of a human BAEpC cell line (BEAS-2B) to infection with Mycobacterium bovis Bacille Calmette Guerin (BCG). Cultured human BEAS-2B cells were experimentally infected with BCG. Uninfected BEAS-2B cultures were included as controls. Following infection, BEAS-2B cells were evaluated by various methods at various time points up to 3 days. Cell proliferation was evaluated by cellular bioreduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. Distribution of cells along the cell cycle was evaluated by FACS analysis of cellular DNA. Apoptotic cells were identified by cell death ELISA and the terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end-labeling method. Eighty-four apoptosis-relevant genes were screened by PCR gene microarray. Translation of Fas, Fas ligand (Fas-L), and Fas-associated death domain (FADD) were evaluated quantitatively by real-time PCR. Expression of Fas and FADD proteins was evaluated by immunofluorescence and Western blot. Activity of caspase-3 and caspase-8 was evaluated by colorimetric assay of their enzymatic activity. BCG infection of BEAS-2B cells inhibits proliferation, induces cell cycle arrest at the G0/G1 phase, causes apoptosis, modulates transcription of multiple apoptosis-relevant genes, promotes translation of Fas, Fas-L, and FADD, upregulates expression of Fas and FADD proteins, and increases activity of caspase-3 and caspase-8. Infection with BCG does not cause any significant change in the secretion of TGF-β. The roles of Fas and FADD as mediators of BCG-induced apoptosis in BEAS-2B cells were tested by partial blockade of Fas and FADD expression with silencing RNA. Partial blockade of Fas or FADD expression results in a decreased apoptotic response to BCG infection. In conclusion, BCG induces cell cycle arrest and apoptosis in BEAS-2B cells. BCG induced apoptosis of BEAS-2B cells via the Fas death receptor pathway.

Bacille Calmette Guerin; Fas; Fas ligand; Fas-associated death domain protein; caspase-3; caspase-8

Tuberculosis (TB) infection is transmitted from person-to-person via respiratory droplets. Common sites of TB disease include the lungs, pleura, central nervous system, lymphatic system, genitourinary system, skeleton, and joints. TB may also be disseminated. The World Health Organization estimates that one-third of the world’s population has been infected with TB. TB is estimated to be responsible for at least 1.5 million deaths annually. Of persons infected with TB ~10% will eventually go on to develop active disease. The mortality rate for active TB is more than 50%. The different manifestations of infection with M. tuberculosis reflect the balance between the bacillus and host defense mechanisms, in which the quality of host defense determines outcome.

Acquired T cell-mediated immunity plays a central role in the elimination of M. tuberculosis. CD4+ T cells exert their protective effect by the production of cytokines, primarily γ-interferon (IFN-γ), and by activation of macrophages and cytotoxic lymphocytes. Other T cell subsets, like CD8+ T cells, likely contribute to defense from the infection by secreting cytokines and lysing infected cells (6, 12). The lysis of infected cells can directly kill intracellular M. tuberculosis (10).

However, T cell-mediated adaptive immunity is not completely protective against infection by M. tuberculosis. In a murine model, acquired T cell immunity protects against disseminated M. tuberculosis infection but does not prevent the initial aerogenous pulmonary infection (9). In humans, naturally acquired M. tuberculosis-specific T cell immunity does not prevent exogenous reinfection of the lung by M. tuberculosis (13). In addition to acquired immunity is also important the resistance against M. tuberculosis infection. Alveolar macrophages are the primary cell type involved in the initial uptake and elimination of the pathogens by intracellular killing. Mycobacteria are able to resist various mechanisms by which macrophages usually achieve intracellular killing. The ability of M. tuberculosis to survive within macrophages partially explains why immuno-competent hosts generally may contain the pathogen without eliminating it.

The initiation of infection with M. tuberculosis occurs when organisms in small droplet nuclei are inhaled into the respiratory tract and lungs (4). Bronchial and alveolar epithelial cells are among the first cells to encounter M. tuberculosis following aerogenous infection. Bronchial epithelial cells are held together by tight junctions and make up a protective barrier against M. tuberculosis infection in vivo. It was demonstrated that M. tuberculosis enters and survives within human alveolar epithelial cells in vitro (3, 8). Epithelial cells may contribute to the local inflammatory response in human M. tuberculosis infection by producing chemokines that attract monocytes, lymphocytes, and polymorphonuclear leukocytes (8).

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Given the importance of bronchial airway epithelial cells (BAEpC) as one of the body’s initial defenses against mycobacterial infection, we were motivated to investigate the interaction between mycobacterial pathogens and BAEpC. We hypothesized that mycobacterial infection induces apoptosis in BAEpC. We tested that hypothesis in a model using a human BAEpC culture (cell line BEAS-2B) experimentally infected with *M. bovis Bacille Calmette Guerin* (BCG) in vitro. Our results show that BCG infection of human BAEpC line (BEAS-2B) cells induces apoptosis of those cells, at least partially mediated by the Fas death receptor pathway.

**MATERIALS AND METHODS**

**BAEpC culture and BCG infection.** BAEpC (BEAS-2B, ATCC no. CRL-9699) cell line was obtained from American Type Culture Collection (ATCC; Rockville, MD) and maintained at 37°C, 5% CO2 in BEGM media (Cambrex Bio Science, Walkersville, MD) according to the guide provided by ATCC. BAEpC were seeded in culture plates at a density of 5 × 10⁴ cells/cm². The cultures were infected with BCG at a ratio of 50 bacilli/cell. The infected cultures were incubated for various lengths of time up to 72 h. After incubation with BCG, BAEpC were washed three times with PBS and subjected to analysis in various experiments.

**Extraction of RNA, reverse transcription, and RT² Profiler PCR microarray.** Total RNA was extracted from cultured BAEpC using high-purity RNA isolation Kit (Roche Applied Science, Indianapolis, IN). The RNA was diluted with RNase-free water to 500 ng/μl. Ten microliters of each sample were mixed with 1 μl of 3.5 μM anchored olig-(d)T23 (Sigma, St. Louis, MO) and were denatured at 70°C for 10 min followed by 2 min incubation on ice. Samples were then mixed with 9 μl of reaction buffer containing 10 nM deoxynucleotides, 20 U of enhanced AMV reverse transcriptase, and 20 U of RNase inhibitor for cDNA synthesis performed for 50 min at 42°C followed by an enzyme inactivation step of 15 min at 70°C. The real-time PCR for microarray assay was performed using the RT² Profiler PCR microarray according to the manufacturer’s protocol (SuperArray Bioscience, Frederick, MD). Gene expression was compared according to the C_T value.

**Assay of BAEpC proliferation.** BAEpC were seeded in 24-well culture plates for 20 h and then infected with BCG for 24, 48, or 72 h. Cell number was counted with a metalized hemacytometer (Haussser Scientific, Horsham, PA). Cell proliferation was measured by cellular bioreduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. A 100-μl reaction volume and incubated at 37°C for 4 h. The fluorescent signal was during the elongation step. The real-time PCR products were confirmed by electrophoresis on a 2% agarose gel.

**Estimation of Fas, Fas ligand, and Fas-associated death domain mRNA by quantitative real-time PCR.** Total RNA from cultured BAEpC was purified and diluted with RNase-free water to 100 ng/μl. Ten microliters of each sample were then reverse transcribed into cDNA. After reverse transcription, 80 μl RNase-free water were added to each sample. Ten microliters of diluted cDNA product were mixed with 25 μl of SYBR Green JumpStart ReadyMix, 0.5 μl of internal reference dye, 13.5 μl H₂O, and 1.0 μl of the corresponding Fas, Fas ligand (Fas-L), or Fas-associated death domain (FADD) oligonucleotide primers (80 nM final concentration) to total 50 μl volume for quantification real-time PCR (Table 1).

The quantitative real-time PCR was performed by SYBR Green method using the Applied Biosystems 7500 Real-Time PCR System with the following profile: 1 cycle at 94°C for 2 min, 40 cycles at 94°C for 15 s, 60°C for 1 min, and 72°C for 1 min. The acquisition of the fluorescence signal was during the elongation step. The real-time PCR products were confirmed by electrophoresis on a 2% agarose gel.

Data analysis was carried out by the ABI sequence detection software using the relative quantification. The threshold cycle (C_T), which is defined as the cycle at which PCR amplification reaches a significant value, is given as the mean value. The relative expression of each mRNA was calculated by ΔC_T method (where ΔC_T is the value obtained by subtracting the C_T value of the internal loading control gene β-actin mRNA from the C_T value of the target mRNA). The amount of the target relative to the β-actin mRNA was expressed as 2^(-ΔΔC_T).

**Estimation of Fas, Fas-L, and FADD proteins by Western blot analysis.** BEAS were lysed in RIPA buffer and 20 μg of total proteins were resolved by SDS-PAGE and transferred to a nitrocellulose membrane. Proteins were detected with antibodies against Fas (1:1,000), Fas ligand (1:500), and FADD (1:1,000; Santa Cruz Biotechnology, Santa Cruz, CA). Blotted antibody was developed by horseradish peroxidase-conjugated secondary antibody and enhanced chemiluminescence detection system. β-Actin was probed as an internal loading control. Density of the Western blotting bands was measured by Image software for relative quantitative analysis. BEAS-2B cells (1 × 10⁵ cells/well) were transfected with Fas siRNA.
or FADD siRNA (50 μmol/well) in a 24-well plate following the manufacturer’s instructions (Santa Cruz Biotechnology). Fresh medium was added after 6 h. Cells were incubated with the siRNA for an additional 36 h. The BEAS-2B cells were then subcultured in fresh culture medium. Cellular levels of Fas mRNA or FADD mRNA were measured by quantitative real-time PCR as described above.

BEAS-2B cells were transfected with Fas siRNA or FADD siRNA as described above. Forty-two hours after transfection, cells were replated with fresh medium, infected with BCG at a ratio of 50 bacilli/cell, and then incubated for 16 h. To evaluate the transfection efficiencies of gene silencing in Fas and FADD siRNA-transfected cells, Fas and FADD protein expression were estimated by Western blot analysis. Apoptosis of the BEAS-2B cells was detected by cell death ELISA as described above.

### Table 1. Details of primers used for quantitative real-time PCR

<table>
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<tr>
<th>Gene</th>
<th>Accession Number</th>
<th>Primer Sequence</th>
<th>Positions</th>
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<tr>
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<td>NM_152877</td>
<td>Forward: 5′-ctccaaggagtggaattga-3′</td>
<td>439–458</td>
<td>196</td>
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<td>200</td>
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<td>829–848</td>
<td>200</td>
</tr>
<tr>
<td></td>
<td></td>
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<td>1009–1028</td>
<td>199</td>
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<tr>
<td>FADD</td>
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<td>671–690</td>
<td>399</td>
</tr>
<tr>
<td></td>
<td></td>
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<td>399</td>
</tr>
<tr>
<td>β-Actin</td>
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<td>Forward: 5′-aaagcacatgccacttactc-3′</td>
<td>1265–1284</td>
<td>399</td>
</tr>
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</table>

FasnL, Fas ligand; FADD, Fas-associated death domain.

Fig. 1. *Bacille Calmette Guerin* (BCG) inhibited the growth of bronchial airway epithelial cell (BAEpC) line (BEAS-2B) cells in vitro. Cultured BAEpC were infected with BCG at 50 bacilli/cell for 1, 2, or 3 days. Cells were counted with a hemacytometer. Compared with the uninfected control group, BCG significantly decreases the population of BEAS-2B cells (A). Proliferation of BAEpC was measured by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The MTT metabolic product is decreased in the BCG-infected BAEpC (B). *P < 0.05 compared with control.

Fig. 2. BCG-induced cell cycle arrest in cultured BEAS-2B cells. Cultured BEAS-2B cells were infected with BCG at 50 bacilli/cell for 1, 2, or 3 days and then cells were fixed and stained with propidium iodide for FACScan analysis. Compared with uninfected control, BCG infection increases the percentage of BEAS-2B at G0/G1 phase (A) and correspondingly decreases the percentage of cells at S phase in cultured BEAS-2B (B). *P < 0.01 compared with control.
Measurement of transforming growth factor-β secretion.

BEAS-2B cell culture was infected with BCG at a ratio of 50 bacilli/cell and then incubated for 16 h. Transforming growth factor (TGF)-β concentration in the culture medium was measured by ELISA (R & D System, Minneapolis, MN).

Statistical analysis. Data were analyzed by SigmaStat statistical software. Results are expressed as means ± SD. The data were analyzed by ANOVA with the Student-Newman-Keuls test for multiple comparisons and were considered statistically significant if P values were <0.05.

RESULTS

Proliferation. The proliferation of BAEpC in the presence of BCG was estimated by direct cell counts using a hemocytometer and by MTT assay. A BEAS-2B that is incubated with BCG grows more slowly than uninfected BEAS-2B. BCG infection significantly decreased BEAS-2B cell proliferation in a time-dependent manner. No significant inhibition of proliferation was noticed at 24 h of infection. Significant inhibition of proliferation is seen at later time points. BCG caused 31.6%
decrease in proliferation of BEAS-2B following 48 h of infection compared with uninfected cells \( (P < 0.001) \). BCG caused 26.9% decrease in proliferation of BEAS-2B following 72 h of infection compared with uninfected cells \( (P < 0.001; \text{Fig. 1A}) \). Similar results were noticed when BEAS-2B proliferation was evaluated by MTT assay. The MTT method confirmed that BCG inhibits BEAS-2B proliferation (Fig. 1B).

**Cell cycle analysis.** Since we noticed that BCG inhibits the BEAS-2B cell proliferation, we further analyzed the cell cycle using propidium iodide staining and FACS analysis. After 24, 48, and 72 h in uninfected cultures, the percentage of cells at the G0/G1 phase were 42 ± 4, 39 ± 4, and 38 ± 4%, respectively, whereas in BCG-infected cultures, the percentage of cells at the G0/G1 phase increased to 72 ± 5, 74 ± 6, and 70 ± 5% at 24, 48, and 72 h, respectively (at all time points \( P < 0.01 \) compared with control). In uninfected cultures, the percentage of cells in the S phase were 45 ± 3, 49 ± 5, and 48 ± 4% after 24-, 48-, and 72-h incubation, respectively. In

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**Fig. 6.** BCG-induced Fas and FADD expression in cultured BEAS-2B cells. Cultured BAEpC were infected with BCG at 50 bacilli/cell for 16 h. The Fas and FADD proteins were detected by immunofluorescence histology. Compared with control group (A and C), BCG significantly increased the Fas (B) and FADD (D)-immuno reactive staining in cultured BAEpC.

**Fig. 7.** BCG-induced Fas, Fas-L, and FADD protein expression in BEAS-2B cells. Cultured BAEpC cells were infected with BCG at 50 bacilli/cell for 16 h. Fas, Fas-L, and FADD proteins were detected by Western blotting. Western blotting band densities were measured by Image J software. \( *P < 0.05 \) compared with control.
BCG-infected cultures, the percentage of cells in the S phase at 24, 48, and 72 h decreased to 20/11006 3%, 16/11006 2%, and 28/11006 3%, respectively (at all time points P value <0.01 compared with control). The data show BCG infection significantly increased the percentage of cells at the G0/G1 phase and significantly decreased the percentage of cells in the S phase at all the time points tested. There was no significant difference in the percentage of cells at the G2/M phase cells when comparing BCG-infected and -uninfected cultures at any time point. BCG infection caused a significant increase in the percentage of cells at the G0/G1 phase (Fig. 2A) and a significant decrease in the percentage of cells in the S phase (Fig. 2B) compared with uninfected cultures at 24, 48, and 72 h. In uninfected BEAS-2B cultures, the distribution of cells along the cell cycle did not change significantly over the time interval from 24 to 72 h.

Apoptosis. We investigated the effect of BCG on BEAS-2B bronchial airway cell apoptosis. We quantified the degree of apoptosis by multiple methods. We used cell death detection ELISA, the TUNEL method, and FACS analysis. We used cell death detection ELISA to detect cytoplasmic histone-bound DNA fragments, the characteristic aftermath of apoptosis. This ELISA utilizes monoclonal antibodies directed against histone-bound DNA fragments (mono- and oligo-nucleosomes). We measured BAEpC apoptosis using 10, 20, and 50 MOI of BCG. We noticed no significant apoptosis in BAEpC with 10 MOI, 20 MOI of BCG; however, there was a significant increase in apoptosis with 50 MOI BCG. Therefore, we used the 50 MOI of BCG in this study. BCG infection significantly increased BEAS-2B apoptosis in a time-dependent manner (Fig. 3). As demonstrated by cell death ELISA, at 24, 48, and 72 h post-BCG infection, there were, respectively, 29, 44, and 67% increases in BEAS-2B apoptosis.

We also estimated the percentage of apoptotic cells by the TUNEL method with FACS analysis. In uninfected BEAS-2B, the percentages of TUNEL-positive cells were 6.2/11006 0.8, 10.3/11006 1.1, and 11.8/11006 1.6% at 24, 48, and 72 h of incubation, respectively, whereas in BCG-infected BEAS-2B cells, the percentages of TUNEL-positive cells were 30.8/11006 2.8, 36.4/11006 3.1, and 40.6/11006 4.2% at 24, 48, and 72 h of incubation, respectively. These results demonstrated significant increases.
in the BEAS-2B apoptosis at 24, 48, and 72 h post-BCG infection (Fig. 4). To evaluate whether BCG-induced BEAS-2B cell death is due to necrosis, we estimated nucleosomes in BCG-infected cultures. Nucleosomes were specifically absent in BCG-infected BEAS-2B cell culture supernatants (data not shown). This suggests that BCG-induced cell death in BEAS-2B cells was not due to necrosis.

**Fas, Fas-L, and FADD expression.** To detect the relative apoptosis genes involved in the BCG-induced apoptosis, we scanned 84 apoptosis-relative genes by RT² Profiler PCR microarray. A wide spectrum of apoptosis-relevant genes was upregulated or downregulated by BCG infection. In particular, the three genes Fas, Fas-L, and FADD were upregulated. Upregulation of Fas, Fas-L, and FADD genes was confirmed by the quantitative real-time PCR (Fig. 5).

We also analyzed the Fas and FADD proteins expression in situ using immunofluorescence (Fig. 6). An immunoreactive positive staining was seen in BCG-infected BEAS-2B demonstrating increased expression of Fas and FADD protein compared with uninfected BEAS-2B, where only a faint fluorescence was appreciated. (Fas-L did not have appreciable immunofluorescence.) We also measured the Fas, Fas-L, and FADD protein expression in BCG-infected and -uninfected BEAS-2B cells by Western blot analysis (Fig. 7). In BCG-infected BEAS-2B cells expression of Fas, Fas-L, and FADD was significantly increased compared with uninfected cells. We also tested BCG-infected and -uninfected BEAS-2B cell culture supernatants for soluble form of Fas-L by Western blot analysis. In these culture supernatants, soluble Fas-L was undetectable (data not shown), indicating that cell membrane-bound Fas-L was not cleaved from BEAS-2B cells in BCG infection. In BCG-infected BAEpC, the Fas, Fas-L, and FADD expression at mRNA levels was much higher than the protein expression noticed by Western blot analysis. Since expression of \( /H9252 \)-actin mRNA is very stable accordingly, it was chosen to serve as the internal control in the quantitative PCR experiments. Measurements of gene expression were relative to \( /H9252 \)-actin mRNA. We believe that the variations in protein and mRNA levels are not related to reaction efficiency in these experiments.

**Caspase-8 and caspase-3 activity.** Using a colorimetric assay, we demonstrated increased activity of caspase-8 and caspase-3 activity in BCG-infected BEAS-2B. After 16-h incubation, BCG infection significantly enhanced the activity of both caspase-8 \(( P < 0.01)\) and caspase-3 \(( P < 0.01)\) compared with uninfected BEAS-2B (Fig. 8).

**Modulation of Fas, FADD translation, and apoptosis by Fas silencing RNA and FADD silencing RNA.** To investigate the role of the Fas death receptor signal pathway in BCG-induced apoptosis, we used Fas silencing RNA and FADD silencing RNA to decrease expression of the Fas receptor and FADD, respectively. Forty-two hours after transfection with Fas silencing RNA or FADD silencing RNA, BEAS-2B cells have significantly reduced levels of Fas mRNA or FADD mRNA transcription, respectively (Fig. 9A). Fas, FADD protein expression was significantly decreased in BEAS cells treated with Fas siRNA, FADD siRNA, respectively (Fig. 9B). However, Fas or FADD siRNA treatment did not completely silence respective protein expression.

When BAES cells transfected with either Fas siRNA or FADD siRNA are incubated with BCG for 16 h, they have
significantly reduced apoptosis compared with control cells not transfected with siRNA or transfected with scramble siRNA (Fig. 10). When CH11 antibody (Fas antibody) was included as positive control, we noticed a significant increase in BEAS cell apoptosis. The Fas-mediated apoptosis was significantly decreased in Fas siRNA-treated (Fig. 10A) or FADD siRNA-treated cells.

Measurement of TGF-β secretion. We investigated the effect of BCG infection on TGF-β expression by BEAS-2B cells. Incubation with BCG for 16 h caused no statistically significant change in the secretion of TGF-β compared with uninfected controls (Fig. 11).

DISCUSSION

The present study shows that BCG infection inhibits BAEPc cell proliferation, induces cell cycle arrest at the G0/G1 phase, and promotes apoptosis in a cultured BEAS-2B cell line. Furthermore, we show that BCG-induced apoptosis is associated with upregulation of the Fas death receptor pathway.

Our findings are significant because this is the first published study to show the effect of mycobacterial infection on human bronchial epithelial cells in terms of gene transcription and expression and regulation of important cell life cycle events, cell cycle arrest and apoptosis. Apoptosis induced by BCG infection is partly due to the Fas death receptor pathway and higher activity of caspase-8 and caspase-3.

Danelishvili and co-workers (3) previously reported studies of M. tuberculosis infection in cultured human alveolar epithelial cells. Using gene arrays they identified four genes (bad, caspase-3, bcl-2, and rb1) with differential expression in infected alveolar epithelial cells compared with uninfected controls. They used RT-PCR to demonstrate that M. tuberculosis infection in cultured human alveolar epithelial cells decreased expression of the pro-apoptotic genes (bad and caspase-3) and increased expression of the anti-apoptotic genes (bcl-2 and rb1). In cultured alveolar epithelial M. tuberculosis infection caused greater necrosis (as demonstrated by release of LDH into the supernatant) than apoptosis (as demonstrated by TUNEL method). They concluded that M. tuberculosis infection protects alveolar epithelial cells against apoptosis. Our results show that BCG infection has the opposite effect in bronchial epithelial cells. We noticed significantly increased apoptosis in BCG-infected BAEPc. Interestingly, necrosis was specifically absent in these cultures. Similar results were noticed by Vernooy and co-workers (14) who showed that in murine bronchial epithelial cells, intratracheal instillation of lipopolysaccharide (LPS) causes apoptosis. This concordance suggests BAEPc may have developed apoptosis as a common defense mechanism against widely divergent insults. Apoptosis may be an important mechanism for the elimination of mycobacteria and other pathogens from the bronchial airways.

Moreover, induction of apoptosis has been demonstrated in the M. tuberculosis-infected macrophages (3). Macrophages are commonly regarded as the primary cell type involved in the initial uptake of M. tuberculosis. Macrophage apoptosis serves as a defense mechanism to prevent the spreading of the mycobacterial infection by sequestering the mycobacteria within apoptotic bodies and by contributing to their demise by activation of newly recruited uninfected macrophages (5).

Apoptosis is a distinct form of cell death controlled by an internally encoded suicide program (16). It seems to be a physiological mechanism for removing cells from the body without inducing inflammation and subsequent damage to contiguous cells. Apoptosis occurs through the activation of an intrinsic cell suicide program. The apoptotic machinery is activated when pro- and anti-apoptosis balance is upset by the withdrawal of survival signals such as growth factors, the intervention of conflicting signals during the cell cycle, or the recognition of a specific molecular at the cell surface.

In general, apoptosis signal pathways are classified into mitochondria-dependent and mitochondria-independent pathways. The Fas death receptor signal pathway is mitochondria independent. Our data demonstrate that activation of Fas death receptor signal pathway contributes to the apoptosis induced by BCG infection in BAEPc. BCG infection upregulates expression of Fas receptor, its ligand Fas-L, and their downstream mediator FADD. In addition, BCG infection also leads to increased activity of caspase-8 and caspase-3. Our results also show the Fas death receptor signal pathway is upregulated at multiple points, emphasizing the importance of this pathway in BCG-induced BAEPc apoptosis. Partial blockade of Fas or FADD resulted in an attenuated apoptotic response to BCG infection. This result indicates that the Fas death receptor signal pathway plays an active role as a mediator of BCG-induced apoptosis. However, in the current study although siRNA transfection effectively decreased Fas or FADD expression in BEAS-2B cells, it was not completely suppressed. It is possible that partial inhibition of apoptosis noticed in BAEPc was due to partial silencing of Fas or FADD expression in the present study.

In conclusion, we believe the bronchial epithelium is an important target of TB infection and may play an important role in the defense against TB infection. This study of human BAEPc experimentally infected by BCG may serve to elucidate that role.

ACKNOWLEDGMENTS

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GRANTS

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