Cytotoxic Potential of Lung CD8\(^+\) T Cells Increases with Chronic Obstructive Pulmonary Disease Severity and with In Vitro Stimulation by IL-18 or IL-15


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Cytotoxic Potential of Lung CD8+ T Cells Increases with Chronic Obstructive Pulmonary Disease Severity and with In Vitro Stimulation by IL-18 or IL-15

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Lung CD8+ T cells might contribute to progression of chronic obstructive pulmonary disease (COPD) indirectly via IFN-γ production or directly via cytolysis, but evidence for either mechanism is largely circumstantial. To gain insights into these potential mechanisms, we analyzed clinically indicated lung resections from three human cohorts, correlating findings with spirometrically defined disease severity. Expression by lung CD8+ T cells of IL-18R and CD69 correlated with severity, as did mRNA transcripts for perforin and granzyme B, but not Fas ligand. These correlations persisted after correction for age, smoking history, presence of lung cancer, recent respiratory infection, or inhaled corticosteroid use. Analysis of transcripts for killer cell lectin-like receptor G1, IL-7R, and CD57 implied that lung CD8+ T cells in COPD do not belong to the terminally differentiated effector populations associated with chronic infections or extreme age. In vitro stimulation of lung CD8+ T cells with IL-18 plus IL-12 markedly increased production of IFN-γ and TNF-α, whereas IL-15 stimulation induced increased intracellular perforin expression. Both IL-15 and IL-18 protein expression could be measured in whole lung tissue homogenates, but neither correlated in concentration with spirometric severity. Although lung CD8+ T cell expression of mRNA for both T-box transcription factor expressed in T cells and GATA-binding protein 3 (but not retinoic acid receptor-related orphan receptor γ or α) increased with spirometric severity, stimulation of lung CD8+ T cells via CD3ε-induced secretion of IFN-γ, TNF-α, and GM-CSF, but not IL-5, IL-13, and IL-17A. These findings suggest that the production of proinflammatory cytokines and cytotoxic molecules by lung-resident CD8+ T cells contributes to COPD pathogenesis. The Journal of Immunology, 2010, 184: 6504–6513.

Chronic obstructive pulmonary disease (COPD), the fourth leading cause of death in the United States (1), is a progressive, debilitating disease that is rapidly increasing in worldwide prevalence. Current therapies have very limited impact on disease progression, making greater understanding of pathogenesis crucial. COPD is an inflammatory condition triggered by oxidant stress, notably tobacco smoke exposure and, in the developing world, indoor biomass fuel combustion. CD8+ T cells have been implicated in the development of COPD because their numbers in lung parenchyma and small airways correlate inversely with lung function (2–5). We (6) and others (7–9) have demonstrated that CD8+ T cells isolated from lung parenchyma in COPD are largely Tc1 cells. Indeed, we previously showed that mRNA transcripts for IFN-γ from unstimulated lung CD8+ T cell correlated directly with disease severity, whereas IL-4 transcripts were essentially undetectable (6). However, two groups who studied cells isolated from the alveolar spaces found evidence of a significant Tc2 component (10, 11), implying that there may be anatomic compartmentalization of the CD8+ T cell phenotype in COPD.

Whether and how lung CD8+ T cells actually contribute to COPD pathogenesis, however, remains undefined. One possibility is that T cell production of IFN-γ fosters lung destruction. Evidence supporting this possibility comes from an inducible transgenic murine system in which local overexpression of IFN-γ led to lung inflammation and emphysema associated with induction of matrix metalloproteinase 12 (12). Another possibility is that CD8+ T cells directly kill lung parenchymal cells that they recognize as altered-self or infected via perforin plus granzymes or Fas ligand (Fasl). The oxidant injury induced by smoking could plausibly lead to antigenic modification that would be recognized by CD8+ T cells in the context of class I MHC. A correlation has been shown between numbers of lung CD8+ T cells and apoptotic cells of all types identified in microscopic sections (13), but to date, no studies have directly proven that CD8+ T cells are responsible for parenchymal cell apoptosis in emphysema. Considerations such as these argue

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Abbreviations used in this paper: AMr, alveolar macrophage; COPD, chronic obstructive pulmonary disease; DC, dendritic cell; F, female; FasL, Fas ligand; FEV1, forced expiratory volume in 1 s; FVC, forced vital capacity; GATA-3, GATA-binding protein 3; GOLD, Global Initiative for Chronic Obstructive Lung Disease; KLRGB1, killer cell lectin-like receptor G1; LTRC, Lung Research Tissue Consortium; M, male; No stim, no stimulation; NS, nonsmoker; ROR, retinoic acid receptor-related orphan receptor; S, smoker with normal lung function; T-bet, T-box expressed in T cells; Tm, T effector memory cell.

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for investigation of how the cytotoxic potential of lung CD8+ T cells correlates with COPD progression.

Although the effector functions of CD8+ T cells are typically assayed after TCR stimulation, multiple recent findings suggest that TCR-independent mechanisms merit special examination in COPD. IL-18, a member of the IL-1 cytokine superfamily, is in combination with IL-12, an important mediator of Ag-independent IFN-γ production by T cells (14, 15). IL-18 is strongly expressed by alveolar macrophages (AMs) of patients with severe COPD (16) and is increased in the peripheral blood of patients with COPD relative to controls (17, 18). In mice, treatment with rIL-18 and rIL-12 drives pulmonary inflammation and lung injury (19). Cigarette smoke-exposed wild-type mice had increased levels of IL-18 mRNA and protein that localized to AMs, and cigarette smoke-induced emphysema was decreased by a null mutation of the IL-18R (17). IFN-γ production by CD8+ T cells has also been reported to be stimulated by IL-15, a key cytokine for the development and maintenance of CD8+ T cell memory (20, 21). Additionally, TCR-independent activation of CD8+ T cell cytotoxicity is potentially relevant in COPD. Short-term cytokine stimulation can induce TCR-independent, non–MHC-restricted cytotoxicity in highly purified human CD8+ T cells (22). Tobacco smoke-induced upregulation on pulmonary epithelium of ligands for the cytotoxic T cell-activating receptor NKG2D (CD314) has recently been implicated in COPD pathogenesis based on a combination of murine and human data (23).

To investigate the capacity of lung CD8+ T cells to participate in COPD pathogenesis, we correlated their phenotype and in vitro function with disease severity, with special attention to expression of IL-18R. For efficient use of limited human tissues, we employed three controls. Subjects with no history of smoking and normal spirometry (e.g., viable cells, mRNA from purified lung CD8 T cells, preserved lung tissue) for specific purposes. Our results show that although lung CD8+ T cells from all COPD subjects have a predominantly effector memory T cell (TEM) phenotype, regardless of disease severity as defined by pulmonary function, their production of mRNA transcripts for perforin and granzyme B and their expression of IL-18R protein increases as the disease progresses. We further show that in vitro stimulation with IL-18 (plus IL-12) in the absence of TCR stimulation very markedly increases production of IFN-γ and TNF-α, whereas IL-15 stimulation increases intracellular perforin expression.

### Table I. Summary of cohort A, B, and C demographics, smoking histories, and spirometry

<table>
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<th>Group</th>
<th>Cohort</th>
<th>Subjects (n)</th>
<th>Sex Ratio (M/F)</th>
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<th>Smoking History (pack. y)</th>
<th>FEV1 (% Predicted)</th>
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<td></td>
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*Data are presented as average (SD) unless otherwise indicated.

F, female; M, male.
number of subjects, gender ratio, and ranges of age and smoking history for each subject group in cohort C. Importantly, because the University of Michigan Healthcare System and the VA Ann Arbor Healthcare System are collectively one of four contributing sites to the LTRC, it is highly likely that some subjects in this cohort are also represented in cohort A; however, no attempt was made to correlate subjects in the two cohorts.

Sample preparation for flow cytometric analysis and in vitro stimulation

Lung sections from cohort A weighing ∼3 g were homogenized using a Waring blender without enzyme treatments, which we have previously shown efficiently produces single-cell suspensions of high viability (6). Cells were filtered through a 70-μm strainer to remove debris and then were used immediately in two types of experiments.

For flow cytometry, cells were resuspended at 10×10^6 cells/ml staining buffer (2% FBS in PBS), incubated at 4°C for 10 min, and then were added in a volume of 100 μl to each flow tube. Monoclonal anti-human Abs against CD45 (H330), CD8 (RPA-T8), CD27 (O323), CD69 (FNS0), IL-18R (H44) (eBioscience, San Diego, CA), and CD62L (Dreg 56) (BD Biosciences, San Jose, CA) were used. Appropriate isotype-matched controls were used in all experiments. Abs were conjugated to either FITC, PE, or PE-cyanine 5 (PE-Cy5). Cells were incubated in the dark with Abs for 25 min at room temperature and then washed. Cells were fixed and stored in staining buffer plus 2% paraformaldehyde prior to being analyzed on the flow cytometer.

Similarly, to measure protein levels from supernatants of cultured cells, a Waring blender without enzyme treatments, which we have previously shown efficiently produces single-cell suspensions of high viability (6). Protein analysis of lung homogenates and culture supernatants was performed using GraphPad Prism (GraphPad, La Jolla, CA). Nonparametric (Spearman) correlation analysis was used to determine the correlation coefficient, r_s. Paired t tests and one-way ANOVA, with Dunn’s post hoc testing, were used to determine statistical differences between treatment groups in vitro experiments. A two-tailed p value of <0.05 was considered to indicate significance. Log transformation was used for analyses of data that did not meet assumptions for normality. PROC GLM with Tukey’s method for multiple comparisons was employed to contrast patient groups using SAS 9.1 statistical software (SAS Institute, Cary, NC). A similar approach was used to examine difference between smoking-status groups (never-smoker, exsmoker, active smoker). Regression analysis was used to examine the relationship of age, gender, percent emphysema, smoking status, smoking exposure (pack-years, time since quitting), presence versus absence of lung cancer as the indication for surgery, and recent infections to the relationship between FEV_1 percent predicted (continuous or categorically defined) and expression of various markers.

Results

Description of clinical cohorts from which tissue was derived

To overcome the inherent limitations of working with human lung tissue samples that are usually small and yield relatively few cells, different samples were obtained from three separate cohorts of human subjects undergoing clinically indicated resection procedures for pulmonary nodules, lung volume reduction surgery, or lung transplantation. The lung tissue from cohort A (n = 47) was used immediately for flow cytometric analyses and CD8^+ T cell culture. Cohort B (n = 22) lung tissue was used immediately to isolate CD8^+ T cells for subsequent real-time PCR analyses. Lung tissue from cohort C (n = 95) was obtained from the Lung Tissue Research Consortium (LTRC) as frozen tissue sections, which we used to extract total protein for cytokine analysis. Table I shows the number of subjects, gender ratio, and ranges of age and smoking history for each subject group in all three cohorts. Because each cohort provided only one type of sample, results of different assays necessarily derive from different subjects, precluding some direct comparisons. It can be seen, however, that the three cohorts are quite comparable in distributions of age, gender, smoking history, and pulmonary function.

CD69 expression on lung CD8^+ T cells correlates with disease severity

To gain insights into the potential function of human lung CD8^+ T cells in COPD, we first used flow cytometry on single-cell lung suspensions from cohort A to analyze their expression of CD62L and CD27. Regardless of disease severity, the majority (∼75% at all stages) of lung CD8^+ T cells were CD62L^-CD27^+ (data not shown). Interestingly, when CD69, a marker of acute activation, was analyzed in the same manner, the fraction of lung CD8^+ T cells expressing CD69 increased significantly with disease severity, expressed either as GOLD stage (r_s = 0.43, p = 0.006) (Fig. 1C, 1D) or FEV_1 percent predicted (r_s = −0.35, p = 0.02; data not shown). Further analyses suggested that the predominant differences were between normal nonsmokers and the COPD groups. CD69 expression also correlated (r_s = −0.47, p = 0.009; data not shown) with decreasing diffusion lung capacity for carbon monoxide (n = 32), but did not correlate with percent emphysema determined by analysis of high-resolution computed tomographs. Similarly, the frequency of CD8^+ T cells failed to relate to percent emphysema. Additional analyses showed no significant correlation between CD8^+ CD69^+ lung T cells and age, gender, pack-years, smoking status, duration since cessation of smoking, presence
versus absence of lung cancer as the final diagnosis from the surgery, recent respiratory infections, or use of inhaled corticosteroids.

We next analyzed RNA transcripts from isolated CD8+ T cells from cohort B for T-bet, GATA-3, and RORγ, transcription factors that drive Tc1, Tc2, and Tc17 responses, respectively. T-bet transcripts were significantly inversely correlated with FEV1 \((r_S = -0.60, p = 0.004)\) (Fig. 1E), in accord with the strong correlation of disease severity with IFN-γ mRNA transcripts that we have previously reported in this cohort (6). Surprisingly, even though we were previously unable to detect transcripts for IL-4 (6), IL-5, or IL-13 (C.M. Freeman, J.L. Curtis, S.W. Chensue, unpublished observations) by CD8+ T cells in this cohort, GATA-3 transcripts were produced by CD8+ T cells and also showed a trend to increase as FEV1 decreased \((r_S = -0.44, p = 0.049)\) (Fig. 1F). By contrast, lung

**FIGURE 1.** Lung CD8+ T cells in COPD are predominately TEM cells at all disease stages, but their expression of CD69 and T-bet increases as FEV1 decreases. CD8+ T cells from lung tissue were either stained and analyzed by flow cytometry (A–D) or isolated using positive selection magnetic beads and processed for real time RT-PCR (E–G). A, Representative histograms showing staining for CD27 (top panel) and CD62L (bottom panel) on gated lung CD8+ T cells of a patient with COPD. Shaded profiles, isotype control; open profiles, specific staining. B, Percentage of CD8+ T cells that display an effector memory phenotype (CD62L− CD27−) stratified by subject group. Spearman nonparametric analysis was used to calculate \(r_S\). Circles represent individual patients; bars represent the mean ± SEM (cohort A, \(n = 42\)). C and D, CD69 expression. C, Representative histograms from a normal smoker without COPD (top panel) and GOLD stage 4 subject (bottom panel). D, The percentage of CD69+ CD8+ lung T cells stratified by FEV1 (percent predicted). E, T-bet. F, GATA-3. G, RORγ. Results are expressed as arbitrary units. Circles represent individual patients (cohort B, \(n = 22\)). Spearman nonparametric analysis was used to calculate \(r_S\). 1–4, GOLD stages of COPD severity; NS, nonsmoker; S, smoker with normal lung function.
CD8+ T cells showed little to no RORγ (Fig. 1G) or RORα (not shown) transcript expression at any disease severity. Purified lung CD8+ T cells also did not express transcripts for IL-17A, IL-17F, or IL-22. Collectively, these data are consistent with the widely held view that lung CD8+ T cells in COPD principally display a Tc1 phenotype, but imply that some might exhibit Tc2 characteristics under appropriate stimulation.

We also examined mRNA transcripts for three receptors that have been used to identify subsets of human CD8+ effector T cells: KLRG1, IL-7R (CD127), and CD57. Driven by elevated levels of T-bet, especially during chronic viral infections, CD8+ T cells can become terminally differentiated, KLRG1high, IL-7Rlow short-lived effector cells (26, 27). CD57-positivity identifies oligoclonally derived cells typically associated with chronic infections or extreme age. CD8+ CD57+ T cells are TEM cells capable of immediate functional activity, producing IFN-γ and TNF-α in response to Ag challenge (28, 29). We found that a large fraction of subjects at all GOLD stages has no detectable mRNA transcripts for any of these receptors. We did not detect any consistent relationship to disease severity (Fig. 2); however, we did detect a correlation between KLRG1 and IL-7R (r_S = 0.46, p = 0.03) (data not shown).

Perforin and granzyme B, but not FasL, transcripts from lung CD8+ T cells correlate with decreasing FEV1

We next analyzed transcript expression from isolated lung CD8+ T cells from cohort A for the cytotoxic molecules perforin, granzyme B, and FasL. These preformed, apoptosis-inducing molecules represent two pathways by which CD8+ T cells kill target cells, typically those which are virally infected or damaged. We found a significant inverse correlation between mRNA expression of perforin (r_S = −0.69, p = 0.001) and of granzyme B (r_S = −0.50, p = 0.02) and pulmonary function, as measured by FEV1 (percent predicted) (Fig. 3A, 3B). Furthermore, expression of transcripts for perforin and granzyme B within individual subjects was strongly correlated (r_S = 0.85, p < 0.0001) (data not shown). The strength of the correlation did not vary when adjusted for age and gender. Based on the known hierarchy of cytotoxic effector molecules expression (30, 31), these results, together with those presented above, imply that the CD8+ T cells that increasingly accumulate within the lungs during progression of COPD are short-term TEM cells, rather than terminally differentiated effectors. By contrast, FasL transcript expression did not correlate with FEV1 (Fig. 3C).

None of these molecules showed any correlation with smoking history, as measured by pack-years (data not shown). Because computed tomography scans are not available for this cohort of patients, correlations between the cytotoxic molecules and percent of emphysema cannot be assessed.

IL-18R expression on lung CD8+ T cells correlates with worsening pulmonary function

Recent human and animal studies have implicated elevated IL-18 levels and IL-18R signaling in the pathogenesis of pulmonary

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**FIGURE 2.** Expression of KLRG1, CD57, and IL-7R by lung CD8+ T cells. Lung CD8+ T cells were isolated for RNA analysis by real-time RT-PCR. A, KLRG1. B, CD57. C, IL-7R. Transcripts are expressed on the vertical axis as arbitrary units versus FEV1 (percent predicted) on the horizontal axis. Circles represent individual subjects (cohort B, n = 22). Spearman nonparametric analysis was used to calculate r_S.

**FIGURE 3.** Expression of perforin and granzyme B by lung CD8+ T cells increases with worsening COPD severity. Lung CD8+ T cells were isolated for RNA analysis by real-time RT-PCR. Perforin (A), granzyme B (B), and FasL (C) were measured and are expressed on the vertical axis as arbitrary units versus FEV1 (percent predicted) on the horizontal axis. Circles represent individual patients (cohort B, n = 22). Spearman nonparametric analysis was used to calculate r_S.
emphysema and COPD (16, 17). Therefore, we used flow cytometry to analyze the expression of IL-18R on human lung CD8+ T cells from cohort A. We found that IL-18R expression showed a significant correlation with disease severity, whether expressed as GOLD stage 4) (Fig. 4A, 4B) or as FEV1 percent predicted (rS = −0.30, p = 0.04; data not shown). IL-18R expression did not show any correlation with pack-years, diffusion lung capacity for carbon monoxide, or percent emphysema, but there was a very strong correlation in individual subjects between expression of IL-18R and CD69 (rS = 0.83, p = 0.0001; data not shown). Although we did not simultaneously measure the two receptors on individual CD8+ T cells, these data suggest that IL-18R is likely expressed on CD69+ lung CD8+ T cells. The correlation persisted after adjustment for age, gender, duration because cessation of smoking, presence versus absence of lung cancer, and history of recent respiratory infections.

To determine whether IL-18 is present in the lungs in COPD, we homogenized frozen lung tissue from 95 subjects from cohort C and analyzed expression of the mature, bioactive form of IL-18 (Fig. 4C). Although IL-18 levels did not correlate with COPD severity, even when adjusted for the same variables as analyzed for IL-18R expression, these data show that IL-18 is present in human lung tissue collected during periods of clinical stability.

**IL-18 stimulation induces lung CD8+ T cells to upregulate production of IFN-γ**

IL-18R expression by lung CD8+ T cells might contribute to COPD pathogenesis by inducing IFN-γ production (14, 15) and thereby activating macrophage production of matrix metalloproteinase-12, which is essential for development of emphysema in a murine model (32), and which some (33, 34) but not all (35, 36) studies have found to be overexpressed in COPD. To test this hypothesis, isolated lung CD8+ T cells from five individual lung samples from cohort A with GOLD stages of 2, 3, or 4 were cultured for 48 h in the presence of various recombinant cytokines, including IL-18, IL-12, and IL-15, but importantly, without any stimulation via TCR. By themselves, none of these cytokines had any effect on IFN-γ production, as assessed by protein concentrations in culture supernatants. However, when IL-18 and IL-12 were used simultaneously, IFN-γ production was very significantly increased (>800-fold) (Fig. 5A). These data extend to the human lungs the previous observation that this cytokine combination can induce IFN-γ in CD8+ T cells (37, 38) and are particularly interesting for the magnitude of the synergistic effect.

Similarly, TNF-α was significantly increased by stimulation by IL-18 plus IL-12 (Fig. 5B), whereas GM-CSF, IL-5, IL-13, and IL-17A showed no changes when stimulated with any of the cytokines (Fig. 5C–F). Taken together, these data demonstrate the capacity of IL-18+ lung CD8+ T cells to produce and secrete proinflammatory Tc1 cytokines in a TCR-independent fashion and further support the potential relevance of AMø elaboration of IL-18 in COPD.

**TCR stimulation of CD8+ T cells does not result in Tc2 cytokine production**

Having demonstrated in Fig. 1F that lung CD8+ T cells display transcripts for the Tc2 transcription factor, GATA-3, we next asked whether TCR stimulation, via anti-CD3ε exposure, would induce the CD8+ T cells to secrete Tc2 cytokines. Isolated lung CD8+ T cells were cultured for 48 h without or with plate-bound anti-CD3ε. All cytokines were below the limit of detection in unstimulated wells. Anti-CD3ε stimulation caused significant increases in production of IFN-γ, TNF-α, and GM-CSF; however, IL-5, IL-13, and IL-17 showed only very slight changes that did not attain statistical significance (Fig. 6).

**IL-15 stimulation induces lung CD8+ T cells to upregulate intracellular perforin production**

To investigate whether cytokine stimulation could also increase the cytotoxic potential of lung CD8+ T cells, we measured intracellular perforin following 48 h of in vitro stimulation. Lung tissue from five individual subjects with GOLD stages of 2, 3, or 4 were used in this experiment. In contrast to the effect on cytokine production, stimulation with IL-18 plus IL-12 had no effect on perforin. However, IL-15 stimulation led to a 3-fold increase in intracellular perforin expression compared with the unstimulated control CD8+ lung T cells (Fig. 7A). IL-15 has been shown to induce the synthesis

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**FIGURE 4.** IL-18R expression on lung CD8+ T cells increases with worsening COPD severity. CD8+ T cells from lung tissue were stained and analyzed by flow cytometry. A, Representative histograms showing IL-18R mAb staining of gated CD8+ T cells from a smoker with normal lung function (top panel) and from a subject with advanced COPD (GOLD stage 4) (bottom panel). Shaded profiles, isotype control; open profiles, specific staining. B, The percentage of CD8+ T cells that express IL-18R stratified by subject group (cohort A, n = 42) as defined in the legend to Fig. 1. C, IL-18 protein levels from human lung tissue stratified by group (cohort C, n = 95). Spearman nonparametric analysis was used to calculate rS and p values. Open circles represent individual patients; bars represent the mean ± SEM. 1–4, GOLD stages of COPD severity; NS, nonsmoker; S, smoker with normal lung function.
The principal findings of this study indicate that lung CD8+ T cells in COPD are activated TEM cells of a Tc1 phenotype, for which CD69 and IL-18R and of mRNA for perforin, granzyme B, T-bet, and GATA-3; 2) demonstration of the functional significance of IL-18R expression by lung CD8+ T cells in COPD, as shown by very significantly augmented secretion of IFN-γ and TNF-α (but not Tc2 or Tc17 cytokines) on stimulation with IL-18 plus IL-12 in the absence of TCR activation; and 3) evidence that IL-15 can prime lung CD8+ T cells for increased cytotoxic potential, as indicated by enhanced intracellular perforin expression. Together with previously published findings (3, 6, 8, 9, 42, 43), these data suggest a model in which the disease stage-dependent recruitment of CD8+ T cells to the lungs under the influence of ligands for CCR5, CXCR3, and CXCR6 and their retention and survival there sets the stage for a bidirectional, potentially TCR-independent, positive-feedback interaction with lung macrophages, leading to progressive lung inflammation and destruction.

COPD is an insidious, highly heterogeneous condition that primarily affects the lungs, but is also associated with significant systemic inflammation (44). Individuals with COPD variably exhibit increased resistance in the conducting airways due to mucus gland hypertrophy and fibrosis and increased parenchymal compliance due to emphysema. These apparently independent processes combine to determine the defining feature of COPD, irreversible airflow limitation measured during forced exhalation (45). Despite important insights derived from animal models (17, 19, 23, 32, 46–49), investigation of human pathological specimens remains essential in understanding this heterogeneity.

The current study is significant because it extends previous seminal studies of CD8+ T cell involvement in COPD (2–5, 7, 8, 13) in several ways: by the size of our primary sample cohorts (cohort A, n = 42; cohort C, n = 95); by the correlations with disease severity, with logistic regression analysis to exclude potentially confounding variables; and by our complementary use of flow cytometric, real-time PCR and in vitro stimulation to analyze lung CD8+ T cells. Because the primary site of pathological changes in COPD is the small (<2 mm diameter) airway (50), we believe that analyses that derive from distal lung parenchyma are more representative of key disease processes than those that depend on sampling of sputum or bronchoalveolar lavage. To overcome the inherent limitations of working with small samples yielding relatively few cells, this study employed several complementary subject cohorts. Hence, results of different assays necessarily derive from different subjects, precluding some direct comparisons.
The findings of increased expression of CD69 and IL-18R on lung CD8^+ T cells with advancing disease raise several interesting questions about their life history. CD69 expression is generally taken to signify acute activation, as it is one of the earliest surface molecules to be upregulated on TCR engagement (51, 52), and it declines when antigenic stimulation is withdrawn (53, 54). Hence, our data imply any of three possibilities: differing in vivo kinetics of CD69 expression from that seen in vitro; a constant turnover of CD69 molecules to be upregulated on TCR engagement (51, 52), and it can activate lung CD8^+ T cells (C.M. Freeman and J.L. Curtis, manuscript in preparation). The very high fraction of CD69^+ lung CD8^+ T cells we found in many subjects suggests to us the likelihood of TCR-independent activation, but a definitive answer will require considerable additional research.

Although it is entirely consistent with a Tc1 phenotype, the correlation of lung CD8^+ T cell expression of IL-18R with COPD severity is intriguing due to the known variable effect of IL-18 on immune responses depending on cytokines coexperienced with it. First identified as an IFN-γ-inducing factor (15), IL-18 is later shown to enhance both type 1 responses (in the presence of IL-12) and type 2 responses (in the absence of IL-12) (reviewed in Ref. 63). Indeed, when stimulated by IL-18 plus Ag, murine memory Th1 cells produced not only IFN-γ and TNF-α but also IL-9 and IL-13 in vitro and induced severe lung inflammation in vivo (64). Therefore, given the strong GATA-3 expression by lung CD8^+ T cells, we found in advanced COPD, the lack of Th2 cytokine production on stimulation with IL-18 (alone or in combination with IL-12) or with anti-CD3ε is noteworthy. Similarly, although transgenic overexpression of IL-18 alone has been shown to induce emphysema associated with increased IFN-γ, IL-5, and IL-13 (65), our data argue that lung CD8^+ T cells are not solely responsible for such an effect in humans with COPD. It is also possible that IL-18 has other actions that favor lung destruction in COPD (e.g., by facilitating colocalization of CD8^+ T cells and lung mononuclear phagocytes). IL-18 has been shown to be a chemoattractant for human CD4^+ Th1 cells (66); although CD8^+ T cells in that study were not responsive to IL-18, they also did not express IL-18R. Additionally, a positive regulatory loop has recently been shown by which IFN-γ and IL-18 signaling accelerate proliferation of memory murine CD8^+ T cells during recall responses to Ag presented by splenic DEC205^+ dendritic cells (DCs) in vitro (67). It will be interesting to see whether a similar effect exists with human lung DC subsets. Thus, the lack of correlation between total lung concentrations of IL-18 and disease severity in the current study, a novel finding, should not be taken to exclude a role for that cytokine in disease progression. It is possible that measuring IL-18 in the whole lung homogenate prevents us from detecting more subtle changes in IL-18 expression that are occurring on a microenvironmental level. Hence, our data should not be construed to negate the potentially more sensitive results of Imoako and colleagues (16), who used morphometric analysis of immunohistochemically stained lung tissue to show a difference in IL-18^+ cell density between nonsmokers or smokers with normal lung function and COPD patients. However, like our results, they also did not find a correlation between IL-18 expression and spirometric severity within patients with COPD. IL-18 is constitutively produced not only by macrophages and DC but by lung epithelium (68) as an inactive propeptide, and its processing and secretion is regulated, like that of IL-1, by caspase-1. We considered the possibility that...
The ab we used might detect both pro–IL-18 and its processed fragment, but rejected it based on the manufacturer’s information about that Ab clone.

Our data demonstrating that CD8+ T cells display increased transcripts for perforin and granzyme B with worsening pulmonary function agree with and extend previous studies of sputum (69) and bronchoalveolar lavage fluid (70). Results of several studies that have shown no increase in the expression of cytokotoxic enzymes in CD8+ T cells in the peripheral blood of patients with emphysema (71, 72) further argue for the local activation of CD8+ T cells within the lungs. Importantly, the study by Hodge et al. (70) found a significant correlation between granzyme B expression and apoptosis of bronchial epithelial cells. The idea that apoptosis of lung structural cells might contribute to emphysema has gained increasing support from basic and clinical data (34, 73). CD8+ T cells can also use the perforin/granzyme system for immunoregulatory function (e.g., to lyse DC, thereby preventing them from migrating to lymph nodes and prolonging immune response). Interestingly, memory CD8+ T cells that secrete TNF-α have recently been shown to block this lysis of DC (74), suggesting the possibility of complex regulatory networks within lung parenchyma.

In summary, we found that lung CD8+ T cells demonstrate increased IL-18R and C69 expression and increased mRNA transcripts for T-bet, perforin, and granzyme B with worsening pulmonary function (increased GOLD stage or decreased FEV1). Furthermore, expression of IFN-γ, TNF-α, and intracellular perforin by CD8+ T cells was increased by in vitro stimulation with either IL-18 plus IL-12 or IL-15, respectively. Our findings support the concept that CD8+ T cells contribute to COPD progression via production of cytotoxic molecules and proinflammatory cytokines.

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Disclosures

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References


