Increased production of TGF-β and apoptosis of T lymphocytes isolated from peripheral blood in COPD

S. J. Hodge, G. L. Hodge, P. N. Reynolds, R. Scicchitano, and M. Holmes

Increased production of TGF-β and apoptosis of T lymphocytes isolated from peripheral blood in COPD.

Submitted 16 December 2002; accepted in final form 8 March 2003

Hodge, S. J., G. L. Hodge, P. N. Reynolds, R. Scicchitano, and M. Holmes. Increased production of TGF-β and apoptosis of T lymphocytes isolated from peripheral blood in COPD. Am J Physiol Lung Cell Mol Physiol 285: L492–L499, 2003; 10.1152/ajplung.00428.2002.—Chronic obstructive pulmonary disease (COPD) is associated with inflammation of airway epithelium, including an increase in the number of intraepithelial T cells. Increased apoptosis of these T cells has been reported in the airways in COPD, and although this process is critical for clearing excess activated T cells, excessive rates of apoptosis may result in unbalanced cellular homeostasis, defective clearance of apoptotic material by monocytes/macrophages, secondary necrosis, and prolongation of the inflammatory response. Lymphocytes are known to traffic between the airway and the peripheral circulation, thus we hypothesized that in COPD, circulating T cells may show an increased propensity to undergo apoptosis. We analyzed phytohemagglutinin (PHA)-stimulated peripheral blood T cells from COPD patients and controls for apoptosis using flow cytometry and staining with annexin V and 7-aminoactinomycin D. As several pathways are involved in induction of apoptosis of T cells, including transforming growth factor (TGF)-β/TGF receptor (TGFR), TNF-α/TNFRI, and Fas/Fas ligand, these mediators were also investigated in peripheral blood samples from these subject groups. Significantly increased apoptosis of PHA-stimulated T cells was observed in COPD (annexin positive 75.0 ± 14.7% SD vs. control 50.2 ± 21.8% SD, P = 0.006), along with upregulation of TNF-α/TNFRI, Fas, and TGFR. Monocyte production of TGF-β was also increased. In conclusion we have demonstrated the novel finding of increased apoptosis of stimulated T cells in COPD and have also shown that the increased T-cell death may be associated with upregulation of apoptotic pathways, TGF-β, TNF-α, and Fas in the peripheral blood in COPD.

apoptosis; blood; cytokine; chronic obstructive pulmonary disease; transforming growth factor-β

CHRONIC OBSTRUCTIVE PULMONARY DISEASE (COPD), a disease of the airways associated with cigarette smoking, is characterized by airway wall inflammation with or without alveolar destruction (emphysema). This inflammation leads to recurring cycles of injury and repair, and disorders in the repair process can lead to tissue remodeling with altered structure and function (41). Apoptosis, or programmed cell death, is important for the regulation of normal cell turnover in the lung and is a key mechanism in the control of the repair process. Activated T cells, which are increased in the airways in COPD, are thought to play an important role in the pathogenesis of the disease, with their numbers in the alveolar wall correlating with the extent of emphysema (31). These T cells must be removed by apoptosis at the end of an inflammatory response to maintain cellular homeostasis (22, 28). Under normal conditions, apoptosis is followed by rapid phagocytosis of apoptotic cells by macrophages/monocytes with minimal inflammatory response. However, increased rates of apoptosis of activated T cells may result in unbalanced homeostasis, leading to an overloading of the local capacity for phagocytosis and defective clearance (27). This could potentially lead to retention of apoptotic material, secondary necrosis, and perpetuation of the inflammatory response.

There have been reports of increased apoptosis of lymphocytes obtained from the airways by bronchoalveolar lavage in COPD (24, 34). Lymphocytes are known to traffic from the bloodstream to the bronchoalveolar space and then may later rejoin the peripheral circulation (21). On the basis of this known trafficking of lymphocytes between the airways and the peripheral blood and the increased rates of apoptosis in airway-derived T cells, we hypothesized that we could detect an increase in the propensity of peripherally derived T cells from COPD patients to undergo apoptosis compared with cells from subjects without this disease.

Several pathways have been reported to be involved in inducing apoptosis of T cells. These include transforming growth factor (TGF)-β/TGF receptor (TGFR) 1, TNF-α/TNFRI, and Fas/Fas ligand (FasL) (22, 36, 37). Increased production of TGF-β and TNF-α has been reported in the airways in COPD (19, 43). We thus hypothesized that increased activation of these proapoptotic pathways might contribute to increased T-cell apoptosis in the airways. In the present study, we investigated production of these apoptotic mediators and expression of their receptors in the peripheral blood from COPD subjects. These factors may contrib-
ute not only to T-cell apoptosis but also to apoptosis of alveolar wall cells, thereby contributing to the development of emphysema (17, 24).

Alterations in lymphocyte subsets in the peripheral blood and airways of patients with COPD have also been reported (32). The CD4/CD8 ratio is significantly decreased with the percentage of CD8 lymphocytes increased. These ratio changes in COPD could be due to a number of factors, including relatively increased rates of apoptosis of CD4 vs. CD8 T cells. Alternatively, the changed ratio could primarily be due to an absolute increase in CD8 T-cell numbers. We therefore specifically investigated apoptosis of the CD4 and CD8 subsets of T cells in the COPD patients and control subjects.

We used multiparameter flow cytometry to determine cytokine, cytokine receptor, and apoptosis levels as previously reported (8, 12, 15). It was important to use whole blood, as we have previously shown that purification of peripheral blood mononuclear cells (PBMCs) results in increased levels of apoptosis (13). By investigating peripherally derived cells in this way, we hope to gain further insight into the role of apoptosis in COPD.

**MATERIALS AND METHODS**

**Study population.** After obtaining informed consent, we collected peripheral blood into sodium heparin (50 IU/ml) as anticoagulant from 18 patients with COPD (mean age 66 yr, 3 females, 15 males). All COPD subjects were reformed smokers with the exception of one current smoker. For 16 nonsmoking volunteers with no history of asthma or hay fever, specimens were obtained and used as controls (mean age 41 yr, 8 females, 8 males; Table 1). We established the diagnosis of COPD using the Global Initiative for Chronic Obstructive Lung Disease criteria of a diagnosis of COPD with a relevant history supported by a postbronchodilator forced expiratory volume in 1 min (FEV1) <80% and FEV1/forced vital capacity <70% (30). The study protocol was approved by the Research Ethics Committee of the Royal Adelaide Hospital following the guidelines of the Declaration of Helsinki.

**Reagents.** Phycoethyrin-conjugated monoclonal antibodies (MAbs) against TGF-β (IQ Products), Fas (CD95), and CD132 (Pharmingen, San Diego, CA); TNF-α (Becton Dickinson BD, San Jose, CA); TNFR1 (R&D); IL-4 (BD), IL-4R (CD124, CD132), and Fas (CD95) were stained as previously reported (9). Briefly, 200-μl aliquots of blood were stained with 3 μl of directly conjugated MAbs to surface markers of interest, lysed with FACSlyse (BD), washed, then acquired immediately by flow cytometry.

**Staining for apoptosis with annexin V.** Staining with annexin V (a natural ligand for phosphatidylserine (PTS)) was used for evaluation of apoptosis. In early apoptosis, membrane alteration exposes phospholipids, such as PTS, on the outer cell membrane. Staining for cell type identification was carried out as described above. Cells were further washed and stained with annexin V as we have previously reported (11, 14).

**Staining for apoptosis with 7-AAD.** Staining with MAbs for cell type identification and 7-AAD for apoptosis was carried out as we have previously reported (14).

**Staining for intracellular cytokine production.** After surface staining as described above, cells were stained for cytokine production as we have previously reported (10). In brief, red blood cells were lysed, then cells were permeabilized with 500 μl of FACSperm (BD), washed, then stained for 20 min with directly conjugated MAbs to TGF-β, IL-4, TNF-α, and isotype-matched controls. Cells were washed, and events were acquired immediately. As TNF-α and Fas have been reported to induce apoptosis of CD8⁺ T cells (23), production of these mediators was investigated for CD3⁺, CD4⁺, and CD8⁺ T cells.

**Flow cytometric analysis.** We carried out flow cytometric analysis using a FACS Calibur flow cytometer (BD) equipped with an air-cooled 488-nm argon ion laser. We collected 10,000 events in list mode using CellQuest software (BD) for analysis. Cells were initially gated on the basis of forward scatter and side scatter characteristics, with gates set to remove debris and platelets (Fig. 1A). Results were expressed as a percentage of cells exhibiting positive fluorescence. Analysis of intracellular cytokine production was carried out on T cells and monocytes, identified by staining characteristics with MAbs (Fig. 1B). T lymphocytes were gated based on known staining characteristics with CD3 PC-5 versus side scatter. CD8⁺ events were identified, then CD4⁺ events gated by CD3⁺CD8⁻ staining characteristics. Monocytes were gated based on known staining characteristics with CD14 PC-5.

**Table 1. Demographic characteristics of the population studied**

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Control Group</th>
<th>COPD</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>16</td>
<td>18</td>
</tr>
<tr>
<td>Age, yr</td>
<td>41</td>
<td>66</td>
</tr>
<tr>
<td>Smoking, pack yr</td>
<td>8.5</td>
<td>90.4</td>
</tr>
<tr>
<td>FVC, % pred</td>
<td>103</td>
<td>47.5</td>
</tr>
<tr>
<td>FEV1, % FVC</td>
<td>100</td>
<td>88.8</td>
</tr>
</tbody>
</table>

Results are expressed as mean values. COPD, chronic obstructive pulmonary disease; FEV1, forced expiratory volume in 1 min; FVC, forced vital capacity; pred, predicted.
ELISA measurement of soluble TGF-β. Plasma from 24-h LPS-stimulated blood was removed after centrifugation at 1,800 g for 5 min. Plasma was stored at −20°C before batch testing. “Active” TGF-β (i.e., nonpolymerized) was quantified with a Quantikine immunoassay kit (R&D), following instructions supplied by the manufacturer.

Determination of TGFR expression by flow cytometry. We obtained PBMCs by diluting peripheral blood with an equal volume of RPMI 1640, layering 7 ml over 3 ml of Lymphoprep (Nycomed Pharma, Oslo, Norway), and centrifuging for 15 min at 500 g. The monolayer was removed and washed twice with 10 ml of RPMI 1640 containing 10% FCS (Gibco). The cells were resuspended in RPMI and incubated for 24 h, at 37°C in 5% CO₂ in air. We quantified TGFR using a sensitive indirect kit (Fluorokine, R&D) following instructions supplied from the manufacturer.

Statistical analysis. The Wilcoxon nonparametric test for paired data was used to analyze the data, using SPSS software. P values ≤ 0.05 were considered significant.

Investigation of the influence of age on apoptosis and cytokine production. There was a difference in the ratio of male-female control subjects (male-female, 8:8) tested in parallel with the COPD subjects (male-female, 15:3) in this study. To investigate the influence of sex, we investigated levels of apoptosis and production of cytokines in peripheral blood from eight normal, nonsmoking volunteers and eleven males as described above.

RESULTS

CD4+ and CD8+ T cells in peripheral blood. The percentage of CD8 T cells was slightly but significantly increased in the peripheral blood of COPD subjects compared with control subjects (31.7 ± 14.3% SD vs. 26.6 ± 11.2% SD, P = 0.049). The percentage of CD4 T cells was not significantly different between the groups (COPD 68.2 ± 14.3% SD vs. 73.1 ± 11.2% SD). The ratio of CD4/CD8 T cells was decreased in the peripheral blood of COPD subjects compared with control subjects (3.1 ± 2.4 SD vs. 4.3 ± 4.1 SD, P = 0.041).
Quantitation of apoptosis by flow cytometry. Apoptosis, identified by staining with annexin V, was higher for PHA-stimulated T cells from patients with COPD than those from the control group (P = 0.006, Table 2). Apoptosis of stimulated T cells was significantly increased in both CD4 and CD8 T cells from COPD subjects (P = 0.043 for both subsets). There was no significant difference in levels of apoptosis between CD4 and CD8 T-cell subsets in COPD patients. To confirm these findings, we carried out 7-AAD staining in parallel. There was good correlation between the two methods (Fig. 2). Apoptosis, identified by staining with 7-AAD, was higher for PHA-stimulated T cells from COPD subjects than from control subjects (COPD 70.0% ± SD 23.1% vs. control 58.2% ± SD 15.9%, P = 0.043).

Expression of Fas. Having found an increase in the propensity of T cells from COPD subjects to undergo apoptosis, we next investigated a number of potential pathways that may explain this. To investigate the role of the Fas pathway in apoptosis of T cells in peripheral blood, we measured Fas (CD95) expression for CD3+, CD4+, and CD8+ T cells, and CD14+ monocytes. T cells from COPD patients expressed Fas (CD95) at a higher frequency than control subjects (Table 2). This difference was significant for both CD4 and CD8 T cells (Table 2). However, Fas expression by monocytes was not significantly different for COPD and control groups.

Quantification of intracellular cytokines and cytokine receptors by flow cytometry. In addition to Fas, TNF-α has also been reported to induce apoptosis of T cells (44), thus we hypothesized that changes in the levels of this mediator in COPD might be involved in the alteration of T-cell apoptosis rates and/or the CD4/CD8 ratio. Using flow cytometry, we found significantly increased production of TNF-α by peripheral blood T cells in COPD (P = 0.008, Fig. 3). There was no significant difference noted for CD4+ or CD9+ T cells (CD4, COPD 17.8 ± 20.1% vs. control 3.6 ± 2.9%; CD8, COPD 15.2 ± 24.0% vs. control 2.0 ± 15.2%, not significant). The effect of TNF-α is dependent on which TNFR is activated, with TNFR1 and TNFR2 having pro- and antiapoptotic roles, respectively. Increased expression of TNFR1 was observed for CD3+ T cells from COPD subjects compared with controls (P = 0.003, Table 3).

Table 2. Apoptosis (positive annexin V staining) and Fas expression for phytohemagglutinin-stimulated peripheral blood from COPD and control subjects measured by flow cytometry

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Marker</th>
<th>Control Group</th>
<th>COPD</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>T cell</td>
<td>annexin V</td>
<td>50.2 ± 21.8</td>
<td>75.0 ± 14.7</td>
<td>0.006*</td>
</tr>
<tr>
<td>CD4+</td>
<td></td>
<td>54.8 ± 27.7</td>
<td>68.5 ± 20.7</td>
<td>0.043*</td>
</tr>
<tr>
<td>CD8+</td>
<td></td>
<td>43.1 ± 25.9</td>
<td>52.0 ± 20.7</td>
<td>0.043*</td>
</tr>
<tr>
<td>T cell</td>
<td>Fas (CD95)</td>
<td>35.5 ± 12.5</td>
<td>44.8 ± 16.3</td>
<td>0.013*</td>
</tr>
<tr>
<td>CD4+</td>
<td></td>
<td>41.1 ± 10.4</td>
<td>53.6 ± 16.0</td>
<td>0.002*</td>
</tr>
<tr>
<td>CD8+</td>
<td></td>
<td>24.2 ± 20.4</td>
<td>36.6 ± 28.0</td>
<td>0.004*</td>
</tr>
<tr>
<td>Monocyte</td>
<td></td>
<td>89.9 ± 17.4</td>
<td>86.0 ± 18.6</td>
<td></td>
</tr>
</tbody>
</table>

Results (% of cells exhibiting positive staining measured by flow cytometry) are expressed as mean values ± SD. NS, not significant. *Significant difference from control group.

Fig. 2. Contour plot of annexin V FITC vs. 7-aminoactinomycin D (7-AAD) staining. Good correlation between annexin V and 7-AAD staining of apoptotic T cells from phytohemagglutinin (PHA)-stimulated peripheral blood control subject, stimulated with PHA for 24 h. Annexin V-positive staining (quadrants Q1 and Q2) 25.9%; 7-AAD staining (Q1 and Q2) 24.5%.

Fig. 3. Intracellular cytokine production by T cells and monocytes from peripheral blood from COPD and control groups. C, control; T, COPD. Note significantly increased production of TNF-α by T cells and monocytes and increased transforming growth factor (TGF)-β production by monocytes. *Significant difference (P < 0.05) between COPD and controls. Arrows denotes values from the COPD subject, who was a current smoker.
There was no significant difference in expression of TNFR2 between COPD and control groups.

We also investigated the role of TGF-β and its receptor in induction of apoptosis of peripheral blood T cells in COPD. Flow cytometric analysis showed low production of TGF-β by activated T cells and no significant difference between COPD and control groups (Fig. 3). However, monocyte production of TGF-β was significantly increased in COPD (Fig. 3). In contrast to TGF-β, TGFR expression was significantly increased for T cells from COPD subjects compared with controls (P = 0.018, Table 3).

Evaluation of secreted, active TGF-β by ELISA. Although we found increased cellular TGF-β levels, TGF-β is produced in a latent form and is biologically inactive and unable to bind to its receptors until it has been activated (2). The flow cytometric method utilized an MAb to both active and latent TGF-β and hence measured total TGF-β. To measure secreted, active TGF-β in the peripheral blood, we applied ELISA techniques to measure plasma levels. Release of active TGF-β in the peripheral blood in COPD was significantly increased compared with the control group (P = 0.015) (Fig. 4).

Investigation of the influence of age on apoptosis and cytokine production. There was no significant difference in the levels of apoptosis and production of IL-4 or TGF-β for the various age groups (Table 4). TNF-α production for peripheral blood-derived T cells increased with age, but the increase did not reach statistical significance (P = 0.087, 55- to 65-yr-old age group compared with the 35- to 45-yr-old group) (Table 4). Monocyte TNF-α production was significantly lower for the 55- to 65-yr-old age group compared with the 35- to 45-yr-old age group (Table 4).

Investigation of the influence of sex of the subjects on apoptosis and cytokine production. There was no significant difference in levels of apoptosis or cytokine production between males and females (Table 4).

**DISCUSSION**

COPD is a complex disease for which current therapies are often inadequate. New insights into the pathogenesis of this disorder are needed, and in recent years there has been considerable interest in the possible role of apoptosis in this and many other diseases. Increased apoptosis of T cells has been reported in the airways in COPD (24, 34), and this may have significance for the disease pathogenesis. We report here the novel finding of increased apoptosis of stimulated T cells from the peripheral blood in COPD. The increase was not age or sex related.

We have previously found that the detectable level of apoptosis of T cells (identified by annexin V staining) in peripheral blood samples is <1% (11). Direct comparisons of unstimulated blood samples between groups of subjects have not previously been reported due to the low levels of apoptosis detectable, which is likely a reflection of the rapid removal of apoptotic cells from the circulation. Such comparisons are thus likely to miss important differences between groups. The present study utilized PHA stimulation of peripheral blood T cells from COPD and control subjects, using flow cytometry and annexin V and 7-AAD staining methods. PHA has been widely reported for studies of the potential of T cells to undergo apoptosis when stimulated (1, 5, 7, 16, 18, 25, 26).

**Table 3. Expression of cytokine receptors by T cells in COPD measured by flow cytometry**

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Control</th>
<th>COPD</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNFR1</td>
<td>0.6 ± 0.8</td>
<td>1.2 ± 1.3</td>
<td>0.002*</td>
</tr>
<tr>
<td>TNFR2</td>
<td>3.8 ± 2.6</td>
<td>3.7 ± 3.2</td>
<td>NS</td>
</tr>
<tr>
<td>TGFβR</td>
<td>5.5 ± 2.8</td>
<td>15.8 ± 16.1</td>
<td>0.018*</td>
</tr>
</tbody>
</table>

Results (% of cells exhibiting positive staining measured by flow cytometry) are expressed as mean values ± SD. TNFR, tumor necrosis factor receptor; TGFR, transforming growth factor receptor. *Significant difference from control group.
Airways or alternatively from apoptotic stimuli in the peripheral blood. The link between T cells in the peripheral blood and the airways is still a controversial area, and further studies are thus warranted. We are currently investigating the changes in intracellular apoptotic pathways in lymphocyte subsets in both airways and peripheral blood in COPD to further clarify apoptotic mechanisms associated with this disease.

Activation of the Fas/FasL pathway induces apoptosis of mature CD4+ T cells after repeated antigenic stimulation (38), thus the raised levels of Fas we report here may be significant for apoptosis induction in the peripheral blood in COPD (42).

Apoptosis of CD8+ T cells can be mediated by either TNF-α or Fas pathways (44). The effect of TNF-α is dependent on which TNFR is activated, with TNFR1 and TNFR2 having pro- and antiapoptotic roles, respectively. In the present study, production of TNF-α and expression of TNFR1 by peripheral blood T cells were increased in COPD. These results are consistent with previous reports of increased circulating TNF-α in the peripheral blood in COPD (39). However, the significance of these findings is not clear, as increased age was found to be associated with increased T-cell production of TNF-α (but not TNF-β or IL-4), although the difference did not reach statistical significance. Therefore, the observed increase in T-cell production of TNF-α in COPD subjects (mean age 66 yr) compared with control subjects (mean age 41 yr) may have been influenced by age differences between the groups. Further studies using age-matched controls are warranted.

In contrast to T-cell production, a significantly greater percentage of monocytes from COPD subjects produced TNF-α compared with control subjects. As production of this cytokine was shown to significantly reduce with age, the results suggest that monocyte production of TNF-α may play a role in increased apoptosis in the peripheral blood in COPD.

Although in absolute terms the level of TNFRs seen was low, the difference between the COPD and control groups was clear. The low levels may be a reflection of the sensitivity of the flow cytometry technique and does not discount the potential significance of the findings (45, 46). Cytokine binding has been shown to occur through receptors that need only to be expressed at low concentrations (~100 molecules per cell) to transmit activation signals (45), which is well below the level of sensitivity of flow cytometry.

TNF-β, unlike the other cytokines investigated in this study, is produced in a latent form and is biologically inactive and unable to bind to its receptors until it has been activated (2). The flow cytometric method utilized a MAb to both active and latent TNF-β and hence measured total TNF-β. To measure active TNF-β, we also applied ELISA techniques to measure plasma levels. The TNF-β/TGFR pathway enhances apoptosis of peripheral blood T cells by inhibiting proliferation at G1–S phase transition (3) and inhibiting IL-2-induced expression of α- and β-chains of IL-2R and IL-2-induced activation of signal transduction molecules Janus kinase-1 and signal transducer and activator of transcription 5 (4). Our findings of increased production of TGFR-β by monocytes, increased release of active TNF-β, and upregulated expression of TGFR-β by T cells suggest that this cytokine may contribute to excess apoptosis of T cells in the peripheral blood in COPD. Interestingly, we have previously reported that plasma-derived factor VIII concentrate has apoptosis-promoting effects on T cells (11). The presence of TNF-β was shown to be a major component responsible for the apoptotic effects seen in PHA-stimulated T cells from hemophilia patients receiving factor VIII prophylaxis therapy (11).

Recent reports have shown that phagocytosis of apoptotic cells by macrophages leads to release of TGFR-β (6). Monocytes may therefore increase their secretion of TGFR-β in the peripheral blood, as a result of ingestion of increased numbers of apoptotic cells, potentially explaining our findings of increased intracellular production of TGFR-β by monocytes and secreted TGFR-β in COPD.

The ratio of CD4/CD8 cells has important implications for the host response to infective and inflammatory stimuli. COPD is associated with a relative increase in CD8+ T cells (32), a finding that was confirmed in the present study. Reasons for this have not been determined. TNF-α and Fas have been shown to induce apoptosis of CD8+ T cells (44). These mediators were therefore investigated for CD4 and CD8 lymphocyte subsets. The increase in CD8+ T cells could not be explained by a relative increase in apoptosis of CD4+ T cells nor by relative changes in TNF-α and Fas. TGFR-β, although well recognized as a growth inhibitor of T lymphocytes, may play a role in increased apoptosis of CD4+ T cells in COPD.
cells, has been demonstrated to be costimulatory for naive CD8+ T cells (20). Thus increased TGF-β in the peripheral blood in COPD, as well as increased expression of TGFR by CD8+ T cells, may partially explain the increase of CD8+ T cells in COPD.

COPD is associated with an increased susceptibility to infection (35). TGF-β has broad inhibitory effects on immune function and may increase susceptibility to opportunistic infections and malignancies (23). We can speculate that elevated TGF-β and TGFR expression in COPD may result in an increase in T-cell apoptosis following an infection. This would lead to a diminished immune response to the infective organism and contribute to the increased frequencies of infection, which are associated with the disease (35).

In conclusion, we have demonstrated the novel finding of increased propensity of peripheral blood T cells in COPD to undergo apoptosis. Whether this finding represents a systemic effect of COPD on peripheral cells or whether these cells have reentered the circulation after passing through the airway epithelium requires further study.

The authors thank the Clinical Trials Unit, Department of Thoracic Medicine, Royal Adelaide Hospital.

DISCLOSURES

This study was supported by a Dawes Scholarship from the Royal Adelaide Hospital.

REFERENCES

30. Pauwels RA, Buist AS, Calverley PM, Jenkins CR, and Hurd SS. Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease. NHLBI/WHO Global Initiative for Chronic Obstructive Lung Disease


