Loss of pro-apoptotic Bim promotes accumulation of pulmonary T lymphocytes and enhances allergen-induced goblet cell metaplasia

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Submitted 7 December 2005; accepted in final form 25 May 2006

Pierce, Joseph, Jules Rir-Sima-Ah, Isaac Estrada, Julie Wilder, Andreas Strasser, and Yohannes Tesfaigzi. Loss of pro-apoptotic Bim promotes accumulation of pulmonary T lymphocytes and enhances allergen-induced goblet cell metaplasia. Am J Physiol Lung Cell Mol Physiol 291: L862–L870, 2006. First published June 16, 2006; doi:10.1152/ajplung.00516.2005.—Bim initiates negative selection of autoreactive T and B cells and shuts down of T cell immune responses in vivo. The present study investigated whether Bim plays a role in the resolution of goblet cell metaplasia (GCM) during prolonged exposure to allergen. Loss of Bim increased T lymphocyte numbers in the bronchoalveolar lavage at 4 and 15 days of allergen exposure. The numbers of pulmonary CD4+8−, CD4+8+, and γδ T cells were significantly higher in naive and allergen-challenged bim−/− mice compared with wild-type (WT) littermates. When activated, pulmonary bim−/− T cells produced increased levels of IFN-γ compared with bim+/+ T cells. No differences were noted in the total numbers of epithelial cells per millimeter of basal lamina between bim+/+ and bim−/− mice, and the rate of resolution over 15 days of exposure was similar in both groups of mice. However, GCM was significantly enhanced and expression of IL-13Rα2 was reduced in bim−/− mice compared with WT mice at 4 days. Furthermore, treatment of bronchiolar explant cultures with increasing IFN-γ levels reduced immunostaining for IL-13Rα2. Collectively, these studies suggest that, during prolonged exposure to allergen, Bim plays no role in the resolution of GCM, but increased IFN-γ levels in bim−/− mice may be responsible for reduced expression of IL-13Rα2 and enhanced GCM despite similar levels of IL-13 in bim+/+ and bim−/− mice.

Asthma is an increasingly common disease that remains poorly understood and difficult to manage. Allergic asthma is a T helper type 2 (Th2) lymphocyte-mediated inflammatory airway disease characterized by airway eosinophilia, increased mucus production caused by goblet cell metaplasia (GCM), and structural remodeling of the airway wall (9, 52). Many studies have shown that, in WT mice, prolonged exposure to allergen causes the removal of Th2 cells, eosinophils, and GCM (27, 41, 54). This transition from antigen sensitization to immunological tolerance is accompanied by a shift in the lymphocyte content in the lung tissue and bronchial lavage fluid (BALF) (6, 54). Antigen-specific regulatory T cells are believed to produce IL-10 transiently and to thereby inhibit the asthma phenotype during the development of tolerance (2, 43). IL-4, a Th2 cytokine, enhances type I dendritic cell maturation and kills type II dendritic cells, a mechanism of mature Th2 cells to selectively inhibit vigorous Th2 responses and enhance Th1 responses by regulating the selective survival of dendritic cell subsets (2, 43). It is, however, not clear whether regulators of apoptosis play a role in the survival or death of regulatory T cells or in the reduction of T cell numbers during prolonged exposure to allergen and the development of tolerance.

The Bcl-2 family of proteins are critical regulators of apoptotic cell death. These proteins contain variable numbers of four conserved Bcl-2 homology (BH) domains, all of which include α-helical segments (29). Anti-apoptotic members, such as Bcl-2 and Bcl-xL, contain all BH domains. The pro-apoptotic proteins are subdivided into multidomain members such as Bax and Bak (containing BH1, -2, and -3) and the BH3-only proteins, is expressed as many isoforms, which are generated by alternative splicing (28), (1) and is found in many cell types including lymphocytes, myeloid cells, epithelial cells, neurons, and germ cells (30). The pro-apoptotic activity of Bim can be regulated by a range of mechanisms such as transcriptional induction by FOXO3A (12, 44), sequestration of the protein to the microtubular dynein motor complex by interaction with dynein-like chain LC8 (32), and Erk-mediated phosphorylation leading to ubiquitination and proteasomal degradation (24). The pro-apoptotic function of Bim can also be inhibited by anti-apoptotic players, such as Bcl-2 or Bcl-xL (5). Deletion of the bim gene in mice produced animals with abnormally elevated numbers of T as well as B cells in all hemopoietic organs (3). In culture, these lymphocytes displayed abnormal resistance to certain apoptotic stimuli, including cytokine deprivation or calcium flux, but they had normal or only slightly reduced sensitivity to other death stimuli such as phorbol ester and DNA damage that could be countered by expression of a Bcl-2 transgene (3, 20). Further studies in vivo demonstrated that Bim is an essential initiator of apoptosis in negative selection of autoreactive thymocytes (4), mature T cells (7), and B cells (11) in shutting down of T cell immune responses to a herpes simplex virus infection (31) or an injected superantigen (20).

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We had observed that allergen-induced GCM is reduced by apoptotic mechanisms during prolonged exposures to allergen (45). Because Bim has been shown to be expressed in epithelial cells (30), the present study investigated whether Bim has a role in the cell death process of metaplastic mucous cells and other airway epithelial cells during the resolution of inflammation after prolonged exposure to allergen. While the resolution of GCM was not affected by the loss of Bim, we found that Bim enhanced allergen-induced GCM because of increased levels of IFNγ that downregulated IL-13Rx2 expression.

MATERIALS AND METHODS

Animals. Male specific pathogen-free wildtype (WT) C57BL/6J mice were purchased from The Jackson Laboratory (Bar Harbor, ME). Mice were housed in isolated cages under specific pathogen-free conditions. After a 14-day quarantine period, mice were acclimatized for 8 days and entered into the experimental protocol at 8–10 wk of age. The bim+/− mice were made available by Dr. Philippe Bouillet (Walter and Eliza Hall Institute); gene targeting was performed in the chambers. Chamber temperatures were maintained at 26 ± 2°C, and lights were on a 12:12-h on-off cycle. Mice were sensitized by injection (ip) with 10 μg of ova albumin (OVA, grade V; Sigma-Aldrich, St. Louis, MO) adsorbed to 2 μg of Alhydrogel (Superfos Biorabbit) in 0.5 ml of sterile water. A booster injection was given on day 7, using the same reagents. Seven days later, mice were exposed 6 h/day to OVA aerosols. OVA exposures were generated by aerosolizing (6 h/day, 5 days/wk) 1% heat-aggregated OVA (chicken egg, grade V; Sigma-Aldrich), diluted with filtered air, and then delivered to whole body exposure chambers. The total mass concentration of OVA was determined by gravimetric analysis of filter samples taken every 2 h during exposure. The mass concentration of OVA was 2.3 mg/m³. Mice were exposed to allergen for 4 or 15 consecutive days and euthanized immediately after the last exposure. Naive mice that were sensitized with OVA and not sensitized served as 0 time point.

Collection of BALF and cell counting. Mice were injected (ip) with 150 μl of heparin (ICN Biomedical, Aurora, OH) 10 min before euthanization with 0.2 ml of 1:10 Euthasol (penytoin sodium; Delmarva Laboratories, Midlothian, VA) and exsanguination via the renal artery. The thoracic content was exposed, and the lungs were perfused in ice-cold PBS, and the BALF was collected. The cells recovered by cardiac puncture with 0.9% saline (wt/vol; McGraw, Irvine, CA) were prepared and stained with Wright Giemsa solution (Sigma-Aldrich). Lungs were minced and incubated with collagenase A (0.7 mg/ml; Roche Applied Science, Indianapolis, IN) in RPMI-1640 (Gibco, Grand Island, NY) with 5% FBS (Hyclone, Logan, UT) and D-Nase I (0.03 mg/ml; Sigma-Aldrich) for 90 min at 37°C with agitation. Digested lungs were passed through a wire mesh cup. Larger particles were removed from the digested lung by gravitational flow through a syringe column plugged with loose nylon wool. Cells in suspension were pelleted and resuspended in RPMI-1640 with 5% FBS, and red blood cells were lysed by incubating the homogenate with red blood cell lysis buffer (0.15 M NH₄Cl, 1 mM KHCO₃, and 0.1 mM Na₂EDTA, pH to 7.4 with 1 N HCl) at room temperature for 1 min. Cells were pelleted and resuspended in RPMI-1640 with 5% FBS. Cellular debris was removed by spinning the homogenate through a 30% Percoll-PBS solution (Amersham Biosciences) and removing the supernatant. The cell pellet was resuspended in RPMI-1640 with 5% FCS, and live cells were stained with Trypan blue and counted with the use of a hemacytometer. To obtain a sufficient number of lung cells for analyses of secreted cytokines and for immunophenotyping of pulmonary T cells, isolated lung cells from three to four mice had to be pooled. We have, therefore, 10–15 mice/group for cell counts and cytokine assays within the BALF but only 3–6/group for immunophenotyping and for analyses of secreted cytokines from isolated lung cells.

Cytokine secretion and ELISA measurements. For cytokine secretion studies, a portion of the isolated lung cells was incubated on 100 × 20 mm tissue culture plates for 2 h at 37°C, 5% CO₂, to remove adherent cells. Nonadherent cells were recovered and resuspended in lung cell medium (2 mM l-glutamine, 100 U/ml penicillin-streptomycin, 1 mM MEM-sodium pyruvate, and 1% MEM nonessential amino acids; all from Gibco) with 5 × 10⁻⁵ M β-mercaptoethanol (Sigma-Aldrich), 1 μg/ml indomethacin (Sigma-Aldrich), 250 U/ml catalase (Sigma-Aldrich), and 10% FCS in RPMI-1640. Cells were either left unstimulated or were stimulated with concanavalin A (5 μg/ml; Sigma-Aldrich) for 72 h at 37°C, 5% CO₂. Culture supernatants were collected, frozen at −80°C, and analyzed for cytokine content at a later time.

ELISA plates (Greiner Bio-Ore, USA Scientific) were coated with capture antibodies diluted in 0.1 M Na₂HPO₄ overnight at 4°C, washed, and blocked with 1% BSA in PBS. Samples were added to the plates after subsequent washes and incubated overnight at 4°C. Detection proceeded the following day by adding biotinylated monoclonal antibodies, streptavidin-horseradish peroxidase (1 μg/ml), and ABTS substrate, and the light absorption was read at optical density (OD)₄₅₀ nm. Pairs of monoclonal antibodies against IFNγ (R46A2 and biotin-XMG1.2) were purchased from BD Biosciences Pharmingen, San Diego, CA. Antibodies specific for IL-13 (38213.11 and biotin-XMG1.2) were purchased from BD Biosciences Pharmingen, Minneapolis, MN. Cytokines were quantified by comparison with standard curves generated using recombinant cytokines (BD Biosciences Pharmingen). Detection limits were assigned as the lowest concentration in the linear portion of the standard curve (IFNγ at 190 pg/ml, IL-13 at 20 pg/ml).

Immunophenotyping by flow cytometry. A portion of the isolated lung cells were subjected to direct immunofluorescence staining for T-lymphocyte markers and then counted by three-colored flow cytometric analysis. All conjugated monoclonal antibodies (Ab) were purchased from BD Biosciences Pharmingen. T cells (1 × 10⁶) from each sample were washed with 1% FCS-PBS, resuspended in 0.1 ml of 0.0125 mg/ml Mouse BD Fc Block, and allowed to incubate at 4°C for 10 min. Samples were washed and resuspended in 10 μg/ml FITC-conjugated hamster anti-mouse γ8 T-cell receptor Ab, 8 μg/ml R-phycocerythrin-conjugated rat anti-mouse CD8a (LY-2) Ab, and 4 μg/ml PerCP-conjugated rat anti-mouse CD4 (L3T4) Ab solution in 1% FCS-PBS and allowed to incubate at 4°C for 30 min. Incubation with these primary antibodies was followed by three washes in 1% FCS-PBS and fixation with 0.5% paraformaldehyde (Sigma-Aldrich) at 4°C. All cells were analyzed in a Becton Dickinson FACSCalibur.
noprecipitation assay (RIPA) buffer (10 mM Tris, pH 7.4, 150 mM NaCl, 1% Triton X-100, 1% deoxycholate, 0.1% SDS, 5 mM EDTA) supplemented with the protease inhibitors PMSF (1 mM), pepstatin (10 μg/ml), aprotinin (2 μg/ml), and benzamidine (2 μg/ml) and analyzed by Western blotting as described (46). The polyclonal rabbit anti-Bim (human, rat, mouse antibody; Stressgen) that detects the three isoforms, BimEL, BimL, and BimS, was used at 1:500 dilution. For detection of IFNγ, the biotinylated rat anti-mouse IFNγ (BD Biosciences Pharmingen) was used at 1:100 dilution (5 μg/ml).

Statistical analysis. Grouped results from at least four different mice were expressed as means ± SE. Data were analyzed using Statistical Analysis Software (SAS) from the SAS Institute (Cary, NC). Results grouped by time point and genotype were analyzed using two-way analysis of variance (ANOVA). When significant main effects were detected (P < 0.05), Fishers least significant difference test was used to determine differences between groups. A P value of <0.05 was considered to indicate statistical significance.

RESULTS

Exposure of mice and rats to prolonged aerosol challenge with allergen is typified by a significant decrease in airway inflammation (54), airway hyperresponsiveness (16, 22), and GCM (41) from an initial peak response level. To study whether cell death and apoptosis regulatory proteins are involved in the decrease of GCM and epithelial cell numbers during prolonged exposures to allergen, we exposed bim+/+ and bim−/− littermates to allergen for 0, 4, or 15 days. Expression of BimEL protein was detected in the lungs from bim+/+ but not from bim−/− mice, while other Bim isoforms were not detected (Fig. 1A). In general, inflammatory cell numbers that were recovered by bronchoalveolar lavage reached maximum levels at 4 days and were reduced by 15 days postexposure (Fig. 1, B–E). No significant differences between bim+/+ and bim−/− mice were observed for the number of neutrophils, macrophages, and eosinophils (Fig. 1). However, significant numbers of lymphocytes were present in the BALF of naive bim−/− but absent in bim+/+ mice (Fig. 1D). The numbers of lymphocytes were significantly increased (~5-fold) in bim−/− compared with bim+/+ littermates at 4 days as well as at 15 days of exposure. Furthermore, while the number of lymphocytes were increased from 0 to 4 days in bim−/− mice, a statistically nonsignificant increase in lymphocytes was found in bim+/+ mice at 4 days that disappeared to background levels at 15 days (Fig. 1D). In bim+/+ mice, IL-13 levels significantly increased from 0 to 4 days and decreased from 4 to 15 days, whereas IL-13 levels in bim−/− mice remained unchanged overall (Fig. 2A). No differences in IFNγ levels were observed between bim+/+ and bim−/− at any time point (Fig. 2B).

Loss of Bim leads to increased numbers of T cells in nonimmunized Bim-deficient mice (3) and inhibits shut down of T-cell immune responses in the spleen (20, 31). However, the role of Bim in peripheral T-cell subsets during prolonged exposure to allergen has not been characterized. The numbers of CD4+8−, CD4+8+, and γδ T cells in the lungs were significantly increased in naive bim−/− mice compared with naive bim+/+ mice (Fig. 3). Although the numbers of CD4+8− and CD4+8+ cells were not different between bim+/+ and bim−/− mice at 4 days of allergen exposure, their numbers were statistically different at 15 days of exposure because of an apparent decrease from 4 to 15 days in bim−/− mice. No apparent difference was observed at 4 days between bim−/− and bim+/+ mice because of a statistically nonsignificant
increase in the numbers of CD4⁴⁺⁻ and CD4⁻⁸⁺ T cells in bim⁺⁺/⁺⁺ mice (Fig. 3, A and B). Most strikingly, there was an approximate fivefold increase in the numbers of γδ T cells in bim⁻⁻/⁻/⁻ mice compared with bim⁺⁺/⁺⁺ mice in nonexposed and allergen-exposed mice. The number of γδ T cells remained unchanged throughout the exposure period in both bim⁺⁺/⁺⁺ and bim⁻⁻/⁻/⁻ mice (Fig. 3C).

We next analyzed the Th1 and Th2 cytokine profiles of bim⁺⁺/⁺⁺ and bim⁻⁻/⁻/⁻ pulmonary nonadherent lung cells to determine the state of T-cell differentiation. Significantly increased levels of IL-13 were produced by cultured T cells from both bim⁺⁺/⁺⁺ and bim⁻⁻/⁻/⁻ mice at 4 days of allergen exposure compared with their naive counterparts (Fig. 4A). Interestingly, at all time points, cultured T cells from the lungs of bim⁻⁻/⁻/⁻ mice produced three- to fourfold more IFNγ compared with T cells from bim⁺⁺/⁺⁺ mice, although at 4 days, this difference was only marginally significant (Fig. 4B). Cells that were not treated with concanavalin A showed a similar pattern of cytokine secretion; however, the levels of IL-13 and IFNγ were in the range of concentration of 0.2–04 ng/ml. This finding was confirmed by analyzing the protein extracts from bim⁺⁺/⁺⁺ and bim⁻⁻/⁻/⁻ lung and finding that IFNγ levels were significantly higher in bim⁻⁻/⁻/⁻ compared with bim⁺⁺/⁺⁺ lungs (Fig. 4C). Densitometric analysis of the bands representing Bim normalized to the actin band showed twofold increased IFNγ levels in bim⁻⁻/⁻/⁻ compared with bim⁺⁺/⁺⁺ lungs (data not shown).

To investigate whether Bim has any role in the resolution of airway epithelial cell hyperplasia, the numbers of total epithelial cells per millimeter BL were counted in naive and allergen-exposed bim⁺⁺/⁺⁺ and bim⁻⁻/⁻/⁻ mice (Fig. 5). A significant increase from ~80 to ~130 cells/mm BL from 0 to 4 days was followed by an apparent decrease to ~100 cells/mm BL from day 4 to day 15 of allergen exposure. No significant differences were observed between bim⁺⁺/⁺⁺ and bim⁻⁻/⁻/⁻ mice, suggesting that Bim does not play a role in the resolution of epithelial cell hyperplasia during prolonged exposure to allergen.

We have previously shown that the resolution of the GCM during prolonged exposure to allergen is in part dependent on the Bax protein (45). Because BH3-only proteins can initiate the cell death pathway upstream of Bax/Bak (33), we tested whether Bim plays a role in the cell death of metaplastic goblet cells in mice exposed to allergen. Interestingly, both the intraepithelial-stored mucosubstances [measured as nl/mm² epithelium (Vs)] and the numbers of mucous cells per millimeter BL increased, resulting in a two- to threefold higher amount of stored mucosubstances as well as mucous cell numbers in bim⁻⁻/⁻/⁻ compared with bim⁺⁺/⁺⁺ littermates at 4 days postallergen exposure (Fig. 6, A and B); see representative photomicrographs (Fig. 6, E and F). There was an enhanced decrease of both volume density (Vs) of stored intraepithelial mucosubstances and mucous cell numbers per millimeter BL in bim⁻⁻/⁻/⁻ mice from 4 to 15 days of allergen exposure compared with bim⁺⁺/⁺⁺ mice (Fig. 6, A and B, and representative photomicrographs in Fig. 5, C–G, for bim⁺⁺/⁺⁺ and Fig. 5, D–H, for bim⁻⁻/⁻/⁻ mice). Although the parameters for GCM were still elevated in bim⁻⁻/⁻/⁻ mice at 15 days, the rate of resolution of mucous cells appeared to be similar in bim⁺⁺/⁺⁺ and bim⁻⁻/⁻/⁻ mice.
levels in the BALF or synthesis from activated pulmonary T cells. However, Bim was not required for the resolution of metaplastic mucous cells during prolonged exposure to allergen.

The number of neutrophils, macrophages, and eosinophils increased over 4 days of allergen exposure and then decreased to background levels from day 4 to day 15 of exposure in both bim<sup>+/+</sup> and bim<sup>−/−</sup> mice, suggesting that the death of neutrophils and eosinophils is not Bim dependent. We have previously reported that the decrease in number of eosinophils during prolonged exposure to allergen is not affected by loss of Bax (45). Others have shown that eosinophils are cleared by apoptosis through the Fas-FasL-dependent pathway (48). The death of neutrophils has been reported to be initiated by reactive oxygen species, which then activate autocrine and paracrine death receptor signaling (38). However, whereas the numbers of lymphocytes were increased ~2-fold in bim<sup>+/+</sup> mice from 0 to 4 days of exposure, their numbers increased by approximately ~10-fold in bim<sup>−/−</sup> mice during this time. This

**DISCUSSION**

The present study shows that bim<sup>−/−</sup> mice develop significantly greater GCM in response to allergen exposure compared with bim<sup>+/+</sup> mice without any evidence of increased IL-13 levels in the BALF or synthesis from activated pulmonary T lymphocytes. However, Bim was not required for the resolution of metaplastic mucous cells during prolonged exposure to allergen.

![Image](http://ajplung.physiology.org/)

Fig. 2. In the BALF of bim<sup>+/+</sup> mice, IL-13 levels are increased at 4 days and decreased at 15 days of allergen exposure. While statistically no different from bim<sup>+/+</sup> mice, IL-13 levels remained unchanged in bim<sup>−/−</sup> mice at all time points. No differences were observed for IFNγ at any time point. Mouse lungs were lavaged, and IL-13 (A) and IFNγ (B) were detected by ELISA. Bars = group means ± SE (n = 10–15/group). *Significantly different as indicated by bars; P < 0.05.

GCM was significantly higher in bim<sup>−/−</sup> mice compared with bim<sup>+/+</sup> mice, despite IL-13 levels being lower in the BALF of bim<sup>−/−</sup> compared with bim<sup>+/+</sup> mice and bim<sup>−/−</sup> pulmonary T cells mice producing similar levels of IL-13. Therefore, we wanted to test the hypothesis that higher levels of IL-13Rα2 expression in bim<sup>+/+</sup> mice inhibit the effect of IL-13. IL-13Rα2 functions as an inhibitor of IL-13 signaling and Stat 6-responsive gene expression, likely by functioning as a decoy receptor (14, 34). T cells showed no immunostaining for IL-13Rα2 in both bim<sup>+/+</sup> and bim<sup>−/−</sup> mice (not shown). However, while immunoreactivity to IL-13Rα2 was present in airway epithelia from both bim<sup>+/+</sup> and bim<sup>−/−</sup> mice that were devoid of GCM, little to no immunostaining was observed with IL-13Rα2 antibodies in bim<sup>−/−</sup> mice in areas with extensive GCM (Fig. 6, I and J).

To further investigate whether increased presence of IFNγ may have affected expression of IL-13Rα2, we treated explant cultures of mouse bronchioles from WT mice with 1 ng/ml IL-13 combined with 0, 1, 10, or 50 ng/ml IFNγ. Immunohistochemical staining showed that expression of IL-13Rα2 decreased with increasing IFNγ levels in the culture medium (Fig. 7, A–D).

![Image](http://ajplung.physiology.org/)

Fig. 3. Increased nos. of CD4<sup>+</sup> (A), CD8 <sup>+</sup> (B), and γδ T cells are found in the lungs of bim<sup>−/−</sup> mice compared with bim<sup>+/+</sup> littermates. Lung tissues from bim<sup>+/+</sup> and bim<sup>−/−</sup> mice were harvested, and T cells were isolated after digestion of the lung with collagen. Nos. of CD4<sup>+</sup> (A), CD8 <sup>+</sup> (B), and γδ (C) T cells were determined by cell counting and flow cytometry analysis. Bars = group means ± SE (n = 3–6/group). *Significantly different as indicated by bars; P < 0.05.
all three isoforms, BimEL, BimL, and BimS, in lung extracts from rats (data not shown).

As expected, bim−/− mice had increased levels of pulmonary CD4+8−, CD4−8+, and γδ T cells. This finding is in agreement with previous reports demonstrating that both the CD4−8− pro-T cells and the mature T cells (CD4−8+ and CD4−8+) were two- to threefold higher in bim−/− compared with bim+/+ littermates (3). Bim is required for deletion of autoreactive thymocytes (4) as well as mature CD4−8+ T cells (7) and of T-cell receptor/CD3-induced killing of semimature (CD4−8+/HSA+) thymocytes (51). In addition, Bim was shown to be required for the shut down of T-cell immune responses in vivo (20, 31).

No differences in IFNγ levels were observed in the BALF of bim+/+ and bim−/− mice, but T cells isolated from the lung tissues of both naive and allergen-exposed bim−/− mice had a capacity to produce approximately fourfold more IFNγ compared with those from bim+/+ mice. Furthermore, immunoblotting of lung extracts showed that IFNγ levels are higher in bim−/− compared with bim+/− lungs. These results suggest that Bim deficiency preferentially sustains γδ T cells that are known to produce IFNγ, indicating that bim−/− T cells are biased toward Th1 cytokine production and that most of the IFNγ in the BALF may be derived from cell types other than T cells, natural killer cells being likely candidates.

The numbers of airway epithelial cells did not differ significantly between bim+/+ and bim−/− mice; however, exposure to allergen did increase the numbers of total epithelial cells per millimeter BL at 4 days of allergen exposure in both sets of animals. No increase in epithelial cells was observed after a single antigen challenge in BALB/c mice (13). Reader et al. (36) reported that after three allergen challenges, each 3 days apart, 10% of mucous cells proliferated. In those studies, the increase in the numbers of mucous cells was accompanied by a compensatory decrease in Clara and ciliated cell numbers (36). The increase in total cell number observed in our study may be due to the repeated daily challenge with allergen for 6 h/day over 4 days, which may cause significant injury to the lung.

While exposure to allergen for 15 days did not completely reverse this increase, the reduction of airway epithelial cells from 4 to 15 days was similar in bim+/+ and bim−/− mice, suggesting that the absence of Bim has no effect on the resolution of airway epithelial cell hyperplasia in allergen-exposed tissues of both naive and allergen-exposed bim−/− mice with a capacity to produce approximately fourfold more IFNγ compared with those from bim+/+ mice. Furthermore, immunoblotting of lung extracts showed that IFNγ levels are higher in bim−/− compared with bim+/− lungs. These results suggest that Bim deficiency preferentially sustains γδ T cells that are known to produce IFNγ, indicating that bim−/− T cells are biased toward Th1 cytokine production and that most of the IFNγ in the BALF may be derived from cell types other than T cells, natural killer cells being likely candidates.

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While exposure to allergen for 15 days did not completely reverse this increase, the reduction of airway epithelial cells from 4 to 15 days was similar in bim+/+ and bim−/− mice, suggesting that the absence of Bim has no effect on the resolution of airway epithelial cell hyperplasia in allergen-exposed tissues of both naive and allergen-exposed bim−/− mice with a capacity to produce approximately fourfold more IFNγ compared with those from bim+/+ mice. Furthermore, immunoblotting of lung extracts showed that IFNγ levels are higher in bim−/− compared with bim+/− lungs. These results suggest that Bim deficiency preferentially sustains γδ T cells that are known to produce IFNγ, indicating that bim−/− T cells are biased toward Th1 cytokine production and that most of the IFNγ in the BALF may be derived from cell types other than T cells, natural killer cells being likely candidates.
exposed mice. A previous study reported low to moderate levels of Bim expression in ciliated epithelia of the trachea and bronchi, but no Bim was found in the alveoli of the lung (30). In this study, we were able to detect Bim in macrophages but not in airway epithelial cells (data not shown). The absence of Bim in epithelial cells is consistent with the observation that Bim deficiency had no effect on the resolution of airway epithelial cells after allergen exposure.

As previously described (41), GCM was significantly increased in WT mice at 4 days and significantly decreased from 4 to 15 days of allergen exposure. Both quantification of intraepithelial-stored mucosubstances (Vs) and the number of goblet cells per millimeter BL showed similar results, indicating that increases were due to GCM and not only enlargement of existing mucous cells. The question of whether increased number of T cells together with increased GCM in bim

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Fig. 7. IL-13Rα2 levels are decreased in IL-13-treated bronchiolar explant cultures treated with increasing levels of IFNγ treatment. Explant cultures were maintained in air-liquid interface cultures and treated with IL-13 at 1 ng/ml together with 0 (A), 1 (B), 10 (C), or 50 (D) ng/ml IFNγ for 7 days. Tissue sections were immunostained with antibodies to IL-13Rα2.
may enhance GCM induced by the Th2 cytokine IL-13, which is the main cytokine to induce GCM in airway epithelia (23, 53, 56, 57). This hypothesis is supported by our finding that expression of IL-13Ra2 was reduced in IL-13-treated organ cultures when explants were co-treated with increasing concentrations of IFNγ. The reduction of IL-13Ra2 expression suggests that, in bim−/− mice, high IFNγ levels may have downregulated expression of this decoy receptor, thereby enhancing the effect of IL-13 to increase GCM. Zheng et al. (55) have reported that IL-13Ra2 levels are increased in both IL-13- and IFNγ-overexpressing transgenic mice. However, they did not study the effect of combined treatment with IL-13 and IFNγ over a long period, as our study did. We have previously reported that IFNγ suppresses allergen-induced GCM (41). Such a double-sided effect of IFNγ (inhibiting some, potentiating others) on IL-13-induced changes in the lungs has been reported by various groups (15, 17, 35).

The mechanism underlying the development of tolerance after prolonged exposure to allergen has been reported to be both dependent (27) and independent (39, 40) of IFNγ and γδ T cells. Results from our studies suggest that, while the deletion of pulmonary T cells is Bim dependent, the mechanisms involved in the death of dendritic and regulatory T cells (37) that may be responsible for inhibiting prolonged Th2 responses are probably independent of Bim.

Acknowledgments

We thank Yoneko Knighton for preparing tissue samples for light microscopic analyses and Dr. James Aden for carrying out the statistical analyses.

Grants

This research was supported by grants from National Institute of Environmental Health Sciences (NIEHS, ES-09237) and National Heart, Lung, and Blood Institute (HL-68111) and by the NIEHS Center (P30-ES-012072), the National Health and Medical Research Council (NHMRC, Canberra, Australia), and the Leukemia and Lymphoma Society of America (SCOR Grant).

References

BIM REDUCES PULMONARY T CELLS


