Oligoclonal CD4⁺ T Cells in the Lungs of Patients with Severe Emphysema

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Rationale: Within the lungs of patients with severe emphysema, inflammation continues despite smoking cessation. Foci of T lymphocytes in the small airways of patients with emphysema have been associated with disease severity. Whether these T cells play an important role in this continued inflammatory response is unknown. **Objective:** The aim of this study was to determine if T cells recruited to the lungs of subjects with severe emphysema contain oligoclonal T-cell populations, suggesting their accumulation in response to antigenic stimuli. **Methods:** Lung T-cell receptor (TCR) V β repertoire from eight patients with severe emphysema and six control subjects was evaluated at the time of tissue procurement (*ex vivo*) and after 2 weeks of culture with interleukin 2 (*in vitro*). Junctional region nucleotide sequencing of expanded TCR-V β subsets was performed. **Results:** No significantly expanded TCR-V β subsets were identified in *ex vivo* samples. However, T cells grew from all emphysema (n = 8) but from only one of the control lung samples (n = 6) when exposed to interleukin 2 (p = 0.0013). Within the cultured cells, seven major CD4-expressing TCR-V β subset expansions were identified from five of the patients with emphysema. These expansions were composed of oligoclonal populations of T cells that had already been expanded *in vivo*. **Conclusion:** Severe emphysema is associated with inflammation involving T lymphocytes that are composed of oligoclonal CD4⁺ T cells. These T cells are accumulating in the lung secondary to conventional antigenic stimulation and are likely involved in the persistent pulmonary inflammation characteristic of severe emphysema.

Keywords: antigens; chronic obstructive pulmonary disease; lymphocytes

Chronic obstructive pulmonary disease (COPD) is the fourth leading cause of death in the United States and is the only major cause of mortality that continues to increase in incidence (1). It is predicted that COPD will become the fifth most common cause of disability worldwide by 2020 (2). In 1993, the cost of disease in the United States was estimated at 24 billion dollars (3). Despite the enormous burden placed on both society and the individual, little advancement has been made in our understanding of the pathogenesis of COPD, leading to a lack of beneficial therapeutic agents.

A smoking-induced inflammation in the airways and lung parenchyma is critically involved in COPD development (4).

Paradoxically, this inflammatory response continues despite smoking cessation (5, 6). Although the etiology of this continued inflammation is unknown, studies have shown a correlation between the number of T cells present in the emphysematous lung and disease severity (7–12). The stimulus responsible for T-cell recruitment into the lungs of these patients remains unknown.

T cells recognize antigen presented in the context of major histocompatibility complex (MHC) class I or II molecules via a surface T-cell receptor (TCR) composed of a disulfide linked α - and β -chain. The $\alpha\beta$ TCR repertoire is generated via the rearrangement of variable to diversity to junctional region gene segments. When all of the different possible segments and rearrangement variability is taken into consideration, the TCR repertoire is enormous, with greater than 10⁷ possibilities (13). Thus, T cells recruited to the lung in response to a nonspecific inflammatory stimulus would express a heterogeneous or diverse TCR repertoire, similar to that expressed by circulating blood T cells. On the other hand, if T cells are trafficking to the lung in response to a specific antigen, those T cells will express a restricted TCR repertoire, composed of a limited number of T-cell clones (i.e., oligoclonal T cells; see the online supplement for a more detailed description of *TCRB* junctional region gene rearrangement).

We hypothesize that, within the lungs of patients with severe emphysema, T cells accumulate in response to conventional antigenic stimulation, and therefore the T-cell population should contain T-cell clones expressing identical and/or related TCRs on their surface. The results show that, unlike T cells from the lungs of normal subjects, T cells from the lungs of patients with emphysema expressed an activated phenotype demonstrated by their ability to consistently proliferate *in vitro* in the presence of interleukin 2 (IL-2). We show, for the first time, that these CD4⁺ T cells consist of oligoclonal T-cell populations, suggesting their recruitment to the lung in response to a particular antigenic stimulus as opposed to nonspecific trafficking to a site of inflammation. These findings support a paradigm shift in our understanding of the inflammation associated with severe emphysema by suggesting a crucial role for antigen-driven cellular-mediated immunity.

METHODS

Study Population

Eight patients diagnosed with smoking-induced emphysema undergoing lung transplantation secondary to severe disability and oxygen dependence were enrolled in this study. All subjects had normal serum α_1 -antitrypsin levels, evidence of alveolar destruction by chest radiography, and severe emphysema by histologic examination of explanted lung tissue. Lung tissue from the patients with emphysema was processed within 8 hours of explantation. Control lung tissue was obtained from subjects who had suffered brain death and was made available through Tissue Transformation Technologies (Edison, NJ; Table 1). For inclusion in the study, control subjects had to have no evidence of active or chronic infection, no signs of lung disease, no history of cigarette use, and less than 48 hours of mechanical ventilation. At the

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TABLE 1. CHARACTERISTICS OF STUDY SUBJECTS

Patient	Age (yr)	Sex	Diagnosis	Smoking (pack-yr)	Years Quit	FEV ₁ (%)	TLC (%)	D _{Lco} (%)
1	65	M	Emphysema	80	4	16	134	20
2	49	F	Emphysema	60	7	27	89	20
3	59	F	Emphysema	45	9	22	129	22
4	54	F	Emphysema	55	4	34	125	37
5	61	M	Emphysema	18	8	16	159	27
6	51	F	Emphysema	40	5	24	110	29
7	65	M	Emphysema	52	7	21	106	24
8	49	F	Emphysema	30	7	24	135	25
9	58	M	CVA	0	NA	ND	ND	ND
10	41	F	CVA	0	NA	ND	ND	ND
11	48	F	Hydrocephalus	0	NA	ND	ND	ND
12	66	F	CVA	0	NA	ND	ND	ND
13	58	F	CVA	0	NA	ND	ND	ND
14	63	F	CVA	0	NA	ND	ND	ND

Definitions of abbreviations: CVA = cerebrovascular accident; D_{Lco} = diffusion capacity for carbon monoxide; F = female; M = male; NA = not applicable; ND = not done or not available; TLC = total lung capacity.

time of procurement, control lungs were flushed with normal saline, placed on ice, and arrived at our laboratory within 3 to 24 hours. Informed consent was obtained from all study participants. The protocol was approved by the Human Subject Institutional Review Board at the University of Colorado Health Sciences Center.

Isolation, Analysis, and Culture of Mononuclear Cells from Lung Tissue and Peripheral Blood

Ten grams of peripheral lung tissue from the upper and lower lobes of each patient were minced and placed in RPMI 1640 (Gibco, Grand Island, NY) containing 100 U/ml collagenase and 1 µg/ml DNase (both from Sigma, St. Louis, MO). The tissue was agitated at 37°C for 90 minutes, passed through mesh, and washed with balanced salt solution. Mononuclear cells were isolated from the pulmonary cell suspension and from heparinized blood using Ficoll-Hypaque density gradient separation as previously described (14–16).

Mononuclear cells obtained from blood and lung were stained with fluorescently labeled monoclonal antibodies (mAbs) directed against CD3, CD4, and CD8 as well as the surface markers CD28, CD45RO, CD62L, and CCR7 (all from BD Biosciences, San Jose, CA). These same cells were stained with mAbs directed against 16 different TCR-Vβ regions as previously described (see online supplement) (16, 17). The labeled cells were analyzed using a FACScalibur cytometer (Becton Dickinson, San Jose, CA).

For tissue culture, small samples of whole peripheral lung tissue (~20 µg) were cultured for 2 weeks in RPMI 1640 supplemented with 10% heat-inactivated human serum (Gemini Bioproducts, Woodland, CA), 20 mM HEPES, 1 mM sodium pyruvate, 100 U/ml penicillin, 100 µg/ml streptomycin, and 2 mM glutamine (all from Life Technologies, Inc., Gaithersburg, MD) and 20 U/ml of rIL-2 (R&D Systems, Minneapolis, MN). The presence of T-cell blasts was detected by light microscopy and by 90° light-scatter patterns on the cytofluorograph. As with *ex vivo* mononuclear cells, TCR-Vβ expression of the blasting T-cell populations was analyzed by immunofluorescence.

Analysis of Expressed TCRB Junctional Region from Cultured Cells and Frozen Tissue at the Time of Explant

CD4⁺ T cells were purified using a magnetic cell sorting (MACS) column (Miltenyi Biotec, Auburn, CA). RNA was isolated using a commercially available kit (RNAid Plus; BIO 101, La Jolla, CA). cDNA was prepared and polymerase chain reaction amplification was performed as previously described (14, 16). Each polymerase chain reaction product was ligated, transformed, and sequenced as previously described (see online supplement) (14, 16).

Statistical Analysis

A Mann-Whitney test was used to determine the significance of differences between subject groups and cell surface marker expression (JMP,

version 4; SAS Institute, Inc., Cary, NC). A *p* value of less than 0.05 was considered statistically significant.

RESULTS

Analysis of T-Lymphocyte Phenotype in Emphysematous Lung

To characterize the pulmonary T cells isolated from emphysematous lung, we performed immunofluorescence staining. For the upper lobe CD3⁺ cells, 57 ± 1.5% (mean ± SEM) expressed a CD4⁺ phenotype, whereas 34 ± 2.4% were CD8⁺ (*n* = 7). There was no difference in CD4 and CD8 expression between the upper and lower lobes. As shown in Figure 1, compared with T cells from the blood, both CD4⁺ and CD8⁺ T cells in the lung demonstrated significantly decreased expression of the lymph node homing receptors CD62L and CCR7 (*n* = 6 for all marker studies). This demonstrated that the majority of T cells isolated from the lung were differentiated memory T cells that had lost receptors necessary for lymph node trafficking. In addition, lung CD4⁺ T cells showed a significant increase in expression of the

Figure 1. Surface markers expressed by T cells isolated from lungs and blood of patients with severe emphysema (*n* = 6). The upper panel demonstrates the percentage of CD4⁺ T cells that express CD28 (a costimulatory receptor), CD45RO (a marker of previous activation), CD62L (a lymph node homing molecule), and CCR7 (a homing receptor specific for the lymph node). The lower panel demonstrates CD8⁺ T-cell expression of these same markers. Data are presented as mean ± SEM. White bars = peripheral blood mononuclear cells; gray bars = upper lung lymphocytes; black bars = lower lung lymphocytes.

activation marker CD45RO. Finally, a significant number of lung CD4⁺ T cells had lost expression of the costimulatory molecule CD28. CD28 engagement is absolutely required for optimum activation of naive T cells. Conversely, memory T cells no longer require CD28-mediated costimulation for effector function (17). This expression pattern of both memory and activation markers confirmed that the T cells accumulating in and isolated from the lungs of patients with emphysema are phenotypically distinct from those circulating in blood and consist of activated effector memory T cells. Despite this difference, we noted a heterogeneous distribution of TCR-V β subsets on lung CD4⁺ and CD8⁺ T cells similar to that observed in blood ($n = 7$; Figure 2).

Ability of Emphysema versus Normal Control Lung T Cells to Proliferate in Response to IL-2

As opposed to bronchoalveolar lavage T cells from healthy subjects, bronchoalveolar lavage T cells from patients with sarcoidosis proliferate in culture medium supplemented with IL-2 (18). The ability of CD4⁺ lung T cells from patients with sarcoidosis to selectively expand in the presence of IL-2 is most likely due to the increased expression of the IL-2 receptor, which allows for further proliferation of these previously activated T cells in culture. Thus, lung tissue from eight patients with severe emphysema and six control subjects was placed in culture media supplemented with IL-2 and harvested after 2 weeks. All lung samples from the patients with emphysema yielded blasting T-cell populations that were readily detectable by light microscopy (Figure 3A) and flow cytometry (Figure 3B), whereas only one of six control lungs yielded a blasting T-cell population under identical culture conditions ($\chi^2 = 10.4$; $p = 0.0013$). In the majority of cases, T cells from control subjects either died in culture (Figure 3A) or remained within the smaller, resting T-cell population (Figure 3B). In Figure 3B, gating on resting (*lower circle*) and blasting (*upper circle*) T-cell populations from a representative emphysema and control sample is shown. The ratio of blasting to resting lymphocytes for all of the emphysema samples was 4.7 ± 2.6 (mean $\pm$ SEM) compared with 0.62 ± 0.24 for the control samples ($p = 0.013$). As shown in Figure

3C, 70% of the CD3⁺ T cells expressed CD4, whereas 20% expressed CD8 in a representative sample. Overall, the percentage of CD3⁺ cells expressing CD4 increased from 63 ± 2.5 to $69 \pm 5.8\%$ ($p > 0.05$). In contrast, a decreased percentage of CD3⁺ cells expressing CD8 (34 ± 2.4 to $28 \pm 4.8\%$; $p > 0.05$) after culture was seen.

TCR-V β Expression in Blasting T Cells from Lungs of Patients with Severe Emphysema

As with the mononuclear cells isolated immediately after explant, we measured TCR-V β expression on cultured T-cell blasts by immunofluorescence staining and cytofluorographic analysis. We compared the V β repertoire of the cultured T-cell blasts (*in vitro*) with that present at explant (*ex vivo*). Similar to previous studies (19), a significant TCR-V β subset expansion within the cultured T-cell population was defined as a greater than twofold increase in the percentage of T cells expressing a particular V β region compared with *ex vivo* expression. We identified a total of seven V β expansions from five of the eight patients with severe emphysema. Two expansions expressed V β 6.7 and V β 17, whereas the others expressed V β 8.1, V β 14, and V β 22. Patient 1 demonstrated the most impressive skewing of the TCR-V β repertoire in the cultured CD4⁺ T-cell population (Figure 4). The upper lobe culture showed a dramatic expansion of CD4⁺ T cells expressing V β 6.7 (75% after culture in IL-2), whereas in the lower lobe, 48% of the CD4⁺ T cells expressed V β 17. Figure 5 shows CD4⁺ TCR repertoires from the additional four subjects with emphysema who demonstrated an expansion of at least one V β subset after culture in IL-2. Patient 2 showed an increased percentage of V β 22-expressing CD4⁺ T cells at 2 weeks compared with the time of explant (24 vs. 4.8%). In Patient 3, 8.3% of the CD4⁺ T cells after culture expressed V β 14 compared with 1.3% *ex vivo*. Patient 3 also demonstrated a V β 17 expansion after culture (9.5 vs. 4.5% *ex vivo*). Culture of lung cells from Patient 4 with IL-2 resulted in an increased percentage of V β 6.7-expressing CD4⁺ T cells from 4.3 (*ex vivo*) to 21.0% (*in vitro*). In Patient 5, expression of V β 8.1 on CD4⁺ T cells increased from 3.2 (*ex vivo*) to 17.8% (*in vitro*). No significant changes in the TCR-V β repertoire for cultured CD8⁺ T cells compared with *ex vivo* findings were seen (data not shown). From the single control subject who demonstrated blasting in culture with IL-2, we also did not detect any significant alteration of the TCR-V β repertoire.

Analysis of TCRB Junctional Region in Pulmonary CD4⁺ T Lymphocytes before and after Culture in IL-2

The expansion of particular V β -expressing CD4⁺ T-cell subsets from emphysematous lung suggests that a population of cells within these subsets had been activated *in vivo*. To determine if these TCR-V β expansions contained oligoclonal T-cell populations suggestive of conventional antigenic stimulation, we sequenced the TCRB junctional regions of the T-cell populations from both the *ex vivo* and *in vitro* samples from four subjects. The deduced amino acid sequences of the different clones are summarized in Figure 6. Only the TCRB sequences found greater than three times in any sample or those present in both *ex vivo* and cultured T-cell populations are shown (Figure 6). For more information on TCRB junctional region rearrangement and the structure of the gene, see the online supplement. In the *ex vivo* lung samples, multiple shared T-cell clones were detected in noncontiguous samples, suggesting that these CD4⁺ T lymphocytes had responded to an antigen present throughout the lung. Importantly, many of the CD4⁺ T-cell clones detected after culture in IL-2 had already been expanded *in vivo*. For example, in Patient 3, one clonal sequence (BV14-LLAGT-BJ2.1) com-

Figure 2. T-cell receptor (TCR) V β region expression on T cells from the blood and lower and upper lung of patients with severe emphysema ($n = 7$). The *upper panel* demonstrates TCR-V β expression on CD4⁺ T cells, and the *lower panel* demonstrates expression on CD8⁺ cells. Data are expressed as mean $\pm$ SEM.

Figure 3. Blasting of lung T cells from subjects with emphysema and normal subjects after 2 weeks in culture with interleukin (IL) 2. (A) Light microscopy of lung tissue culture reveals large clusters of proliferating lymphocytes in a representative emphysema culture (*left panel*), which are distinctly absent in a representative control culture (*right panel*). (Original magnification: 100 $\times$) (B) Forward-versus side-scatter density plots are shown for representative emphysema (*left panel*) and control (*right panel*) lung tissue culture. Blasting lymphocytes are found within the upper gate with the resting lymphocytes in the lower gate. The percentage of blasting or resting lymphocytes per total cells collected is shown. (C) A density plot of CD4 versus CD8 expression on CD3⁺ T cells from a representative subject with emphysema after *in vitro* culture in the presence of IL-2 is shown.

prised 11 (5/44) and 13% (5/38) of the upper and lower lung *TCRBV14* sequences, whereas this sequence made up 25% (11/44) of the blasted upper lobe CD4⁺ T cells expressing V β 14. In contrast to TCR expression in the lung, we observed a diverse *TCRBV14* repertoire in unstimulated CD4⁺ T cells from the blood of this subject (data not shown). In the lung, we rarely observed clones with related *TCRB* junctional regions. The sole example of related clones was found in Patient 1 (BV17-ATGTSLG-BJ2.3 and BV17-ASGTRGD-BJ2.7). These distinct clones shared identical *TCRB* junctional region length and encoded for amino acids in similar positions, implying stimulation and expansion by a similar, if not the same, antigen. Taken together, our findings demonstrate that the CD4⁺ T cells in the lungs of patients with severe emphysema include a subset of previously activated, oligoclonal CD4⁺ T cells.

DISCUSSION

The results reported here demonstrate the presence of activated CD4⁺ T cells in lungs of patients with severe emphysema. We

hypothesized that these T cells accumulate secondary to conventional antigenic stimulation and should therefore consist of oligoclonal T-cell populations. Although the TCR-V β repertoire of lung T cells from patients with severe emphysema mimicked that found in blood, T-cell blasts could be cultured from diseased tissue of all subjects with emphysema but from only one of the control lungs. Within the CD4-expressing T-cell blasts, clonal populations were identified that had already been expanded *in vivo*. In addition, these CD4⁺ T-cell clones could be identified in both *ex vivo* and *in vitro* samples from distant lung sections but not in blood, implying accumulation of these T cells secondary to an antigen distributed throughout the lung. These findings suggest that activated oligoclonal CD4⁺ T cells may play a role in the persistent inflammation characteristic of emphysema.

As opposed to bronchoalveolar lavage or endobronchial biopsy, lung homogenization captures lymphocytes from throughout the lung parenchyma. We were able to document that these cells were phenotypically distinct from those present in blood. Despite this difference, the TCR-V β repertoire of the lung

Figure 4. Density plots for CD4 versus TCR-Vβ6.7 and TCR-Vβ17 from a representative emphysematous lung tissue culture with IL-2. Representative density plot from Patient 1 demonstrating increased expression of TCR-Vβ6.7 and TCR-Vβ17 on cultured CD4⁺ T-cell blasts derived from the upper and lower lobes is shown.

T cells was heterogeneous and similar to that expressed in blood. This is not altogether surprising because, even in a disease characterized by an intense CD4⁺ T-cell alveolitis such as chronic beryllium disease, we were only able to identify CD4⁺ T-cell expansions in the bronchoalveolar lavage of one-third of those subjects (19). In another CD4⁺ T-cell-mediated disease, sarcoidosis, the proportion of CD4⁺ T cells expressing particular TCR-Vβs in blood and bronchoalveolar lavage was also remarkably similar in nearly all subjects studied (18). This heterogeneous lung T-cell repertoire may reflect the influx of nonspecific T cells to the inflammatory site, which obscure the presence of antigen-specific, pathogenic T cells. For example, after immunization of mice with myelin-basic protein, little enrichment of the antigen-specific subset in inflammatory brain lesions was observed, although the antigen-specific T cells drive the inflammation (20). In addition, pathogenic T cells in this murine model of multiple sclerosis could be enhanced by culture in the presence of IL-2, lending support to our experimental approach in emphysema (21).

Regarding human disease, previous studies of sarcoidosis have shown that important TCR-Vβ subsets and T-cell clones could also be identified by culturing T cells in the presence of IL-2 (18). Importantly, this approach failed to expand lung T cells from normal subjects. In other diseases characterized by the absence of a known antigenic stimulus, such as scleroderma and rheumatoid arthritis, IL-2-induced T-cell expansion has been used to study TCR repertoire (22, 23). Given that pathogenic T cells in the emphysematous lung may be obscured by nonspecific influx of lymphocytes *in vivo*, we cultured emphysematous lung tissue with IL-2 to selectively expand activated lung T cells. Several observations support the validity of using this experimental technique to expand disease-relevant T cells in emphysema. First, T cells from control lung tissue did not reliably expand when cultured under identical conditions. Only one of six control lung samples yielded blasting T-cell populations as identified by light microscopy and scatter analysis on the cytofluorograph. Thus, this culture technique does not expand any lung T-cell population. This differential proliferative capacity may be due to the quantity of T cells present in the tissue, qualitative differences between the T cells, growth factors present in the emphysematous lung, or the inflammatory environment surrounding these cells. Importantly, IL-2 acts as a T-cell growth factor for cells that have previously been activated through their TCR and that express the IL-2 receptor (24). Thus, one explanation for the sensitivity of emphysematous lung T cells to IL-2 is their previous activation by an antigen *in vivo*. Second, the oligoclonal T-cell populations that were selectively expanded in culture were also expanded in the *ex vivo* lung tissue. For example, a

Figure 5. TCR-Vβ repertoire in cultured CD4⁺ T cells. TCR-Vβ repertoire of cultured CD4⁺ T cells compared with *ex vivo* expression in four subjects with emphysema who demonstrated an expansion (> twofold) in the lung after IL-2 exposure in culture compared with *ex vivo* expression. Data are expressed as the mean percentage of CD4⁺ T cells expressing a particular Vβ. Asterisks denote the expanded TCR-Vβ region in the individual patients.

TCR-Vβ14-expressing CD4⁺ T-cell clone (BV14-LLAGT-BJ2.1) accounted for 11 and 13% of the *TCRBV14* sequences in the *ex vivo* upper and lower lobes, and the percentage was further increased after culture in the presence of IL-2.

Within the cultured T-cell blasts of five of the eight (63%) patients with emphysema sampled, we found skewing of the TCR-Vβ repertoire expressed on the CD4⁺ T cells. In sarcoidosis, Forrester and colleagues (18) demonstrated a similar percentage of patients with an increased expression of at least one *TCRBV* gene using identical experimental conditions. In both diseases, the TCR-Vβ expansions were composed of oligoclonal T cells, and importantly, the clones were also expanded in the *ex vivo* samples. Given the enormous diversity of the human TCR repertoire (i.e., $> 1.0 \times 10^7$ possible TCR combinations) (13), the identification of oligoclonal T-cell populations strongly suggests that these CD4⁺ T cells are being actively recruited to the lung in response to conventional antigenic stimulation. Of note, we rarely observed clones with related *TCRB* junctional region sequences and never found clones with nucleotide variation leading to an identical amino acid sequence. Detecting

Patient	TCRB Junctional Region			TCRBJ		Ex vivo		Cultured	
	TCRBV	NBDN	BJ			Upper	Lower	Upper	Lower
Patient 1	BV6.7	NBDN	BJ						
	CASS	S R G M S	TEAFFG	1.1		0/42	2/39	2/45	0/48
	CASS	SKWASRG	TEAFFG	1.1		0/42	3/39	0/45	0/48
	CASS	D G T H D	YGYTFG	1.2		10/42	2/39	0/45	4/48
	CASS	PPPRDIVP	GELFFG	2.2		0/42	0/39	0/45	4/48
	CASS	R T G F	TDTQYFG	2.3		1/42	3/39	2/45	13/48
	CASS	YAPLA	DTQYFG	2.3		0/42	3/39	0/45	0/48
	CASS	YRGGGA	STD TQYFG	2.3		0/42	0/39	0/45	6/48
	CASS	APGQ	SYEQFG	2.7		0/42	0/39	41/45	0/48
	BV17	NBDN	BJ						
	CASS	P P P A G	TEAFFG	1.1		2/23	18/36	0/38	17/39
	CAS	SRTGN	NSPLHFG	1.6		0/23	0/36	8/38	0/39
	CAS	A T G T S L G	DTQYFG	2.3		7/23	1/36	0/38	5/39
	CASS	A S G T R G D	EQYFG	2.7		0/23	1/36	20/38	5/59
	CASS	G S T G R	YEYFG	2.7		0/23	3/36	0/38	2/39
	CASS	PGTGQGI R	EQYFG	2.7		0/23	0/36	0/38	3/39
Patient 2	BV22	NBDN	BJ						
	CASS	EVGGKN	TDTQYFG	2.3		0/29	0/25	3/37	ND
	CAS	R G P Q D	YEYFG	2.7		5/29	3/25	13/37	ND
	CAS	VWGS	EQYFG	2.7		0/29	3/25	0/37	ND
Patient 3	BV14	NBDN	BJ						
	CASS	Q G A	YGYTFG	1.2		2/44	0/38	19/40	ND
	CASS	RGTGTA	NYGYTFG	1.2		3/44	3/38	0/40	ND
	CASS	QTTGG	SNQPQHFG	1.5		4/44	1/38	0/40	ND
	CASS	L L A G T	NEQFFG	2.1		5/44	5/38	11/44	ND
	CAS	RPGGVV	TQYFG	2.3		5/44	3/38	0/44	ND
	CASS	FSTG	TQYFG	2.3		0/44	0/38	4/44	ND
Patient 4	BV6.7	NBDN	BJ						
	CASS	E G A S	SYNSPLHFG	1.6		3/19	ND	27/32	12/15

Figure 6. Analysis of deduced *TCRB* junctional region amino acid sequences expressed in *ex vivo* T cells and cultured CD4⁺ T cells from the lungs of four patients. *TCRB* sequences found three times or more in any sample or present in both *ex vivo* and cultured T-cell populations are shown. For each T-cell clone, the entire *TCRB* junctional region is shown, extending from the 5' end of the selected *TCRBV* family gene, including the highly rearranged *NBDN* gene segment, and ending at the selected *BJ* gene segment. The column *TCRBJ* notes for each clone which *BJ* gene family member was selected during genetic rearrangement. For more detail on the gene rearrangement of *TCRB* junctional region, see the online supplement. The *NBDN* region of sequences found in both *ex vivo* and cultured T-cell populations are shown in *bold*. The number of identical sequences (defined at the nucleotide level) is shown over the total number of sequences analyzed for a given anatomic site or culture sample. These sequence data are available from GenBank under accession numbers AY726734 to AY726754.

T-cell clones with unique nucleotide sequences, yet coding for identical or nearly identical amino acid sequences, would have provided further evidence for T-cell accumulation in response to the same or related antigenic stimulus (14, 25). It is important to emphasize that our panel of anti-TCR mAbs only covers approximately 30 to 35% of the CD4 TCR-V β repertoire, which could have contributed to the absence of expanded populations in three of the eight subjects with emphysema. Finally, immunofluorescence staining for the percentage of cells expressing a particular TCR-V β may have missed small but expanded T-cell populations expressing the same or related TCR junctional regions.

We observed that different TCR-V β -expressing CD4⁺ T-cell subsets were expanded in the patients with emphysema. Of note, V β 6.7⁺ and V β 17⁺ T cells were each increased in the lung of two patients with emphysema, whereas no other TCR-V β subset was increased more than once. The different usage of V β subsets may reflect either different antigens or different major histocompatibility complex (MHC) presenting molecules being recognized at the site of pathology. Because these subjects were undergoing lung transplantation, MHC class II serologic typing was available. However, no shared MHC class II type was identified. A more detailed HLA-DR and HLA-DQ molecular typing in a larger cohort of subjects with severe emphysema may identify a relationship between HLA type and TCR-V β usage. It is also possible that T cells in the emphysematous lung are being recruited in response to a complex antigenic stimulus with potentially multiple T-cell epitopes.

Despite reports suggesting a more robust association between CD8⁺ T cells and emphysema (9–12), skewing of the TCR repertoire after culture was only present in the CD4⁺ T-cell population. Other recent reports also highlight the importance of CD4⁺ T cells in emphysema. Hogg and colleagues (8) reported an association between disease severity and small airways containing CD4⁺ T cells. In bronchial biopsies from patients with mild to moderate COPD, Di Stefano and colleagues (26) reported an increase in IFN- γ and signal transducer and activator of transcription 4 (STAT4)-expressing cells as compared with control samples. Nuclear STAT4 expression was mostly found

in the CD4⁺ rather than CD8⁺ T cells. Thus, in COPD, CD4⁺ T cells likely play an important role in STAT4 overexpression, leading to increased IFN- γ production and Th1-polarized inflammation. Grumelli and colleagues (27) also demonstrated that T cells from the lungs of subjects with emphysema secrete increased amounts of Th1-associated cytokines. In addition, these cytokines were shown to induce macrophage production of matrix metalloproteinase 12, thus linking T-cell activity to alveolar destruction. Finally, Taraseviciene-Stewart and colleagues (28) have recently published the first animal model for emphysema involving the adaptive immune system. In this model, rats injected with human umbilical vein endothelial cells break immunologic tolerance to a receptor on their own pulmonary microvascular endothelial cells and develop emphysema. CD4⁺ T cells from injected animals are both necessary and sufficient to cause disease in nonimmunized animals. Our findings do not detract from the possible importance of CD8⁺ in the pathogenesis of COPD. In preliminary experiments using explanted lungs from patients with severe emphysema due to α_1 -antitrypsin deficiency, culture of lung tissue in the presence of IL-2 resulted in the outgrowth of oligoclonal CD8⁺ T cells and not CD4 cells, suggesting their role in the pathogenesis of disease (A.K.S., personal communication, October 14, 2004). Using polymerase chain reaction amplification followed by spectratyping to compare oligoclonality in the lung versus blood in subjects who smoke, Korn and colleagues (29) found evidence of increased oligoclonality in the lung versus blood for both CD4⁺ and CD8⁺ T-cell populations. Thus, both T-cell populations likely play a crucial role in the continued inflammatory response present within the emphysematous lung.

In summary, reports continue to characterize the T-cell-mediated inflammation associated with emphysema. This continued inflammation despite smoking cessation may result from latent infections because several pathogens have been identified in the lungs of patients with severe emphysema (6, 30, 31). Alternatively, other authors have proposed an autoimmune mechanism as the cause of these T-cell accumulations (32–34). All individuals who smoke develop lung inflammation characterized by the accumulation of macrophages and neutrophils, re-

sulting in proteolytic damage and oxidative stress. This chronic inflammation may alter native protein expression and possibly create neoproteins that are subsequently recognized as nonself. The end result would be the recruitment of antigen-specific T cells to the lung. Regardless of the source of the antigen, we offer, for the first time, evidence that the lungs of patients with severe emphysema contain highly activated oligoclonal T cells. These findings strengthen the hypothesis that cellular-mediated immunity plays a critical role in the pathogenesis of severe emphysema and broaden our understanding of the immunologic basis of severe emphysema.

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