

Increased T-cell survival by structural bronchial cells derived from asthmatic subjects cultured in an engineered human mucosa

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Background: Interaction between lymphocytes and structural cells has been proposed as a key factor in regulating inflammation in asthma.

Objective: This study was designed to investigate the effect of epithelial cells and fibroblasts on T-lymphocyte survival by using a 3-dimensional tissue-engineered model.

Methods: Engineered human bronchial mucosal tissues were produced by using fibroblasts, epithelial cells, and autologous T cells from asthmatic and healthy donors. T-cell apoptosis and apoptotic marker expression by T cells were evaluated by using the terminal deoxynucleotidyl transferase biotinylated d-UTP nick end-labeling technique and immunofluorescence, respectively. Cytokines implicated in T-cell survival were measured by means of ELISA in culture supernatants.

Results: We demonstrated histologically that we were able to generate a well-structured engineered bronchial mucosa by using epithelial cells, fibroblasts, and T cells cultured from healthy and asthmatic subjects. Structural cells from asthmatic subjects cultured in this model induced a significant decrease in the ability of T cells to undergo apoptosis represented by a decrease in DNA fragmentation and proapoptotic molecule expression (Bcl-2-associated X protein and Fas ligand). Structural cells from healthy control subjects have no effect. Among cytokines measured in the supernatants, only TGF- β_1 was significantly increased in the model derived from cells of asthmatic subjects.

Conclusion: These results support the concept that bronchial structural cells might play a critical role in the regulation of inflammation in asthma by increasing the survival of T lymphocytes. The results also further validated the model as a tool for investigating the interaction between inflammatory and structural cells.

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Key words: Asthma, apoptosis, T cells, engineered human bronchial mucosa, cytokines

In asthma, bronchial mucosal tissues are chronically infiltrated by activated inflammatory cells (mainly lymphocytes and eosinophils) that have the capacity to produce a wide range of cytokines (IL-4 and IL-5) and mediators (leukotrienes and prostaglandins). These molecules might significantly change the functional and phenotypic behavior of structural cells (epithelial cells, fibroblasts, and smooth muscle cells).¹ Structural cells are an important source of cytokines and play an active role in modulating the immune response, which suggests that they might be involved in the maintenance and chronicity of the inflammatory response.²

Activated T cells generated during an immune response are cleared by a tightly regulated process of apoptosis. The family of Bcl-2 proteins is localized in the mitochondrial membrane and comprises a number of proteins that play critical roles in the regulation of apoptosis. Some members, such as Bcl-2-associated X protein (Bax), promote apoptosis, and others (Bcl-2 and Bcl-X_L) inhibit apoptosis.³⁻⁵ The apoptosis of T cells can be actively induced by ligation of Fas/Fas ligand (FasL), by binding of TNF to its receptor, or, passively, by removal of cytokines.^{6,7} Cytokines such as IL-15 and IL-7 prevent apoptosis by upregulating genes that code for antiapoptotic protein relative to Bax, including Bcl-2 and Bcl-X_L.^{8,9}

Cell death is necessary to maintain T-lymphocyte homeostasis, and failure to clear activated cells prolongs the immune response and promotes chronic inflammation.¹⁰ Asthma is a chronic inflammatory disease characterized by activation of T cells in the peripheral blood and airway tissue. There is a direct correlation between the number of activated T lymphocytes in the bronchial mucosa and the severity of asthma.^{11,12} On allergic challenge, CD4⁺ T lymphocytes are selectively recruited to the bronchial mucosa. This recruitment is accompanied by a decrease in the number of circulating T lymphocytes.¹³

Cross talk between T lymphocytes and tissue microenvironments in the airways might be one of the potential mechanisms involved in the persistence and survival of activated T cells. Most of the studies published thus far on the interaction between T cells and structural cells were performed on nonasthmatic cells and in a 2-dimensional culture. In this work, we generated engineered human bronchial mucosa (EHBM) models from asthmatic and healthy subjects that mimicked the *in vivo* microenvironment (structural cells and extracellular matrix). These models were used to investigate the role of structural cells in regulating T-lymphocyte survival.

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Abbreviations used

Bax: Bcl-2-associated X protein
 EHBM: Engineered human bronchial mucosa
 FasL: Fas ligand
 PI: Propidium iodide
 TUNEL: Terminal deoxynucleotidyl transferase
 biotinylated d-UTP nick end-labeling

METHODS**Bronchial epithelial cell and fibroblast cultures**

Primary fibroblasts and epithelial cells were isolated from bronchial biopsy specimens of asthmatic ($n = 6$) and healthy ($n = 6$) subjects. The asthmatic subjects fulfilling the American Thoracic Society criteria for asthma¹⁴ were recruited from the Asthma Clinic at Hôpital Laval (Quebec, Canada). They used an inhaled β_2 -agonist only on demand. None used inhaled or systemic corticosteroids. All asthmatic subjects (mean age, 28 years; mean FEV₁, $85\% \pm 3.1\%$; mean PC₂₀, 4.0 ± 2.2 mg/mL) were atopic nonsmokers. Healthy subjects (mean age, 29 years; mean FEV₁, $98.2\% \pm 5.1\%$; mean PC₂₀, 99.4 ± 1.2 mg/mL) were nonatopic nonsmokers with no history of asthma or systemic disease. Bronchial biopsy specimens were obtained by means of bronchoscopy from asthmatic and healthy subjects. The study was approved by the Ethics Committee at Hôpital Laval, and written informed consent was obtained from all subjects before entry into the study.

Fibroblasts and epithelial cells were isolated from bronchial biopsy specimens, as reported previously,¹⁵ and characterized by means of immunofluorescence and flow cytometry with anti-cytokeratin, anti-vimentin, and a mouse anti-human fibroblast antigen Ab-1 antibody (Calbiochem, San Diego, Calif) that showed no cross-reactions with epithelial cells, endothelial cells, smooth muscle cells, or many other cell types. This characterization confirmed the purity of the cultured cells, as described previously.¹⁶ During the culture period, the media of both cell types were changed 3 times a week. When the cultures reached 70% to 80% confluence, the cells were detached, expanded, and used to produce EHBM.

T-lymphocyte isolation

Healthy volunteers and asthmatic subjects from whom structural bronchial cells were derived donated the blood used as a source of T lymphocytes. Mononuclear cells were isolated from the peripheral blood by using Ficoll-Paque (GE Healthcare, Buckinghamshire, United Kingdom [UK]) gradient. Adhering cells were removed by means of incubation in a culture flask for 1 hour at 37°C. Nonadhering cell suspensions were then filtered through a Nylon column. Cells were stained with anti-CD3 and anti-CD19 (BD Bioscience, San Diego, Calif), and flow cytometric analysis showed that 90% of the purified cells were CD3⁺ T lymphocytes and 10% were B cells (CD19⁺ cells).

Preparation of EHBM models including T lymphocytes

Human bronchial mucosa was constructed by means of tissue engineering, as described previously.¹⁵ Four different models were produced to evaluate the effect of each cell type on T-cell survival. First, fibroblasts (3×10^5 cells) and autologous T cells (2×10^6) were incorporated into bovine type I collagen gel (2.7 mg/mL, type III; Sigma) and poured into a Petri dish (35-mm diameter) containing a filter as an anchorage. Forty-eight hours later, epithelial cells (2×10^6) were seeded onto the gel. This model is referred to as EHBM. Second, fibroblasts and autologous T cells were included in the collagen gel. Third, epithelial cells were seeded onto autologous T cell-populated collagen gels. Fourth, the last model was constructed with T cells ($2 \times 10^5/\text{cm}^2$) included in the collagen gel. These models were cultured for 5 days.

Histologic assessment of EHBM

After 5 days of culture, engineered models from healthy and asthmatic subjects were cut and fixed in 4% paraformaldehyde and embedded in paraffin

or frozen in OCT medium (Miles, Elkhart, Ind). Thin microtome sections (5 μm) were prepared, and hematoxylin-and-eosin staining was used to evaluate the structure of the different tissues.

TUNEL

Apoptosis of T cells cultured in EHBM from healthy or asthmatic subjects was studied by using the terminal deoxynucleotidyl transferase biotinylated d-UTP nick end-labeling technique (TUNEL). Sections were dewaxed, and protease was digested and incubated with terminal transferase mixture according to the manufacturer's instructions by using an ApoTACS kit (Trevigen, Inc, Gaithersburg, Md). TACS nuclease-treated samples were used as a control for labeling reaction, and slides stained without terminal deoxynucleotidyl transferase enzyme were used as negative controls. Apoptotic and total cells were counted from entire sections, and the percentage of apoptotic T cells was calculated.

Immunofluorescence

Immunofluorescence was performed by using cryostat sections (8 μm) from EHBM specimens embedded in OCT and frozen in liquid nitrogen. Slides were double-stained with anti-CD3/anti-Bcl-2, anti-CD3/anti-Bax, anti-CD3/anti-Fas, and anti-CD3/anti-FasL (BD Biosciences, San Diego, Calif). Before staining, slides were fixed in Cytfix (PharMingen, San Diego, Calif) for 10 minutes, washed with PBS, and overlaid with specific antibody diluted with PBS containing 1% BSA (B.D.H. Chemicals, Toronto, Ontario, Canada) for 1 hour at room temperature. Sections were then rinsed 3 times with PBS and overlaid with the corresponding fluorescein-conjugated antibody for 1 hour, followed by mounting, as explained in the ProLong Antifade kit (Molecular Probes, Eugene, Ore), to stabilize fluorescence. For a negative control, the irrelevant isotype antibody was used. The number of CD3/Bcl-2-, CD3/Bax-, CD3/Fas-, and CD3/FasL-positive cells in each section from each model was counted with a Nikon Optiphot fluorescence microscope.

Cytokine measurements

IL-2, IL-7, IL-15, and TGF- β_1 levels were measured in culture supernatants by using Quantikine HS Human ELISA Kits (R&D Systems, Inc, Minneapolis, Minn).

Apoptosis assay

The apoptosis/survival assay was performed by using the BD Bioscience Annexin V-fluorescein isothiocyanate detection kit (San Jose, Calif). T lymphocytes were incubated with medium alone, different concentrations of TGF- β_1 , or the supernatant of EHBM for 24 hours. Lymphocytes were suspended in binding buffer and incubated with Annexin V-fluorescein isothiocyanate and propidium iodide (PI), according to the manufacturer's instructions. Analysis was performed by using the Beckman Coulter Epics Elite ESP cytometer. Cells were categorized as follows: viable, Annexin V negative and PI negative; apoptotic, Annexin V positive and PI negative; necrotic, Annexin V positive and PI positive.

Statistical analysis

Values are presented as means $\pm$ SEMs. The data were analyzed by using a crossed-nested design to compare groups and conditions. When interactions between groups and conditions were significant, each group was analyzed by using a randomized block design. *A posteriori* multiple comparisons were performed by using the Tukey-Kramer multiple comparisons test. All reported *P* values were 2-sided and declared significant at .05 levels. The data were analyzed with the SAS statistical package program (SAS Institute, Inc, Cary, NC).

RESULTS**Histologic assessment of EHBM models including T lymphocytes**

As shown in Fig 1, we were able to generate *in vitro* bronchial mucosal tissues using cells isolated from healthy and asthmatic

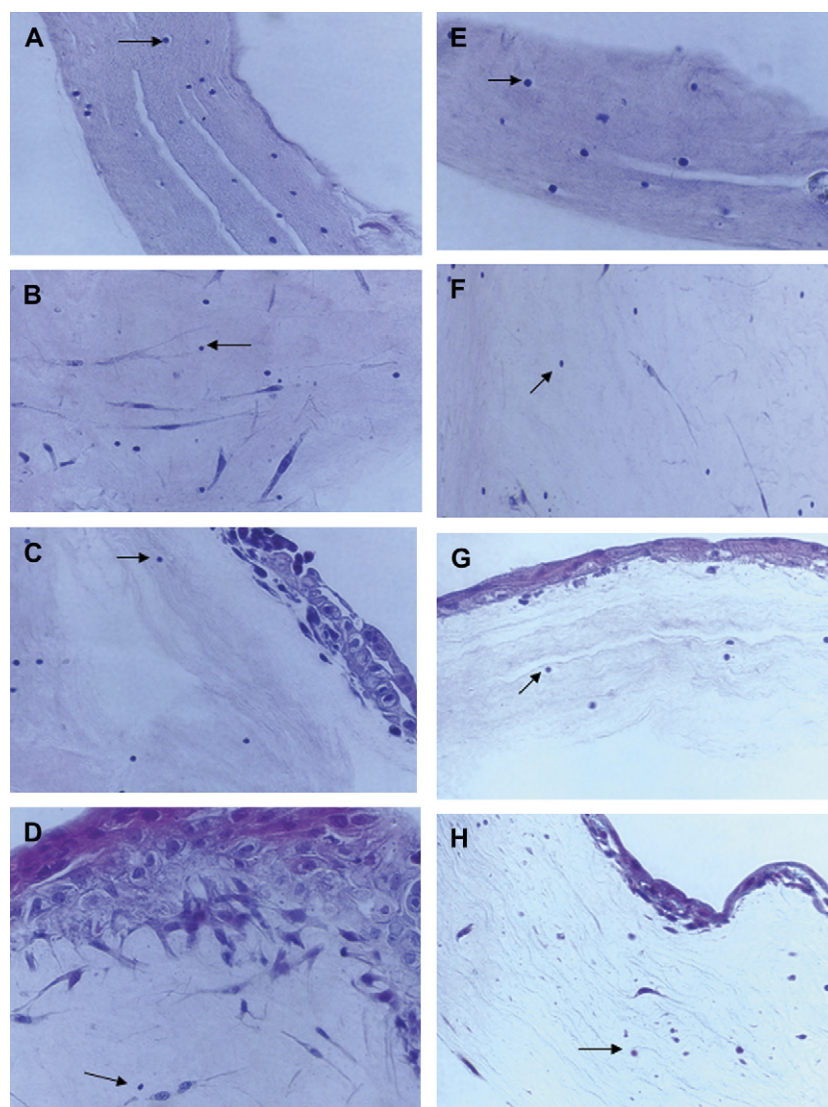


FIG 1. Tissue structure and cell distribution in EHBM. An engineered model containing only T lymphocytes from healthy control subjects (**A**) and asthmatic patients (**E**), T cells and fibroblasts from healthy control subjects (**B**) and asthmatic patients (**F**), a model with epithelial cells and T lymphocytes from healthy control subjects (**C**) and asthmatic patients (**G**), and T-cell fibroblasts and epithelial cells from healthy control subjects (**D**) and asthmatic patients (**H**) are shown. Arrows indicate T lymphocytes.

subjects. In the EHBM containing only T lymphocytes from healthy control and asthmatic subjects (Fig 1, *A* and *E*, respectively), the histologic analyses showed a collagen matrix populated with T lymphocytes. These cells were distributed in the gel, covering all the matrix structure. The addition of fibroblasts from both control and asthmatic subjects into autologous T lymphocyte-populated gel (Fig 1, *B* and *F*, respectively) did not modify T-lymphocyte distribution in the collagen matrix. Epithelial cells from both control and asthmatic subjects were seeded onto autologous T lymphocyte-populated collagen gels (Fig 1, *C* and *G*, respectively). Epithelium from asthmatic subjects showed a less well-organized structure than normal epithelium. However, the presence of epithelial cells did not modify distribution of T cells in the collagen. In an EHBM mucosa model with structural and immune cells, the histologic analyses showed a better structural organization of the epithelium in the model from healthy

control subjects (Fig 1, *D*) compared with the model from asthmatic subjects (Fig 1, *H*). Fibroblasts were found close to the epithelium in both models, suggesting a possibility of interaction between these cell types. Intraepithelial lymphocytes were also observed in some conditions (data not shown). The data at best pointed out the usefulness of EHBM in investigating physiologic and pathologic mechanisms involving structural and immune cell interactions.

Effect of structural cells on T-cell apoptosis

Tissue sections obtained from each EHMB model were stained for apoptotic T cells by using the TUNEL technique. Fig 2 shows the percentage of apoptotic T cells in each model from healthy and asthmatic subjects. There was no significant change in the level of apoptotic T cells from healthy control subjects when they were cultured in different models obtained from healthy

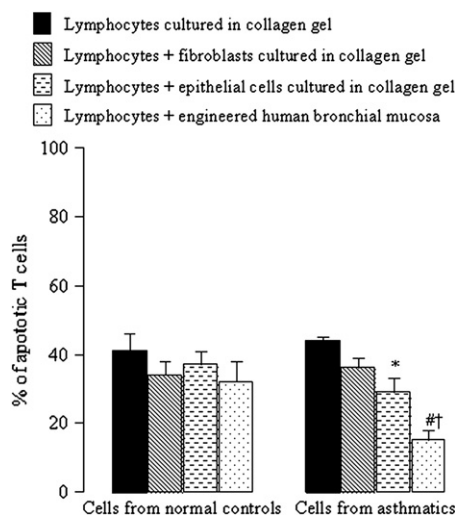


FIG 2. Percentage of apoptotic T cells cultured in EHB from asthmatic and healthy subjects. Tissue sections obtained from different models were stained with the TUNEL technique. Stained T cells were counted, and the percentage of apoptotic T cells in different models obtained with bronchial cells from healthy ($n = 6$) and asthmatic ($n = 6$) subjects was determined. * $P = .048$, † $P = .005$, and # $P = .05$, comparison between EHB from asthmatic and control subjects.



FIG 4. Expression of Bcl-2 by T cells included in EHB from healthy control subjects and asthmatic subjects. The percentage of T cells expressing Bcl-2 in different models obtained with bronchial cells from healthy ($n = 6$) and asthmatic ($n = 6$) subjects is shown. # $P = .006$, ## $P = .01$.

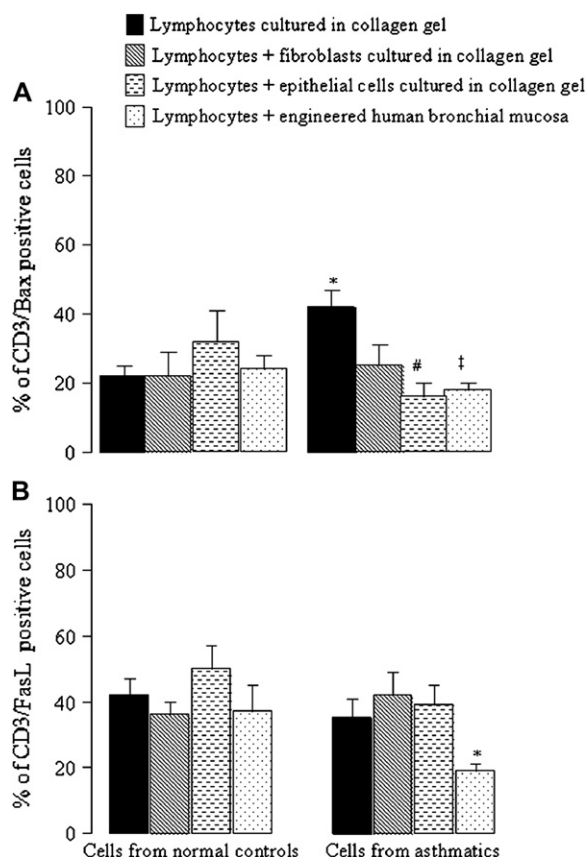


FIG 3. Expression of Bax and FasL by T cells included in EHB from asthmatic and healthy subjects. **A**, Percentage of T cells expressing Bax in different models obtained with bronchial cells from healthy ($n = 6$) and asthmatic ($n = 6$) subjects. * $P = .001$, # $P = .003$, and † $P = .005$. **B**, Percentage of T cells expressing FasL in different models. * $P = .012$.

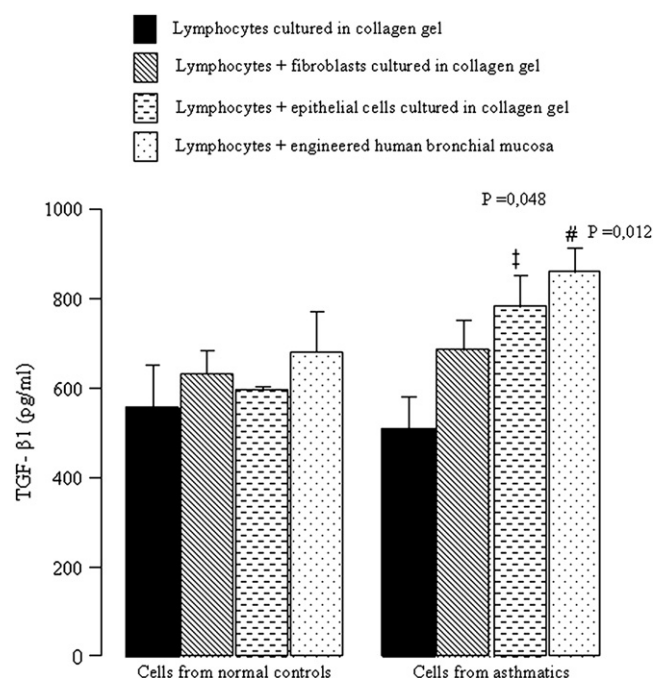
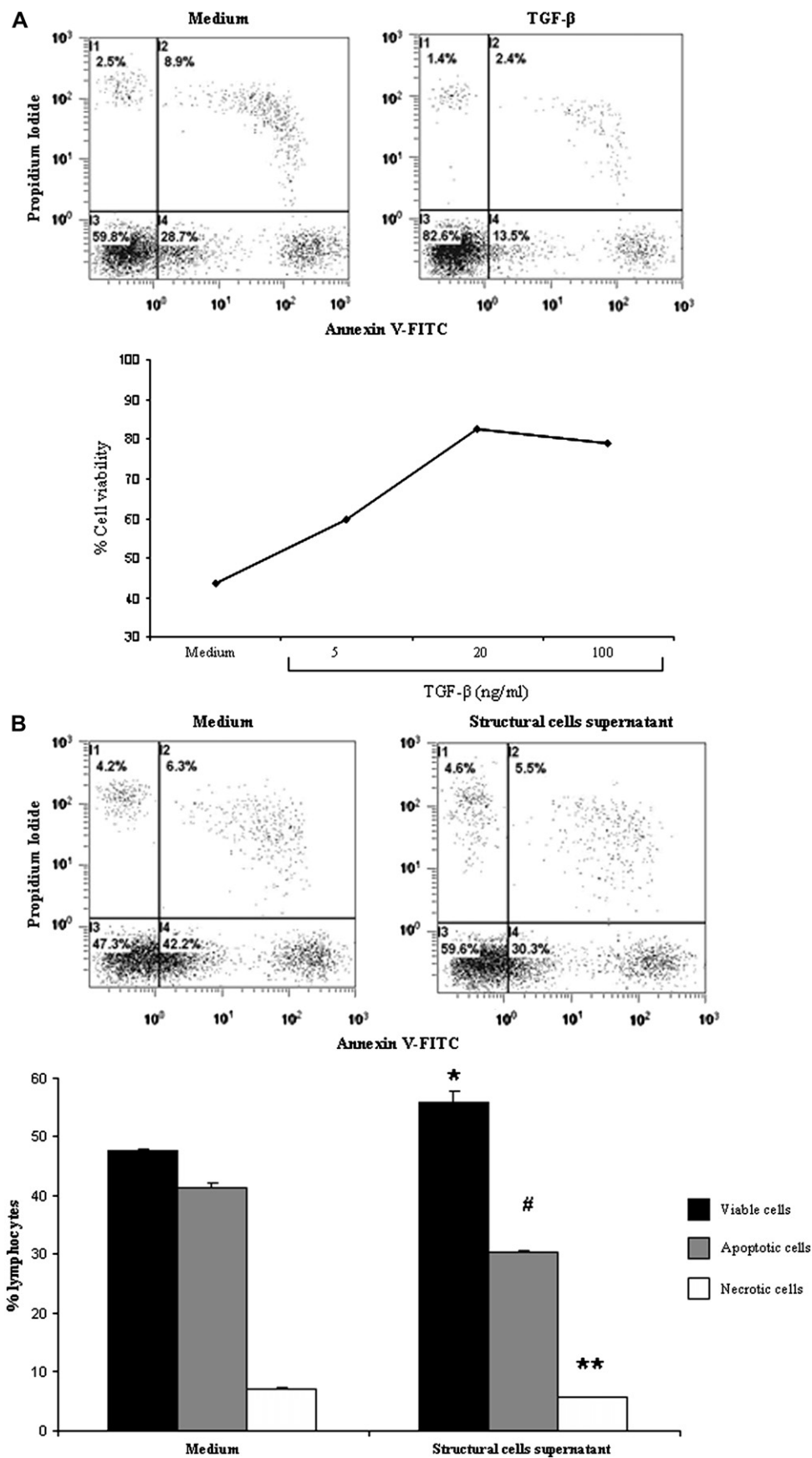


FIG 5. TGF- β 1 production by T cells and structural cells cultured in EHB. TGF- β 1 was measured by means of ELISA in supernatants from different models obtained with bronchial cells from healthy ($n = 6$) and asthmatic ($n = 6$) subjects. † $P = .04$, # $P = .012$.

subjects. However, a significant decrease in the percentage of apoptotic T cells was observed when T cells from asthmatic subjects were included in the model containing epithelial cells and fibroblasts from asthmatic subjects compared with T cells included



in collagen gel ($15\% \pm 1\%$ vs $44\% \pm 3\%$, $P = .005$). Interestingly, this effect was obvious mainly when epithelial cells and T cells, rather than fibroblasts, were seeded because the percentage of apoptotic T cells was significantly less in the model with epithelial cells compared with the model with T cells in gel only ($29\% \pm 4\%$ vs $44\% \pm 3\%$, $P = .048$). Fibroblasts seemed to have no significant protective effect on T cells compared with collagen gel ($36\% \pm 3\%$ vs $44\% \pm 3\%$). The number of apoptotic T cells was significantly lower in EHBM from asthmatic subjects compared with the number from healthy control subjects ($15\% \pm 1\%$ vs $32\% \pm 5\%$, $P = .05$).

Effect of structural cells on proapoptotic and antiapoptotic molecule expression by T cells

Apoptosis induces expression of a wide range of molecules. In this study we evaluated the number of T cells that express Bax, FasL, and Fas as an example of proapoptotic molecules. Fig 3, A, shows that the percentage of Bax-positive T cells from asthmatic subjects included in collagen gel is significantly higher compared with that from healthy control subjects ($P = .001$). When T cells were cultured with EHBM from asthmatic subjects, the percentage of Bax-positive cells decreased significantly compared with that seen in lymphocyte-populated collagen ($18\% \pm 2\%$ vs $42\% \pm 5\%$, $P = .005$). Although fibroblasts seem to have no significant effect, epithelial cells decreased the expression of Bax by T cells from asthmatic subjects significantly ($16\% \pm 4\%$ vs $42\% \pm 5\%$, $P = .003$) compared with that seen in collagen gel. EHBM from healthy control subjects had no significant effect on Bax expression compared with normal lymphocyte-populated collagen ($29\% \pm 5\%$ vs $22\% \pm 5\%$). We then evaluated Fas and FasL expression by T cells in the 4 models. No changes in Fas expression in any culture conditions were observed (data not shown). However, there was a significant decrease in FasL expression by T cells cultured in the EHBM of asthmatic subjects compared with T cells alone ($19\% \pm 2\%$ vs $35\% \pm 6\%$, $P = .012$). There were no significant changes in the healthy models (Fig 3, B).

We evaluated the expression of Bcl-2, an antiapoptotic protein, in T cells cultured in different models. Fig 4 shows that Bcl-2 expression significantly increased when T cells were cultured in EHBM from asthmatic subjects ($72\% \pm 5\%$ vs $42\% \pm 6\%$, $P = .01$). Fibroblasts from asthmatic patients significantly upregulated Bcl-2 expression ($75\% \pm 6\%$ vs $42\% \pm 6\%$, $P = .006$), whereas epithelial cells had no significant effect. Bcl-2 expression did not change in the healthy cultures.

Cytokine production

We measured some IL-2 receptor γ chain signaling cytokines (IL-2, IL-7, and IL-15) and TGF- β_1 by using ELISA. IL-2 and IL-7 levels detected in the models were very low (data not shown). IL-15 was detected only in models with fibroblasts and lymphocyte cells from both healthy control and asthmatic subjects.

IL-15 levels were significantly higher in cells from asthmatic subjects compared with those in cells from healthy control subjects (8.22 ± 1.9 vs 2.14 ± 0.8 pg, $P = .016$).

TGF- β_1 was detected in all tested conditions. Fig 5 shows release of TGF- β_1 by T cells and structural cells cultured in the engineered models. There was no significant variation in TGF- β_1 production by structural cells derived from healthy control subjects. However, TGF- β_1 production was significantly higher in the model containing epithelial cells and T cells compared with that in T cells cultured in the collagen gel (781 ± 91 vs 507 ± 73 pg, $P = .05$). When T cells were cultured with epithelial cells and fibroblasts from asthmatic subjects, the production of TGF- β_1 was significantly increased (856 ± 67 vs 507 ± 73 pg, $P = .012$).

Effect of TGF- β_1 and structural cell supernatants on T-lymphocyte viability

Incubation of lymphocytes with TGF- β_1 significantly increased the percentage of viable cells (Fig 6, A, quadrant I3) and reduced the proportion of apoptotic cells (quadrant I4) and necrotic cells (quadrant I2). TGF- β_1 improved cell viability in a dose-dependant manner (Fig 6, B).

Fig 6, C, shows that incubation of T lymphocytes isolated from asthmatic subjects with EHBM supernatants significantly improved cell viability ($47.5\% \pm 0.2\%$ vs $55.6\% \pm 2.1\%$, $P = .05$; quadrant I3) and reduced apoptosis ($41.4\% \pm 0.9\%$ vs $30.3\% \pm 0.3\%$, $P = .006$; quadrants I4) and necrosis ($6.8\% \pm 0.5\%$ vs $5.5\% \pm 0.1\%$, $P = .015$; quadrant I2).

DISCUSSION

Infiltration of the airways by inflammatory cells, particularly T cells, is a predominant feature of asthma.¹⁷ A defect in the apoptotic process can contribute to the persistence of T cells in the airways and corresponding chronicity of this disease. Structural cells from the human airways and microenvironment surrounding the lymphocytes play a crucial role in regulating T-cell function. In this study we used engineered bronchial mucosa models to test the possibility that structural bronchial cells might play a critical role in regulating T-cell survival.

T lymphocytes obtained from asthmatic and healthy donors were cultured in EHBM derived from the same donors to determine the contribution of epithelium, mesenchyme, and matrix in T-cell survival. We used autologous cells to generate different engineered bronchial mucosa to avoid any confounding parameters. Our results showed that T-cell survival increased when they were cultured in EHBM from asthmatic subjects compared with that from healthy control subjects.

We evaluated antiapoptotic (Bcl-2) and proapoptotic (Bax and Fas/FasL) protein expressions. We found that bronchial structural cells from asthmatic subjects increased the expression of Bcl-2 and decreased Bax and FasL expression. Interestingly, Bax seems to be modulated more by epithelial cells than by fibroblasts. However, fibroblasts appear to be more effective than epithelial

FIG 6. TGF- β_1 and EHBM supernatant effect on T-lymphocyte survival: *quadrant I2*, Necrotic cells; *quadrant I3*, viable cells; *quadrant I4*, apoptotic cells. **A**, Dose effect of TGF- β on T-lymphocyte survival. *Upper panels* represent an example of flow cytometric analysis. **B**, Effect of asthmatic structural cell supernatants on T-lymphocyte survival. *Upper panels* represent flow cytometric analysis. *Lower panels* represent data obtained from 3 different experiments with medium alone or structural cell supernatants. * $P = .05$, # $P = .006$, and ** $P = .015$ with medium alone.

cells in upregulating Bcl-2. This latter observation is consistent with what has been reported in patients with rheumatoid arthritis, in whom synovial fibroblasts were shown to increase T-lymphocyte survival by upregulating antiapoptotic molecules.¹⁸

Previous studies have indicated the increase of Bcl-2 expression in induced sputum mononuclear cells from asthmatic subjects.¹⁹ Vignola et al²⁰ showed that Bcl-2-positive T cells in bronchial biopsy specimens of asthmatic subjects were increased compared with those of control subjects. This is consistent with our finding. Spinozzi et al²¹ reported hypoeexpression of Fas and FasL by T cells isolated from the bronchoalveolar lavage fluid of asthmatic subjects. Moreover, stimulation of CD4⁺ cells with T_H2 cytokines had an inhibitory effect on Fas mRNA.²² However, Krug et al²³ demonstrated that there was an upregulation of FasL on T cells in bronchoalveolar lavage fluid after allergen challenge, but this increase did not promote T-cell apoptosis. Jayaraman et al²⁴ reported comparable levels of cell-surface Fas expression by peripheral blood T cells from asthmatic and nonasthmatic subjects. We also observed that there was no change in this expression by T lymphocytes obtained from both asthmatic and nonasthmatic subjects. However, T cells from asthmatic subjects failed to undergo the normal degree of apoptosis induced by Fas receptor ligation. Melis et al²⁵ showed that there was no difference in spontaneous apoptosis of peripheral blood T lymphocytes from asthmatic and healthy subjects and that they expressed the same baseline level of Bcl-2. There is compelling evidence indicating that in asthma the imbalance toward a T_H2 response is mainly due to the increase in the apoptosis of T_H1 cells.²⁶ In the present study we evaluated the survival of CD3⁺ cells without reference to their cytokine profile. We do not exclude the possibility that the increase in survival we observed might be due to an increase in the apoptosis of T_H1 cells.

The microenvironment in tissues provides cells with a complex of cytokine and growth factors, triggering a cascade of events that promotes cell survival. In the asthmatic airways, the microenvironment surrounding T cells is modified. This might change the behavior of T cells. Adhesion of lymphocytes to extracellular matrix components might also play a critical role in maintaining T cells at the inflammatory sites and decreasing their apoptotic stress. Extracellular matrix components, such as fibronectin, have been shown to increase T-cell survival.²⁷ It has been shown that engagement of integrin inhibits FasL expression and cell death in T cells.²⁸ Integrin signaling blockage with RGD peptides inhibits cell death of activated T cells and blocks the expression of FasL.²⁹ We have shown that fibroblasts and epithelial cells cultured in an engineered model have the capacity to produce extracellular matrix components,¹⁶ such as fibronectin.

T-cell apoptosis can be delayed by cytokines, such as IL-2, IL-4, IL-7, IL-15, and type 1 interferon.^{30,31} In our models we did not detect IL-2 and IL-7. IL-15 was detected only in the model containing fibroblasts and T cells from both healthy control subjects and asthmatic patients. Interestingly, IL-15 levels were significantly higher in the model derived from asthmatic patients compared with those from healthy control subjects. Epithelial cells and fibroblasts, particularly those derived from asthmatic subjects, are sources of different cytokines, mediators, and matrix proteins that might have a prosurvival effect. We showed that addition of culture supernatants from these cells increased the viability of T cells *in vitro*. We found that TGF- β ₁ was produced

extensively by structural cells derived from asthmatic patients compared with cells from healthy control subjects. TGF- β has been shown to protect T cells from apoptosis by maintaining the integrity of the mitochondria. The absence of TGF- β in T cells results in the disruption of mitochondrial membrane potential and a release of death factors.³² TGF- β inhibits FasL expression in T cells and their subsequent apoptosis.³³ We showed that TGF- β ₁ added to culture media increased T-cell viability. Collectively, these findings suggest that in our system TGF- β might play an important role in the increased survival of T cells observed with engineered human mucosa derived from asthmatic subjects.

In conclusion, our findings provide evidence that the tissue environment can play a crucial role in chronic inflammation in asthma and that cooperation between structural cells helps to create a specific milieu that sustains activation and survival of immune cells in the inflamed tissue.

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Clinical implications: Structural cells amplify bronchial inflammation and can be considered as specific targets for new therapeutic approaches.

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