Investigating autoimmunity in chronic obstructive pulmonary disease (COPD).

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Abstract:

Chronic obstructive pulmonary disease (COPD) involves lung inflammation caused by smoking. It is a major health problem worldwide. We are investigating whether smoke-induced self-destruction contributes to lung damage. In COPD patients, lower levels of inflammatory markers were detected in blood, but higher levels in the lungs, compared to controls. The depletion of inflammatory markers from the blood of COPD patients could be due to these markers binding to tissue components in smoke-damaged lung. These lung-bound markers might then promote further tissue damage. This may illustrate the involvement of the body's own defence mechanisms in destroying lung tissue exposed to smoke.

Key words:
Chronic obstructive pulmonary disease, autoimmunity, autoantibodies., T-cells, inflammatory lung disease.

Introduction:

Chronic obstructive pulmonary disease (COPD) is the only major chronic disease that has exhibited a continuous increase in prevalence and mortality rates worldwide [1-2] [3]. Currently it is the fifth commonest cause of death, and is expected to be the third most common fatal disease worldwide by 2020 [2].The impact of this chronic disease exerts an enormous financial burden on health resources worldwide [4].

COPD is a heterogeneous disorder characterized by progressive airflow limitation that is not fully reversible, and associated with an abnormal inflammatory response of the lungs to noxious particles or gases [5]. Patients with COPD typically present with cough, sputum production, and dyspnea on exertion [5, 6]. The chronic airflow limitation characteristic of COPD is caused by a mixture of small airway disease (obstructive bronchiolitis) and parenchymal destruction (emphysema).

Risk factors for COPD include: host factors such as genetic background (e.g. alpha-1 anti-trypsin deficiency), airway hyper-responsiveness, and lung growth. Additionally, environmental factors such as tobacco smoke, occupational dusts and chemicals, indoor and outdoor air pollution, infections, and socioeconomic status contribute to the risk. Interaction between host factors and environmental factors reflects the potential risk of COPD development [5].

Smoking is the dominant identifiable aetiological factor in COPD [7] [8]. However, only 10-20 per cent of smokers develop a clinically significant form of the disease [9]. Additionally, COPD does occur in non-smokers [10] (for instance, in α1-anti-trypsin deficiency).Patients who stop smoking

continue to exhibit marked inflammation of the bronchial wall for many years after smoking cessation [11] [12]. This indicates that smoking may initiate a pathogenic process which is then self-perpetuating, therefore preventing the normal resolution of the inflammatory response [13] [10]. Moreover the mechanisms by which exacerbations occur during the course of the illness remain not fully explained. Thus, many uncertainties still exist about the pathophysiological pathways leading to this disease. However it was shown that COPD shares many clinical and pathological features with several autoimmune diseases, such as rheumatoid arthritis [10].

In this project it is proposed that smoking initiates an acquired immune response to newly created or altered epitopes. This may lead to loss of tolerance to self antigens [14] and would qualify COPD as an autoimmune disorder. A possible model where COPD is considered to be an autoimmune disease triggered by smoking is presented (Figure 1). Constituents of tobacco smoke may be responsible for initiating an innate immune response by stimulating 'danger' signals in epithelial cells and promoting an initial neutrophilic and macrophage inflammation. By diverse mechanisms (proteases, oxidation, etc.) it could damage lung cells producing peptides and modified proteins that function as autoantigens. These neo-antigens and possibly the release of anatomically sequestered antigens would not go unrecognised, as they have the potential to act as antigenic determinants. Recognition by dendritic cells (DC) and other antigen presenting cells (APC) could lead to presentation to T-cell (CD8+ and CD4+), which would be autoreactive, inducing their activation and proliferation. The activation of autoreactive B-cells would then be inevitable and consequently production of antigen-specific autoantibodies by plasma cells may possibly contribute to lung injury.

The current view of COPD as a chronic inflammatory condition [15] does not fully explain the pathogenic processes accompanying this disease. Additionally, the available anti-inflammatory treatments do not have a consistent effect on its inflammatory progression [16]. Therefore, the proposed role of autoimmunity, if true, could add significantly to the understanding and management of COPD. The urgent necessity of developing new therapeutic strategies for COPD might be met by the development of new immunomodulatory drugs to control the autoimmune process.

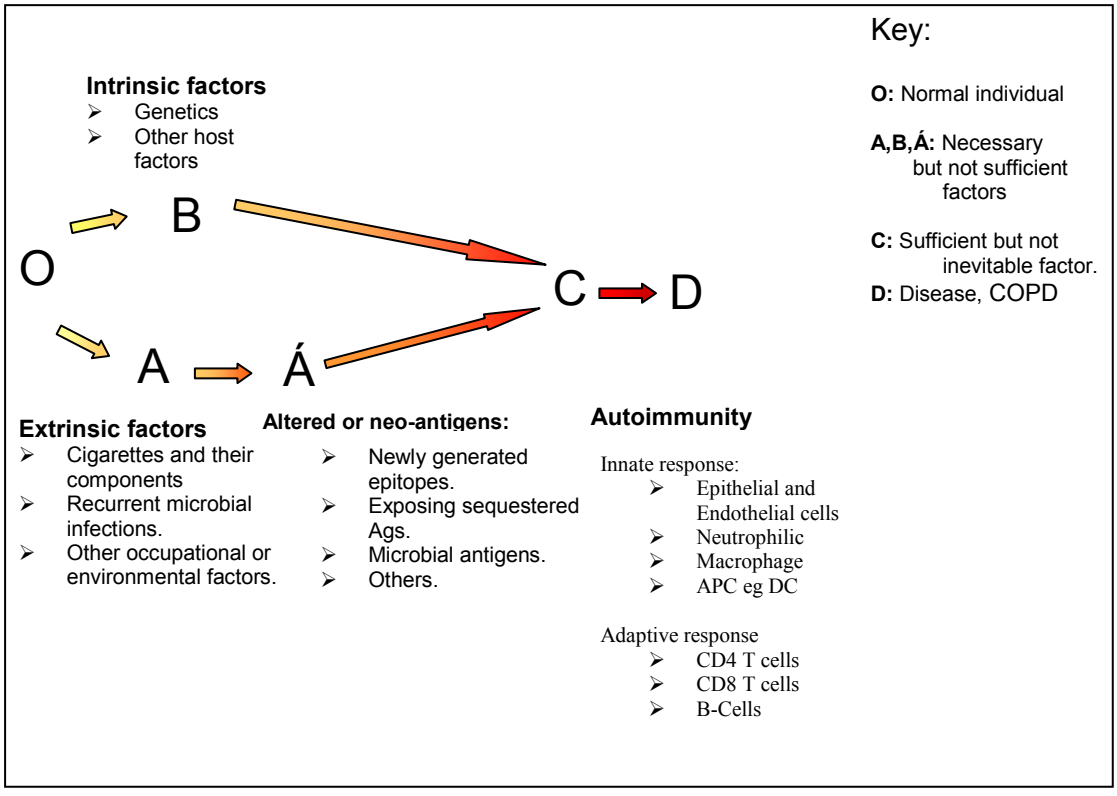

Figure 1: A possible model where Chronic obstructive pulmonary disease COPD is considered to be an autoimmune disease triggered by smoking.

Aim:

In this study, the over all aim is to examine the possibility of an autoimmune response being involved in the pathogenesis of COPD. Our hypothesis suggests an autoimmune component that contributes -at least partly- to the progressive and continuous lung injury. Firstly, by investigating the presence of autoantibodies against lung tissue in serum samples from smokers with COPD compared to controls.

Methods:

Sample population

Peripheral blood samples were obtained from smokers with chronic obstructive pulmonary disease (COPD), healthy smoker (H-S) and healthy non-smoker (H-NS) participants (6 of each) Patients with COPD were recruited from the Department of Respiratory Medicine, Queens's Medical Centre, Nottingham, UK. Healthy smoker and healthy non-smoker volunteers were recruited as controls through advertisement. Five sets of lung samples were obtained from COPD patients, H-S and H-NS. All samples were generously donated by GSK group.

Procedures

- Lung homogenisation.
- Enzyme-Linked ImmunoSorbent Assay (ELISA)
- Western blots
- Nepelometry
- Indirect Immunofluorescence.

Results and conclusions:

I. Serum samples

1. Investigating the presence of natural autoantibodies in the 18 human serum samples of by ELISA:
   The 96 well ELISA plates were coated with human lung homogenates set-1 from COPD patient, H-S and H-NS. Anti-poly Igs, and anti-IgG were used as detection antibodies. Results showed that, in COPD patients' sera, there were reproducibly less antibodies to lung tissue homogenates detected than in H-S and H-NS, with significant differences between COPD and H-NS sera. Wells coated with bovine serum albumin (BSA) were used in each experiment as negative controls and showed no significant difference between groups. The depletion of antibodies from the circulation of COPD patients could be due to antibodies (Ab) binding to tissue components in smoke-damaged lung (hypothesis; Ab sequestered to the lungs).

2. Measuring the total immunoglobulin levels in participants' sera by Nephelometry:
   Nephelometry was used for measuring total poly-Igs, IgG, IgM and IgA immunoglobulin levels. Results showed no significant difference amongst all groups. These results suggest that the reduction of lung reactive serum antibody levels in COPD patients' was not due to a state of immune suppression in COPD patients.

3. Investigating the presence of antibodies in human sera against known antigens:
   To investigate the presence of antibodies in human sera against known antigens, the 96 well ELISA plates were coated with the candidate antigens mentioned in (Table-1).They were assayed against sera from smokers with chronic obstructive pulmonary disease COPD, H-S and H-NS participants, using anti-poly Igs, IgG, IgM and IgA antibodies. Results illustrated that antibody levels in COPD patients' sera were selectively lower to the lung associated antigen collagen-1and elastin but not to antigen of non-lung origin i.e. thyroglobulin. This may indicate selective autoantibody affinity to lung antigens.

II. Lung samples

1. Detection of immunoglobulins in human lung homogenates and pellets by western blots and chromogenic immunodetection.
   The results above repeatedly indicated that there were less detectable circulating antibodies in the sera of smokers with COPD than controls, to antigens in lung homogenates or lung associated antigen (collagen type-I). We hypothesised that this is may be due to antibodies being sequestered to the lungs by binding to their possible target antigens. Western blots were therefore performed to investigate the presence and the types of immunoglobulins in human lung tissue set 1 & 2 homogenates and pellets with chromogenic immunodetection. Immunoglobulins were detected using anti- poly-Igs, anti-IgM, anti-IgG, and anti-IgA enzyme conjugates.
   Results showed a band at around 75 kD that represents μ chain seen most prominently with COPD patients' homogenates and pellets that was detected with anti-Human IgM enzyme conjugate antibody.

2. Investigating immunoglobulins eluted from whole lung tissue using glycine HCl for reactivity against lung homogenates.
   As a screening assay, eluted antibodies from COPD lung samples were examined for reactivity against corresponding lung tissue homogenates. Results indicated that eluted antibodies bound more prominently to antigens in COPD lung homogenates compared to smoker and healthy lung homogenates. This was done using anti-poly-Igs, IgG and IgA detecting antibodies.

3. Quantitative measure of antibodies present in human lung samples (by ELISA) using Glycine-HCl buffer to dissociate tissue associated antibodies against candidate antigens.
   Eluted antibodies from COPD lung samples are more reactive –higher anti-poly-Igs, IgG and IgA antibodies- to some candidate antigens mainly collagen-1, and to a lesser extent to collagen-5, elastin, vitronectin, vimentin, and Cytokeratin-18. This is consistent with the hypothesis stated above that, in COPD, circulating lung-reactive antibodies are depleted as they may be sequestered to the lungs.

General conclusions:

Evidence of antibody involvement in the inflammatory process in COPD.
Suggest two levels of involvement:

1. Sequestration of natural Abs in the lung and depletion from circulation.
2. Active production of autoantibodies against target antigens.

Future work:

- Strengthen the evidence of humoral immunity being involved in the pathogeneses of COPD by
  - Increase sample size (Serum, Lung,)
  - Use Bronchoalveolar lavage (BAL)) samples

Coating:	**Primary Antibodies are from the Elutes Immunoglobulins from Whole lung**				
	Secondary Antibodies s used:				
Known antigens	**Anti-poly_Igs Ab**	**Anti-IgG Ab**	**Anti-IgM Ab**	**Anti-IgA Ab**	**Anti-IgE Ab**
Collagen I	√	√	√	√	
collagens II	√	√	√	√	
collagens ш	√	√	√	√	
collagens IV	√	√	√	√	
collagens V	√	√	√	√	
Thyroglobulin	√	√	√	√	
Fibronectin	√	√	√	√	

Laminin	√	√	√	√	
Elastin peptides	√	√	√	√	
Vitronectin	√	√	√	√	
vimentin	√	√	√	√	
Cytokeratins-18	√	√	√	√	
Cytokeratins-8	√	√	√	√	

Table-1: List of candidate antigens used for the detecting of antibodies in human sera using anti-poly_Igs, IgG, IgM and IgA antibodies.

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