Oxidative Stress–induced Antibodies to Carbonyl-modified Protein Correlate with Severity of Chronic Obstructive Pulmonary Disease

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Rationale: There is increasing evidence for the presence of autoantibodies in chronic obstructive pulmonary disease (COPD). Chronic oxidative stress is an essential component in COPD pathogenesis and can lead to increased levels of highly reactive carbonyls in the lung, which could result in the formation of highly immunogenic carbonyl adducts on “self” proteins.

Objectives: To determine the presence of autoantibodies to carbonyl-modified protein in patients with COPD and in a murine model of chronic ozone exposure. To assess the extent of activated immune responses toward carbonyl-modified proteins.

Methods: Blood and peripheral lung were taken from patients with COPD, age-matched smokers, and nonsmokers with normal lung function, as well as patients with severe persistent asthma. Mice were exposed to ambient air or ozone for 6 weeks. Antibody titers were measured by ELISA, activated complement deposition by immunohistochemistry, and cellular activation by ELISA and fluorescence-activated cell sorter.

Measurements and Main Results: Antibody titer against carbonyl-modified self-protein was significantly increased in patients with Global Initiative for Chronic Obstructive Lung Disease stage III COPD compared with control subjects. Antibody levels inversely correlated with disease severity and showed a prevalence toward an IgG1 isotype. Deposition of activated complement in the vessels of COPD lung as well as autoantibodies against endothelial cells were also observed. Ozone-exposed mice similarly exhibited increased antibody titers to

AT A GLANCE COMMENTARY

Scientific Knowledge on the Subject

There is increasing evidence for the presence of autoantibodies in chronic obstructive pulmonary disease (COPD), but it is yet unclear as to how this is linked to one of the major pathogenic drivers present in COPD, chronic oxidative stress.

What This Study Adds to the Field

The detection of antibodies in COPD against novel carbonyl-modified self-antigens provides a plausible mechanistic link between chronic oxidative stress and the development of a potentially destructive autoimmune component in COPD.

carbonyl-modified protein, as well as activated antigen-presenting cells in lung tissue and splenocytes sensitized to activation by carbonyl-modified protein.

Conclusions: Carbonyl-modified proteins, arising as a result of oxidative stress, promote antibody production, providing a link by which oxidative stress could drive an autoimmune response in COPD.

Keywords: COPD; autoimmunity; oxidative stress; carbonyl

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Chronic obstructive pulmonary disease (COPD) is currently a leading cause of morbidity and mortality worldwide (1), with the main cause being long-term cigarette smoking in the western world (1, 2). Inflammation and remodeling of the small airways are major determinants for the progression and severity of COPD, as defined by the decline in FEV₁ (3). Accumulation of inflammatory mucous exudates in the lumen and infiltration of the wall by innate and adaptive inflammatory immune cells, such as CD4⁺ cells, CD8⁺ cells, B cells, macrophages, and neutrophils, and the formation of lymphoid follicles are all features of the observed inflammation that correlate with the severity of COPD (3, 4).

Previous studies have suggested that autoimmune mechanisms may contribute to the pathogenesis of COPD. Serum autoantibodies against elastin (5) and bronchial epithelial cells along with corresponding IgG and complement (C3) deposition (6) have been observed in COPD lung. It has therefore been proposed that cigarette smoke–derived antigens may be responsible for driving this disease process in COPD (6–9), but until now this has not been investigated. In addition, complement activation in the lung, which is usually direct evidence of autoimmune activation, has not been examined in COPD, particularly in progressive disease (1, 10).

Oxidants, which are a major constituent of cigarette smoke, can cause the formation of carbonyl adducts on proteins (11). They are formed *in vivo* as a result of lipid peroxidation, and then in turn modify proteins, but can also be directly incorporated into proteins through direct oxidation of amino acid side-chains as well as oxidative cleavage of proteins (12). These have been implicated in the pathogenesis of many chronic inflammatory and/or autoimmune diseases (11, 13). In patients with COPD, carbonyl adducts have been found both within the lung (13) and in the circulation (14), and their levels correlated with disease severity, measured by the decline in FEV₁.

We hypothesized that carbonyl stress arising as a result of chronic exposure to oxidants in cigarette smoke drives the production of potentially damaging neo- or autoantibodies to carbonyl-modified “self” protein in COPD. To test this hypothesis, we modified self-proteins with a number of different carbonyl adducts known to be present in COPD, which were then used to screen sera from patients with COPD and control subjects for antibodies against self- and carbonyl-modified self-protein. In addition, the ability of carbonyl-modified self-proteins to trigger lymphocyte activation *in vitro* was also examined. The presence of IgG deposition and of complement activation were examined in lung tissue of patients with COPD and control subjects. Finally, in a chronic animal model of oxidative stress-induced lung inflammation, we examined whether an immune response against carbonyl-modified self-protein could also be triggered.

METHODS

Reagents

Unless otherwise stated, all biochemical reagents used in this study were purchased from Sigma Aldrich Inc. (St. Louis, MO). Research-grade cigarettes (reference code 2R1/1R3F) were obtained from the University of Kentucky. 4-Hydroxynonenol (4-HNE) was obtained from Calbiochem (Nottingham, UK). Bis-malonyldialdehyde (MDA) was acquired from Alpha Diagnostics Intl. (San Antonio, Texas). Antibodies: peroxidase-labeled monoclonal anti human IgG1, 2, 3, and 4; anti-human IgM; and peroxidase-labeled polyclonal goat anti-human IgG (#A6029) were all acquired from Sigma Aldrich Inc. Murine IL-2 ELISA kit was purchased from R&D Systems (Abingdon, UK). Ultra-culture medium was obtained from Biowhittaker (Wokingham, UK).

Clinical Samples

Subjects were recruited from the Section of Respiratory Medicine of the University Hospital of Ferrara, Italy, with approval by the local Ethics Committee. Serum and tissue samples were acquired after written informed consent was obtained, and pulmonary function tests were performed as previously described (15). Predicted values for the different measures were calculated from the regression equations published by Waalkens and colleagues (16). COPD was defined according to international guidelines (post-bronchodilator FEV₁/FVC ratio < 70%), and the severity of COPD was classed according to current Global Initiative for Chronic Obstructive Lung Disease (GOLD) criteria (1). Serum and lung tissue was processed as detailed in the online supplemental, and subject details are summarized in Tables 1 and 2, respectively.

Animals and Treatments

Pathogen-free, 6- to 8-week old male BALB/c mice purchased from Harlan (Wyton, UK) were exposed to 2.5 ppm ozone for 3 hours in a sealed Perspex container either once (acute) or every 3 days for 6 weeks (chronic) (17). Control animals were exposed to air over the equivalent period. Twenty-four hours after the last exposure, the mice were killed and the lung, spleen, lymph nodes, and blood removed. Dendritic cells and lung macrophages were purified by positive selection using MACS (Miltenyi Biotech Ltd, Bisley, UK). Further details are described in the online supplement.

TABLE 1. PATIENT DETAILS (SERUM)

	Age (yr)	M/F	Pack-years	FEV ₁ /FVC	% Predicted FEV ₁
Nonsmokers	51 ± 2	8/5	N/A	0.98 ± 0.03	105 ± 4
Smokers	59 ± 2	14/8	28 ± 3	0.84 ± 0.03	86 ± 3
GOLD I	66 ± 2	1/4	56 ± 20	0.66 ± 0.01	88 ± 8
GOLD II	72 ± 2	10/2	43 ± 7	0.59 ± 0.02	62 ± 2
GOLD III	74 ± 2	6/4	34 ± 7	0.50 ± 0.03	40 ± 1
Severe asthma	51 ± 4	3/9	0	0.85 ± 0.02	70 ± 8

Definition of abbreviations: F = female; GOLD = Global Initiative for Chronic Obstructive Lung Disease; M = male.

Data are given as mean ± SD. FEV₁/FVC ratio is post bronchodilator for subjects with COPD but not smokers or nonsmokers.

Antigen Preparation, ELISA, Immunohistochemistry, Western Blot Analysis, and Flow Cytometry

Human or mouse serum albumin (Sigma; cat# A3782) was modified with either acrolein, 4-hydroxynonenol, malonyldialdehyde (MDA), or cigarette smoke condensate for 24 hours at 37°C. Patient or mouse serum was screened for antibodies against carbonyl-modified proteins by ELISA using 96-well Nunc Maxisorb immunoplates coated with the carbonyl-modified protein prepared above. Bound antibodies were assessed for either total IgG or specific class and isotype using appropriate secondary antibodies. Murine IL-2 was assessed using a commercial ELISA from R&D Systems. Immunohistochemical staining for activated C4d complement was performed using rabbit anti-human C4d (Oxford Biosystems Ltd, Oxford, UK). Lung tissue carbonylated proteins were detected by Western blot using the oxyblot assay (Millipore UK Ltd, Watford, UK). Activated dendritic cells and lung macrophages were assessed by flow cytometry after staining for CD11c, CD80, CD86, CD54 (all from BD Biosciences, Oxford, UK), and F4/80 (Caltag-Medsystems Ltd, Buckingham, UK). Further details are described in the online supplement.

Statistical Analysis

Differences between patient groups were calculated with GraphPad Prism software using a nonparametric Kruskal-Wallis test with Dunn multiple comparison post-test analysis or Mann-Whitney test as indicated. All data are expressed as mean ± SEM and differences were considered significant if *P* was less than 0.05.

RESULTS

Autoantibodies to Carbonyl Modified Self-Protein Are Present in COPD

Serum from patients with stable COPD with different stages of severity, as well as from control subjects, were screened against various forms of carbonylated-modified self-protein to establish the presence of autoantibodies against carbonyl-modified epitopes (Figure 1). Human serum albumin was chosen as a generic self-protein as it was easily available, not expected to have an autoimmune response directed against it, and could therefore be used to screen against carbonyl epitopes. There was a significant increase in antibody titer against carbonyl-modified protein by GOLD stage III (Figure 1), which was

TABLE 2. PATIENT DETAILS (LUNG TISSUE)

	Age (yr)	M/F	Pack-years	FEV ₁ /FVC	% Predicted FEV ₁
Nonsmokers	68 ± 3	4/10	NA	0.80 ± 0.02	104 ± 4
Smokers	64 ± 2	17/3	28 ± 4	0.78 ± 0.01	100 ± 4
COPD	67 ± 2	14/2	45 ± 6	0.59 ± 0.02	69 ± 5

Definition of abbreviations: COPD = chronic obstructive pulmonary disease; F = female; M = male.

Data are given as mean ± SD. FEV₁/FVC ratio is post bronchodilator for subjects with COPD but not smokers or nonsmokers.

Figure 1. Antibodies to carbonyl-modified protein in human serum from patients with chronic obstructive pulmonary disease (COPD), smokers, and non-smokers. Human serum was screened for immunoreactivity toward carbonyl-modified human serum albumin by ELISA and titers determined as detailed in METHODS. Antibody titers against human serum albumin that had been left (a) unmodified or had been modified by (b) acrolein, (c) malonyldialdehyde (MDA), (d) 4-hydroxynonenal, or (e) cigarette smoke extract are shown. (f) The cumulative titers against all the different carbonyl-modified HSAs tested for each patient group are shown. Results are expressed as a box and whiskers plot and displaying the mean for each patient group. Sera from 12 patients with severe persistent asthma (SA) are used as a disease control. Statistical analysis was performed using a nonparametric Kruskal-Wallis test with Dunn multiple comparison post test analysis. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ compared with nonsmokers (NS). # $P < 0.05$, compared with smokers.

inversely correlated with FEV₁ (% predicted), particularly for MDA- and acrolein-modified protein (MDA: $r^2 = 0.31$, $P < 0.01$; Acrolein: $r^2 = 0.27$, $P < 0.01$; 4-hydroxynonenal: $r^2 = 0.17$, $P < 0.05$; cigarette smoke extract (CSE): $r^2 = 0.17$, $P < 0.05$). There was also a small, but significant, increase in autoantibody titer against unmodified human serum albumin (HSA) in subjects with GOLD III COPD only. There was no significant increase in antibody titer against MDA- and acrolein-modified HSA in smokers. In contrast, apart from a significant response to acrolein-modified HSA ($P < 0.001$), there was no significant increase in antibody titer to unmodified, MDA-, 4HNE-, or CSE-modified HSA in sera from patients with severe persistent asthma. Overall, subjects with GOLD III COPD exhibited a highly significant ($P < 0.001$) increase in total antibody titer levels compared with asymptomatic nonsmokers (Figure 1f). Patients with severe asthma had similar total anti-carbonyl titer levels as asymptomatic smokers.

Numerous carbonyl-modified proteins were present in the lung parenchyma from patients with COPD as determined by Western blotting (Figure 2A, lanes 1, 3, and 5). The most abundant carbonyl-modified protein had a molecular weight similar to that for HSA. In contrast, no nonspecific bands were observed in the control lanes (Figure 2A, lanes 2, 4, and 6), although protein loading was similar across all six lanes (Figure 2B).

A significant increase in IgG1 isotype responses in smokers and patients with COPD compared with nonsmokers (see Figure E1 in the online supplement) was observed. Moreover, there was a trend toward increasing immunoreactivity for this isotype response with increased disease severity. Except for a significant increase in IgG2 immunoreactivity against carbonyl-modified protein in GOLD stage III COPD (Figure E1b), there were no significant responses against the other immunoglobulin isotypes tested (IgG3, IgG4, and IgM). In

contrast to carbonyl-modified HSA, significant immunoreactivity toward unmodified HSA was only observed in patients with GOLD stage III COPD, and was of an IgG2 isotype (Figure E1g). No significant response was observed against HSA for IgG1, IgG3, IgG4, and IgM.

Figure 2. Carbonyl-modified proteins are present in parenchymal lung tissue of patients with chronic obstructive pulmonary disease (COPD). Solubilized parenchymal lung tissue from three patients with COPD was analyzed by (A) Western blotting for carbonyl modified proteins after dinitrophenylhydrazine (DNPH) derivatization of carbonyl epitopes, and (B) Coomassie staining for total protein. In lanes 1, 3, and 5, protein samples were derivatized with DNPH before sodium dodecyl sulfate polyacrylamide gel electrophoresis; lanes 2, 4, and 6 were left underderivatized. Further details are available in the online supplement.

Chronic Ozone Exposure Leads to Activated Immune Response to Carbonyl-Modified Self

The effect of 6 weeks' exposure to ozone, an oxidant stress, on antibodies against carbonyl-modified proteins in mice was also determined. Ozone exposure led to a significant increase in antibody titer against MDA-modified murine serum albumin compared with a control group of mice exposed to air only (Figure 3). In contrast, acute exposure to ozone (one exposure) showed no increase in titer above control.

Splenocytes isolated from chronic ozone-exposed mice when treated with MDA-modified albumin exhibited significantly greater proliferation and increased release in IL-2, when compared with splenocytes isolated from control air-exposed mice (Figure E2). In contrast, unmodified murine serum albumin had no significant impact on splenocytes from either air-exposed or chronic ozone-exposed mice. Finally, chronic ozone exposure led to activation of lung antigen presenting cells (APCs), from both within the lung (Figure E3) and draining lung lymph nodes (Figure E4) as demonstrated by increased expression of the surface expression markers CD80, CD86, and CD54.

Complement Deposition and Immunoreactivity against Endothelial Cells in the Peripheral Lung of Stable Patients with COPD

Immunohistochemical staining for C4d was mainly confined to lung vessels, and the number of C4d-positive lung vessels was significantly increased in smokers, with or without COPD, compared with nonsmokers (Figure 4). However, there were no significant differences between subjects with GOLD II COPD and control smokers. In addition, autoantibody levels against endothelial cells were significantly elevated in subjects with COPD compared with healthy nonsmokers (Figure 5). There was no significant increase in anti-endothelial cell autoantibody levels in smokers compared with nonsmokers.

Figure 3. Antibodies to carbonyl-modified self-protein in mice chronically exposed to ozone. Murine serum was screened for immunoreactivity toward carbonyl-modified murine serum albumin by ELISA and titers determined by ELISA as detailed in METHODS. Murine serum from mice either acutely exposed (1 d) or chronically exposed (6 wk) to air or ozone was screened on ELISA plates coated with murine serum albumin (MSA) that had been carbonyl-modified with malonyldialdehyde (MDA). Chronic ozone exposure results in a significant increase in antibody titer against carbonyl-modified protein. Results are expressed as the mean $\pm$ SEM for the titer determination from 6 to 8 mice in each treatment group. Statistical analysis was performed using a nonparametric Kruskal-Wallis test with Dunn multiple comparison post test analysis. * $P < 0.05$, compared with control mice exposed to air.

DISCUSSION

We have shown in patients with stable COPD, the presence of circulating antibodies against carbonyl epitopes formed on proteins as a result of exposure to chronic oxidative stress. The increased antibody titer against carbonyl-modified protein correlated with disease severity in stable COPD and was highly significant in GOLD stage III COPD. Interestingly, antibodies against carbonyl-modified protein also showed a trend toward an IgG1 phenotype. Moreover, we observed the presence of a numerous carbonyl-modified proteins in peripheral lung tissue from patients with COPD. These antigenic carbonyl adducts on self-proteins and their plasma levels have previously been shown to be associated with disease severity in stable COPD (18). Similar findings were observed in mice after chronic exposure to ozone for 6 weeks. Antibodies against carbonyl-modified protein were elevated and splenocytes isolated from ozone-exposed mice became activated in response to stimulation with carbonyl-modified protein. This was accompanied by a greater antigen-presenting cell activation (both macrophages and dendritic cells) in murine lungs as demonstrated by the increased expression of the activation markers CD80, CD86, and CD54 on these cells. Finally, we demonstrate the presence of anti-endothelial cell antibodies and activated complement localized to the endothelium of lung vessels from patients with COPD.

Carbonyl adducts, such as 4-HNE, have been observed in both the lung and systemically in muscle fibers of subjects with COPD (13, 14). Other examples of reactive carbonyl adducts include acrolein, MDA, methyl glyoxal, and numerous others.

Figure 4. Complement (C4d) activation in peripheral lungs of chronic obstructive pulmonary disease (COPD) and control subjects. Photomicrographs showing immunostaining for activated complement C4d with a polyclonal rabbit IgG antibody on peripheral lung from (A) a nonsmoker, (B) a healthy smoker with normal lung function, (C) a patient with mild/moderate COPD, and (D) the negative control wherein the primary antibody is substituted for a nonspecific polyclonal rabbit IgG antibody on COPD lung. C4d+ cells are identified by a brown immunostain. Results are representative of those from 14 nonsmokers, 20 smokers with normal lung function, 16 with mild/moderate COPD. A significant increase in staining between nonsmokers versus healthy smokers and COPD groups was observed ($P < 0.05$ as determined using the Kruskal-Wallis test). Further methodological details are described in the online supplement.

Figure 5. Autoantibodies to endothelial cells in human serum from patients with chronic obstructive pulmonary disease (COPD), smokers, and nonsmokers. Human serum was screened for immunoreactivity toward human endothelial cells by ELISA and titers determined as detailed in METHODS. Plates were coated with live human umbilical vein endothelial cells, then treated with serum from COPD, smokers, or nonsmokers before detecting bound antibody. Results are expressed as the mean $\pm$ SEM for immunoreactivity in each patient group. Statistical analysis was performed using a nonparametric Kruskal-Wallis test with Dunn multiple comparison post test analysis. * $P < 0.05$ compared with control nonsmokers.

A key characteristic of this group of molecules is that they are able to covalently modify proteins nonenzymatically by targeting nucleophilic sites, such as amines or sulfhydryl groups, on proteins (19–21). Consequently, carbonyl-induced protein modifications have been shown to be markers for oxidative stress-derived tissue damage in a number of diseases (22). Protein carbonylation can also affect protein function as demonstrated by us (19, 23) and others (12). Furthermore, carbonyl-modified proteins are highly immunogenic and lead to the formation of neoantigens in the form of carbonylated self-protein (24–26). This in turn leads to the formation of autoimmune-type responses as a result of altered self-antigens (26–28). Protein carbonylation is a heterogeneous event, and it is unlikely that only one particular carbonyl modification will impart antigenicity to a protein, as demonstrated here by the immunoreactivity toward protein modified by several different carbonylating species. Carbonylated proteins can also modulate adaptive immune responses (29, 30) as well as activate T cells and promote Th1-type responses through their uptake and presentation by antigen-presenting cells (31).

We have demonstrated that there are a large number of carbonyl-modified proteins present in the peripheral lung tissue of subjects with stable COPD (GOLD stages I and II) that could act as potential epitopes for the generation of the anti-carbonyl antibodies. HSA was used as a generic screening antigen, because there should be no immune response against it under physiological conditions. However, because of its abundant nature it is very likely to be affected by oxidative stress and hence be modified. A major modified band corresponds to the molecular weight of HSA, which has recently been reported to be the major carbonyl-modified protein in human lung tissue (32). HSA was postulated to be acting as a sacrificial, scavenger antioxidant, but prolonged exposure to oxidative stress may lead to autoantibody production. Clearly, further detailed studies will be required to ascertain the identities and function of these other carbonyl-modified proteins.

The presence of autoantibodies in COPD has been controversial. The increased prevalence of anti-elastin antibodies in a subset of patients with COPD with severe pulmonary emphysema (5) has been contradicted by more recent publications (33–36). However, autoantibodies to several unidentified epithelial

cell proteins (6) and general tissue proteins (37) have also been demonstrated, as well as antibodies in the serum of patients with COPD directed against a number of peptide epitopes from a peptide array (8). In addition, a very recent publication has reported the increased presence of anti-endothelial cell autoantibodies in COPD (9). However, none of the publications addressed how these autoantibodies might have arisen through the breakdown of immune tolerance, apart from speculating that components in cigarette smoke were somehow involved. We have demonstrated that carbonyl adducts, which are potent antigens formed as a result of exposure to chronic oxidative stress (27), such as cigarette smoking or ozone exposure, can trigger an antibody-mediated immune response. Using *in vivo* models, two groups have shown that oxidant stress and carbonyl-modified protein can lead to autoimmunity (28, 31) with specific T cell activation and corresponding antibody production, a prerequisite for any immune response. Similarly, we found that oxidant-stressed mice after chronic ozone exposure exhibited higher antibody titers against carbonyl-modified protein as well as increased activation of their splenocytes in response to stimulation with carbonyl-modified protein. Our unpublished observations also showed increased proliferation of peripheral blood mononuclear cells from subjects with COPD in response to stimulation with carbonyl-modified protein as determined by BrdU uptake, but this needs to be confirmed in a more extensive study using fluorescence-activated cell sorter analysis to determine cell specificity and the extent of proliferation. Allison and Fearon (24) demonstrated that carbonyl modified self-protein or peptides were able to break immune tolerance *in vivo*. The immune response to oxidatively, or more correctly, carbonyl-modified neoantigens is not unique to COPD. Kurien and Scofield (27) highlighted how oxidative stress in other chronic diseases associated with high oxidative stress, such as rheumatoid arthritis, systemic lupus erythematosus, scleroderma and Behçet disease, gave rise to autoimmune responses to carbonyl-modified self-antigens. Furthermore, they highlighted how these carbonyl-modified epitopes could give rise to epitope spreading, a key feature of many autoimmune diseases. Not surprisingly, we also found an anti-carbonyl antibody response in patients with severe asthma, another chronic inflammatory disease that has also been associated with the presence of oxidative stress (38). Interestingly, the response was not as widespread across those carbonyl-modified antigens tested and moreover was not as strong as that observed in subjects with GOLD III COPD. This may simply reflect the greater oxidative burden present in the COPD lung compared with the severe asthmatic lung. Another key feature of many autoimmune diseases is the presence of antinuclear antibodies. It is interesting to note, therefore, that antibodies against adducts of 4HNE, a carbonyl known to be elevated in COPD (13), have dual specificity against both DNA and 4HNE-modified protein leading to an antinuclear antibody-type response (39).

Our immunohistochemical analysis of lung resection samples showed evidence of complement activation around the vascular endothelium in peripheral lung from healthy smokers and patients with GOLD II COPD and not in the lungs from control nonsmokers. We also observed elevated anti-endothelial cell and anti-carbonyl-modified protein titers in smokers and subjects with GOLD II COPD compared with nonsmokers, although significance was only seen in the COPD group. However, antibody titers between the smokers and subjects with GOLD II COPD were similar and may therefore explain the similar level of complement activation. Interestingly, serum levels of C4 were negatively correlated with the degree of pulmonary emphysema in patients with COPD and chronic

bronchitis (40). Other studies have reported similar findings. Miller and colleagues showed decreased levels of serum C3 and C4 proteins in patients with stable COPD compared with control subjects (41). Moreover, they found a correlation between the degree of the reduction of these serum proteins and the presence of symptoms of chronic bronchitis (41). In contrast, Marc and colleagues demonstrated that sputum levels of both C3a and C5a were significantly increased in patients with moderate to severe stable COPD compared with control healthy smokers and nonsmokers (42). Together this would suggest that as complement is sequestered toward the lung where it is activated and consumed, serum levels would decline. Combined with our data, it would suggest that the oxidant stress seen in both smokers and patients with COPD is directing an antibody-mediated autoimmune response against the endothelium. In keeping with these data, triggering an autoimmune response against lung endothelial cells in a murine model led to endothelial-cell death and pulmonary emphysema (43). Similarly, Karayama and colleagues have reported the increased presence of anti-endothelial antibodies in patients with COPD (9). We hypothesize that a similar mechanism is occurring in smokers and to a much greater extent in COPD lungs, where the chronic exposure to oxidative stress leads to carbonyl adducts being formed on lung endothelial cells. Why this should only occur on endothelial cells is not clear, and what is the identity of the carbonyl-modified endothelial-specific antigen remains to be resolved.

In conclusion, this pilot study has demonstrated for the first time the presence of antibodies against carbonyl-modified protein neopeptides in both COPD and a murine model of chronic exposure to oxidative stress. It is also proposed that these antibody responses to carbonyl-modified protein may be targeting endothelial cells in the lung parenchyma of subjects with COPD, leading to lung destruction. We recognize that one of the important limitations of this study is the low numbers of subjects screened in each group. Therefore, it would be important to confirm our findings here in larger cohorts of control subjects and patients with COPD.

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