T-cells in COPD to help or harm? A study of T-cells in COPD patients and related aspects of T-cell function in vitro

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T-CELLS IN COPD
TO HELP OR HARM?

A study of T-cells in COPD patients and related aspects of T-cell function *in vitro*

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Front picture shows $CD8^+$ T-cells in a bronchiole of a COPD patient

To my parents

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APPENDIX (PAPER I-IV)

LIST OF ORIGINAL PAPERS

This thesis is based on the following papers, referred to by their Roman numerals.

I Glader P, Löfdahl C-G, von Wachenfeldt K
αEβ7 expression on $CD8^+$ T-cells in COPD BAL fluid and on TGF-β stimulated T-cells *in vitro*
Lung 2005; 183: 123-138*

II Glader P, Möller S, Lilja J, Wieslander E, von Wachenfeldt K
Cigarette smoke extract modulates respiratory defence mechanisms through effects on T-cells and airway epithelial cells
Accepted for publication in *Respiratory Medicin*e

III Glader P, von Wachenfeldt K, Löfdahl C-G
Systemic $CD4^+$ T-cell activation is correlated with FEV_1 in smokers
Accepted for publication in *Respiratory Medicine*

IV Glader P, Löfdahl C-G, von Wachenfeldt K, Erjefält J
Distribution of lymphocytes and lymphoid aggregates in peripheral lung tissue from COPD patients
In manuscript

Some data not included in the papers are added in this thesis.

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ABBREVIATIONS

APC	Antigen presenting cell
BAL	Bronchoalveolar lavage
BALT	Bronchus associated lymphoid tissue
CB	Chronic bronchitis
CD	Cluster of differentiation
COPD	Chronic obstructive pulmonary disease
CSE	Cigarette smoke extract
FACS	Fluorescence-activated cell sorting
FEV_1	Forced expiratory volume in one second
FVC	Forced vital capacity
ICAM	Inter-cellular adhesion molecule
IEL	Intraepithelial lymphocyte
IL	Interleukin
INF-γ	Interferon-gamma
LFA-1	Leukocyte functional antigen-1
MHC	Major histocompatibility complex
PBMC	Peripheral blood mononuclear cell
TCR	T-cell receptor
TGF-β	Transforming growth factor-beta
TIA-1	T-cell intra-cytoplasmic antigen

INTRODUCTION

Chronic Obstructive Pulmonary Disease, COPD

Symptoms, causes and treatments

Chronic obstructive pulmonary disease (COPD), as defined by the Global initiative for chronic obstructive lung disease (GOLD), is *a disease state characterized by airflow limitation that is not fully reversible. The airflow limitation is usually both progressive and associated with an abnormal inflammatory response of the lungs to noxious particles or gases* (1). Patients experience to varying extents symptoms of chronic and productive cough and daytime or nocturnal dyspnoea. These symptoms are the result of pathological changes in different segments of the airways, including centrally located chronic bronchitis, peripheral bronchiolitis and parenchymal emphysema. Inflammation and excessive mucus production in the central and peripheral airways are distinctive features of bronchitis/bronchiolitis, leading to cough and sputum production, while the destruction of alveolar structures in emphysema results in reduced gas exchange (2, 3).

The diagnosis of COPD is based on spirometric measurements. Forced expiratory volume per one second (FEV_1) is the amount of exhaled air in one second and forced vital capacity (FVC) is the total volume of exhaled air. Both measurements are performed during forced expiration. Airway obstruction is established when $FEV_1/FVC < 0.7$. Severity is then based on FEV_1 values related to predicted normal values, ranging from $\leq$ 80% in mild COPD to $\leq$ 30% at a very severe stage, according to GOLD standards (1). In addition to the classical symptoms associated with stable disease, these patients from time to time experience disease exacerbations with increased dyspnoea, increased production of purulent sputum, increased cough and a fall in lung function (4). Such exacerbations, which often lead to hospital admissions, occur at different frequencies in different patients and have been correlated with an accelerated decline in lung function over time and a higher mortality (5, 6). Airway infections of bacterial and/or viral origin often trigger these exacerbations (7-10) and patients are therefore often treated with antibiotics.

The most important risk factor for developing COPD is cigarette smoking (11). However, exposure to air pollutants and wood smoke, passive smoking and a genetic mutation leading to deficiency in α_1-anti trypsin also contributes to a small number of cases (12-14). In the year 2000 COPD was ranked among the six leading causes of death in the world (cardiovascular diseases, tumours, injuries, lower respiratory infections and HIV/AIDS being the other five) (15), and since smoking is increasing in the world today (16) and COPD is a disease with a slow onset, the prevalence of COPD is believed to increase in the future (1).

The most effective treatment for COPD today is smoking cessation, which slows down the decline in lung function (17). Drug therapies in COPD are otherwise focused at symptomatic relief rather than cure. Current treatment with bronchodilators such as beta-2-agonists and anti-cholinergics in combination with broad anti-inflammatory drugs like corticosteroids, and in the near future probably also phosphodiesterase-4 (PDE4) inhibitors, are used to improve airflow and to some extent suppress the chronic inflammation (18, 19). New potential targets in the therapeutic area focus on the recruitment and protease production of neutrophils and macrophages, key players in COPD further discussed below. Inhibitors of leucotrienes like LTB4, and inhibitors of chemokine receptors such as CXCR1 and 2 are currently being developed to prevent neutrophil recruitment, and protease inhibitors may have the potential to inhibit some of the tissue degradation seen in emphysema patients. In addition to drug therapy lung volume reduction surgery, which increases the lung elastic recoil, and oxygen treatment are other options for extending the lives and improving life quality of these patients (20, 21).

Airway pathology

The pathological changes in the lungs of COPD patients are very heterogeneous both within and between patients with varying degree of bronchitis/bronchiolitis, emphysema, and airway remodelling at different sites of the lung. Biopsies from both central and peripheral airways as well as resected lung parenchyma are often examined in COPD studies (Figure 1).

Bronchitis and bronchiolitis are characterized by mucosal and submucosal inflammation and oedema present at the site of bronchi and bronchioles. Bronchial glands are often hypertrophic and produce excessive amounts of mucus. The accumulation of large amounts of mucus and inflammatory cells can sometimes lead to plugging of the airways (Figure 1C), with reduced airflow as a result (22-24).

Emphysema is characterized by destruction of the alveolar structure due to rupture of alveolar septa (Figure 1E). This results in enlarged alveolar spaces and reduced gas exchange as the surface area of the respiratory zone decreases. The reduced gas exchange in combination with decreased elastic recoil, due to the destruction of alveolar walls, results in reduced respiratory function, which makes emphysema patients experience dyspnoea (12). An imbalance between the level of proteases and antiproteases, due to an increase in neutrophil and macrophage derived proteases, (e.g. neutrophil elastase and matrix metalloproteinases), is a probable cause of tissue destruction (25). The imbalance of proteases and antiproteases is clearly demonstrated in patients with α_1-anti trypsin deficiency, where the lack of protection against proteases causes emphysema in these patients (26).

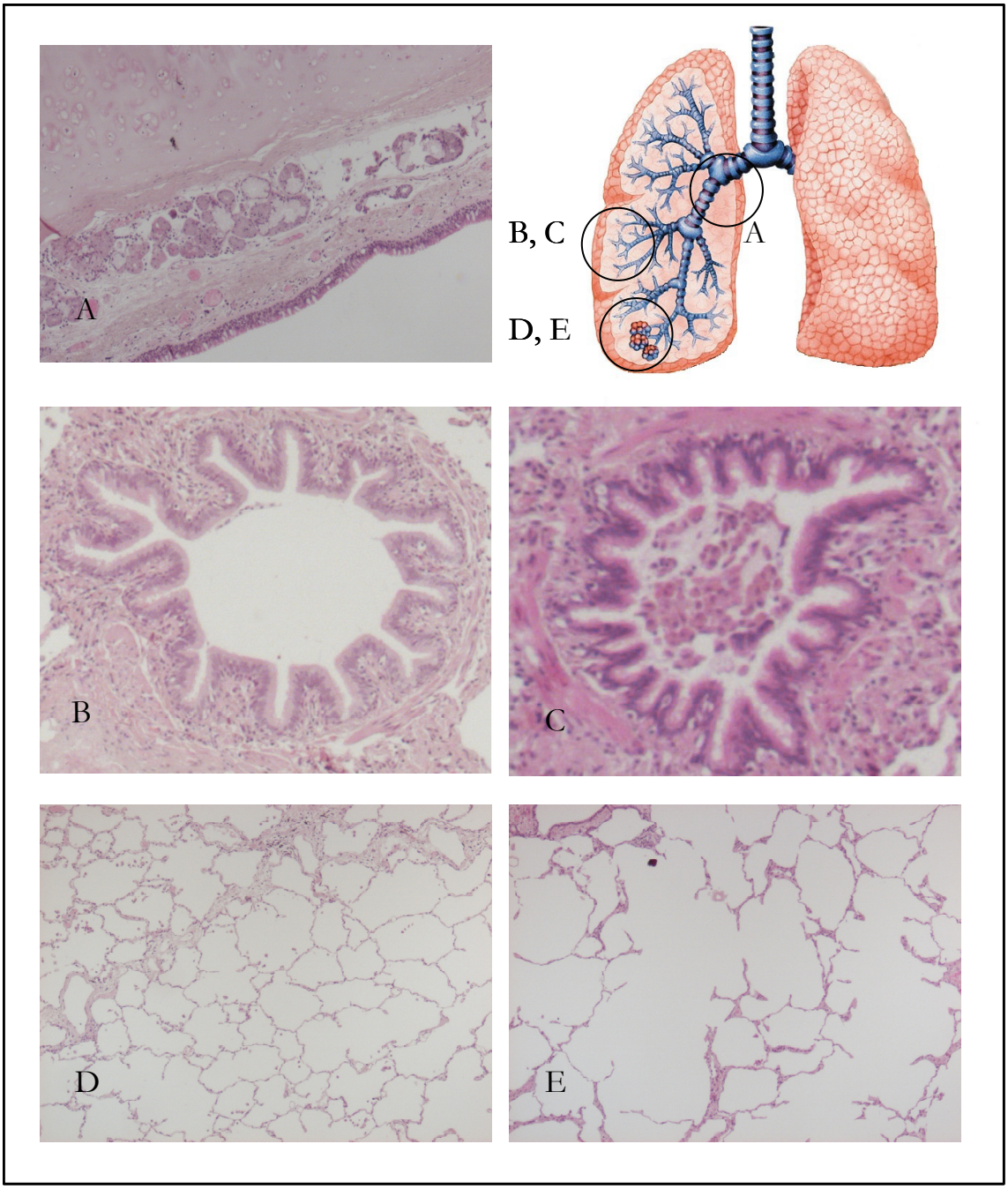

Figure 1 Bronchial biopsies sampled to study the central airways contain cartilage, smooth muscle and submucosal glands and are lined with ciliated epithelium (A). Bronchioles in the peripheral airways lack cartilage (B) and are in COPD patients often inflamed and sometimes also plugged by mucus and cell debris (C). The lung parenchyma is responsible for the respiratory gas exchange, which benefits from the large area of the alveoli (D). In emphysema the alveolar structure is destroyed due to tissue degradation leaving large sacs with a higher volume/area ratio (E). (IHC photos taken by P. Glader, lung drawing is a free picture from Oxford illustrated science encyclopaedia)

Airway remodelling in COPD occurs at different sites throughout the lung. Goblet cell hyperplasia and squamous cell metaplasia in the bronchial epithelium is sometimes seen and submucosal gland hypertrophy and impaired epithelial integrity can also be present (27, 28). In smaller airways goblet cell metaplasia results in pathological mucus production in small bronchioles. The increased mucus production provides a suitable environment for bacterial colonisation, which is often seen in the lower airways of COPD patients and smokers (29, 30). The small airways are also subjected to smooth muscle increase. In the parenchyma fibrosis and thickening of alveolar septa reduces the elasticity of the lung tissue (28).

Airway inflammation

COPD is largely considered to be driven by chronic inflammation of the lung. Inflammatory cells are normally recruited to the site of injury when a trauma has occurred or an infection is present. In the case of COPD, cigarette smoke exposure could provide the "trauma" that initiates the influx of inflammatory cells. When the epithelium is exposed to cigarette smoke an increase in the production of chemokines like interleukin-8 (IL-8) follows (31), which orchestrate the recruitment of neutrophils that are among the first cells to be on site of the injury (32) followed by macrophages and lymphocytes. Usually after an injury the inflammation will resolve with time. In COPD, however, the inflammation persists and becomes a chronic destructive process. The reason for this is currently not known.

Neutrophils, macrophages and epithelial cells are along with $CD8^+$ T-cells, which will be further discussed below, thought to be major players in the COPD inflammation (33). These cells have been found in increased numbers at different sites of the lungs in COPD patients and the mediators they produce are believed to drive the COPD pathogenesis forward (34). Neutrophils have been demonstrated in large numbers in sputum, BAL and tissue and have been correlated to a decline in lung function (35-37). Macrophages are distributed throughout the lungs and ordinarily harbour the alveolar lumen as a defence against airborne particles and pathogens, which they remove by phagocytosis. In smokers and COPD patients the macrophages are large and filled with coal particles after having phagocytosed particles from the cigarette smoke (38). One theory regarding COPD pathogenesis suggests that these macrophages are "over fed" with particles due to the cigarette smoking and that this would limit their ability to phagocytose apoptotic cells, which in turn could lead to secondary necrosis of the apoptotic cells (39, 40). This hypothesis is supported by a study on bronchoalveolar lavage macrophages showing reduced phagocytosis of apoptotic airway epithelial cells in COPD patients compared to non-smokers (41). The presence and engulfment of necrotic cells rather than apoptotic cells may have the potential to enhance inflammation instead of hampering it (39). Macrophages are also found in the mucosa and in the bronchiolar epithelium where intra epithelial macrophages have been correlated to a decline in lung function (42, 43).

Neutrophils, macrophages and also epithelial cells produce a range of different proteases, cytokines and chemokines that will affect the environment of the lung. As stated above, neutrophil and macrophage proteases have the potential to degrade lung tissue. If not balanced by anti-proteases this may lead to emphysema (25). Chemokines and cytokines like IL-8, granulocyte macrophage colony stimulating factor (GM-CSF) and tumour necrosis factor-α (TNF-α) are involved in the regulation of the inflammation and are produced both by inflammatory cells and by the epithelium (44, 45). Another cytokine that may be involved in COPD is transforming growth factor-β (TGF-β) (46, 47). This cytokine is produced by both epithelial cells and macrophages and has a wide range of biological functions, including pro-fibrotic and anti-inflammatory properties and is involved in differentiation of T-cells into αEβ7 expressing intra epithelial T-cells, which will be further described below.

T-cells

T-cell origin and maturation

The T-cells in the lung constitutes a large part of the total T-cell population of the body (48). As other leucocytes, T-cells are derived from progenitor cells in the bone marrow from which they later migrate to the thymus where they mature.

During maturation in the thymus T-cells are differentiated into $CD4^+$ and $CD8^+$ T-cells, defined by their expression of the co-receptor molecules CD4 and CD8. Both $CD4^+$ and $CD8^+$ T-cells recognize antigens that have been processed and presented on the surface of antigen-presenting cells (APCs). $CD4^+$ T-cells, also called T-helper cells, recognize antigens presented on the protein MHC class II, present on so called professional APCs, which constitutes mainly of monocytes/macrophages and dendritic cells. MHC class II presents extra-cellular antigens, like bacterial peptides. $CD8^+$ T-cells, also called cytotoxic T-cells, recognize antigens presented by MHC class I, present on all cells in the body. MHC class I presents intra-cellular antigens like viral or tumour proteins (49, 50) (Figure 2).

T-cells are part of the acquired immune system and the specificity of the T-cells lies in their T-cell receptor (TCR). The TCR is a multimeric protein complex of immunoglobulin like proteins and is present on the cell in association with CD3. The TCR is composed of two disulfide-linked chains, either αβ or γδ. Combinations of variable domains within the TCR generate the specificity. T-cells recognizing MHC are selected through a positive selection process. The risk of having T-cells directed against self-antigens, potentially creating autoimmunity, is decreased through a subsequent negative selection process where T-cells that react with MHC expressing self-peptides are eliminated (49). The $CD4^+$ and $CD8^+$ T-cells described above belong to the αβ T-cell subset. The γδ T-cells are much less studied and exist in fewer numbers. In blood they constitute only a few percent of the T-cells, whereas in some

parts of the body, including the intestine, the γδ T-cells are more common (51) and they are also, to a less extent, present in the airways (52).

Once T-cells have matured in thymus with regard to both TCR specificity and $CD4^+/CD8^+$ subtype they start circulating the blood and the lymph by which they are transported to the different tissues in the body in search for their specific antigen.

T-cell activation and effector functions

Once T-cells encounter an antigen-presenting cell with the appropriate MHC molecule and peptide, they need two signals in order to become activated and proliferative. Signal one is delivered by binding of TCR/CD3 complex to the antigen/MHC complex and signal two is provided by binding of the T-cell to co-stimulatory molecules on the antigen-presenting cell. Two common ways of delivering the second signal include binding of T-cell CD28 to CD80/CD86 on the antigen-presenting cell or by binding of leukocyte functional antigen-1 (LFA-1) to inter-cellular adhesion molecule-1 or -2 (ICAM-1, 2). As mentioned above, $CD4^+$ cells are also known as T-helper (Th) cells. When activated, they produce either IL-2 and INF-γ, which promotes T-cell proliferation and $CD8^+$ T-cell cytotoxicity or they produce IL-4, IL-5 and IL-10 that facilitates B-cell antibody production (Figure 2).

Because of their cytotoxic effector function, $CD8^+$ T-cells are also called cytotoxic T-cells, Tc. In order to accomplish their cytotoxic function, $CD8^+$ T-cells inserts perforin into the membrane of the target cell. This pore-forming protein allows the transport of cytotoxic T-cell granule proteins into the target cell. Among these granule proteins are granzymes and T-cell intra-cytoplasmic antigen-1 (TIA-1), which activates caspase mediated DNA fragmentation leading to apoptosis of the target cell. Another mechanism for $CD8^+$ T-cells to induce apoptosis is by binding of FAS-ligand to FAS, called the death receptor, on the target cell (Figure 2). In addition to their cytotoxic activity $CD8^+$ T-cells also produce cytokines similar to the $CD4^+$ T-cells (e.g. INF-γ, IL-4 and IL-5) (49, 50).

$CD4^+$ T-cells producing INF-γ and IL-2 are often called Th1 cells and $CD8^+$ T-cells producing the same cytokines are called Tc1 cells, while cells producing IL-4, IL-5 and IL-10 are called Th2/Tc2 cells. Both $CD4^+$ and $CD8^+$ T-cells produce other cytokines like TNF-α, IL-8, GM-CSF, IL-16, IL-17 and TGF-β that are all involved in the orchestration of inflammation.

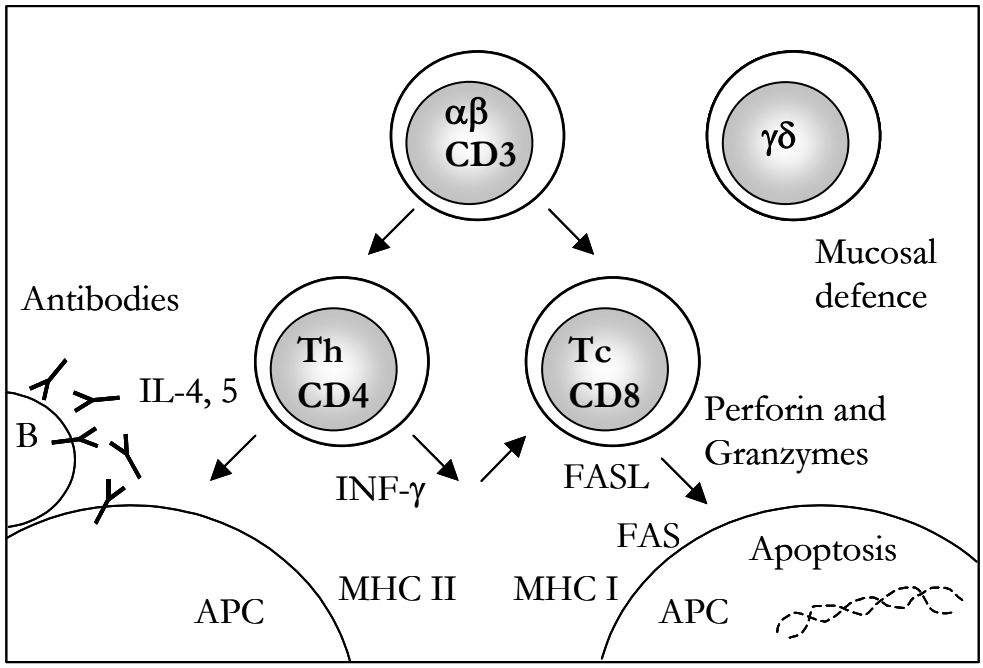

Figure 2 T-cells can express T-cell receptor αβ or γδ. They are further divided into $CD4^+$ T-helper cells recognizing extracellular antigens presented on MHC class II and $CD8^+$ T-cytotoxic cells recognizing intracellular antigens presented on MHC class I.

T-cell activation markers

During T-cell activation the protein production is altered. Some proteins are up-regulated and others are down-regulated. One way to follow the course of T-cell activation is to look at up- and down-regulations of certain membrane bound proteins referred to as activation markers. Many T-cell activation markers have been given names using the cluster of differentiation, CD, nomenclature (53, 54), and a few of these will be described here. The costimulatory molecule CD28 has been described above and is important in the signalling for IL-2 production and proliferation (55). One of the earliest up-regulated T-cell activation markers is CD69. This protein is present also on other leucocytes and mediates down stream cell signalling, yet with an unknown function (56). CD29 is the beta chain of very late antigens, VLAs, which are present on T-cells (e.g. VLA-4) and a variety of other cell types and bind to extra cellular matrix molecules like fibronectin, collagen and laminin and are late activation markers. CD45RA and CD45R0 represents two different splice forms of CD45 and discriminate between naïve and memory T-cells respectively (57). Naïve T-cells are those which have not yet encountered their specific antigen and have not been activated before, while the opposite is true for memory T-cells. Antibodies directed against all of these activation markers, and many other CD markers, are commercially available and can be used to phenotype T-cells in the laboratory.

Intraepithelial T-cells

Intraepithelial T-cells are situated in the mucosal epithelium throughout the body (58, 59). These cells are often recognised by their expression of CD103, which is the α-subunit of the integrin αEβ7 (60-63). This integrin is involved in the retention, stimulation (64-66) and perhaps also recruitment (67, 68) of T-cells to the epithelium, where it binds to its ligand E-cadherin that is expressed at the basolateral side of epithelial cells (69-71) (Figure 3). Intraepithelial T-cells have mainly been studied in the intestinal mucosa, in which they are very frequent in number, but they are also well documented in the lung (59). While approx. 30% of the intestinal intraepithelial T-cells express γδ-T-cell receptor (58), the airway intraepithelial T-cells mainly express αβ-T-cell receptor (72). The exact function of intraepithelial T-cells in the lung is so far not known, although a most probable role is involvement in the host defence against airborne pathogens invading the mucosa, where the IELs would constitute a persistent (72) T-cell pool reacting fast in response to antigens presented by the epithelial cells and by antigen presenting cells present in the epithelium (59). It has been shown that the intestinal epithelium down-regulates T-cell proliferation and cytokine production through signals mediated by cell-to-cell contact between the epithelium and the T-cells (73). If such an interaction occurs also in the airway mucosa the intraepithelial T-cells in the lung could be involved in the tolerance towards non-pathogenic airborne antigens.

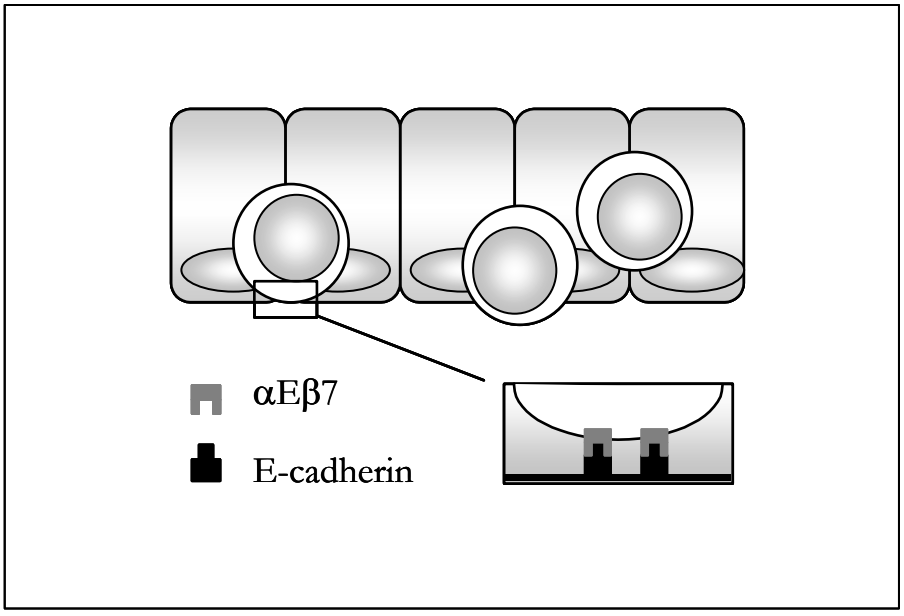

Figure 3 The integrin αEβ7 is expressed by intraepithelial T-cells and binds to E-cadherin on the epithelial cells.

Bronchus associated lymphoid tissue, BALT

Mucosal defence consists not only of the mucosal barrier with the epithelium and its intraepithelial leukocytes, but also of the mucosal associated lymphoid tissue (MALT) (74). MALT is called G(gut)ALT in the intestine and B(bronchus)ALT in the lung. Whereas GALT is present in all adults, the development of BALT in humans has been put forward as a response towards infections and is suggested not to be present in healthy adults (75, 76). This argumentation has however been questioned and data are evolving showing the presence of BALT in healthy adults (77). It seems rather clear though, that BALT is not present in humans at birth but develops as the lung is exposed to airborne antigens (78, 79). The BALT structure, described and reviewed in several publications (80, 76, 81), is facing the bronchial lumen with a lymphoepithelium consisting of non-ciliated or ciliated cuboidal epithelial cells and intraepithelial lymphocytes. Below the epithelium lies an organised structure of lymphocytes and antigen presenting cells. A B-cell follicle, sometimes with a germinal centre, is surrounded by a perifollicular structure consisting of T-cells of mainly $CD4^+$ origin. Dendritic cells are situated in between the T-cells to transport and present luminal antigens to the T-cells. The lymphocytes are thought to be entering BALT through high endothelial venules (HEV), which are present in the lymphoid structure (77).

Other lymphoid aggregates, not fulfilling all the criteria of BALT, are sometimes seen throughout the lung. These assemblies of lymphocytes have not yet gained a common name and are referred to as lymphoid nodules (82), or lymphoid follicles (83) in different publications. These lymphoid structures are not always in contact with the bronchial epithelium and can be found in the parenchyma more adjacent to the alveolar lumen than to bronchial or bronchiolar lumens. They are loosely organised with a B-cell centre surrounded by mainly $CD4^+$ but also by scattered $CD8^+$ T-cells. These lymphoid aggregates seem to be more frequently present in smokers and may be caused by recurrent inhalation of irritants such as cigarette smoke (82).

T-cells in COPD

T-cell recruitment in COPD

As the lung is located at the interface between the sterile environment of the body and an external environment with high antigen and microbial load, the lung relies on an efficient defence and therefore normally harbours T-cells, ready to respond to an early infection. When the lung is exposed to increased loads of harmful substances such as cigarette smoke or pathogens, an increased number of T-cells are recruited to the lung. Cigarette smoke induced T-cell production of IL-16, a cytokine that has been shown to be involved in $CD4^+$ T-cell recruitment and to be up-regulated in BAL fluid of cigarette smokers (84, 85), may be an important mediator of T-cell recruitment in COPD. Other chemottractants may include chemokines like CCL3 (macrophage

inflammatory protein-1 alpha, MIP-1α) and CCL5 (regulated upon activation T-cells expressed and secreted, RANTES), which attract T-cells to the site of injury by binding to the receptor CCR5 on the T-cells. The presence of $CD3^+$ cells coexpressing $CCR5^+$ have been shown in bronchial biopsies from COPD patients and the number of these cells correlated with the number of both $CD4^+$ and $CD8^+$ T-cells (86). Another receptor/ligand pair shown to be up-regulated in COPD patients is CXCR3 expressed on the T-cells and its ligand CXCL-10 (interferon-gamma-inducible 10 kD protein, IP-10) expressed by the bronchiolar epithelium and in the wall of the pulmonary arteries (87). Another although speculative theory put forward for T-cell recruitment and activation in COPD is that self-peptides, produced when the lung epithelium is exposed to cigarette smoke, are recognized by T-cells creating a state of autoimmunity (88-91).

T-cell occurrence in lung tissue of COPD patients

Even though both $CD4^+$ and $CD8^+$ T-cells are recruited to the lung, the $CD8^+$ T-cells are often focused upon when discussing lymphocytes in COPD, and studies have been made at different levels of the lung with regard to their occurrence. As further described below, many of these studies have shown increased numbers of $CD8^+$ T-cells in COPD patients and smokers, and the number of these cells has been correlated with a decline in lung function. Because of this, $CD8^+$ T-cells are believed to be involved in the pathogenesis of COPD. Not all studies however, find increased numbers of $CD8^+$ T-cells in COPD patients and in some studies the presence of these cells seems to be an effect of cigarette smoking rather than COPD per se, as reviewed below.

The perhaps most cited study regarding large airways was made by O'Shaughnessy et al., and examined lymphocyte populations in the subepithelial zone of bronchi in non-smokers, chronic bronchitis (CB) patients and COPD patients. In the O'Shaughnessy study, the number of $CD3^+$ and $CD8^+$ T-cells was significantly increased in COPD patients in comparison to non-smokers, while the number of $CD4^+$ cells did not differ between the groups, making the CD4/CD8 ratio lower in the patients than in non-smokers (38). Moreover, a significant negative association ($rho= -0.42$, $p<0.05$) was found between the number of $CD8^+$ T-cells and FEV_1 % of predicted in a joined group of COPD and CB patients. This correlation has been cited in many studies as evidence of $CD8^+$ T-cell involvement in lung function deterioration. Another study of large airways made by Lams et al. showed no differences in numbers of $CD3^+$ or $CD4^+$ cells between non-smokers, smokers and COPD patients, but the number of $CD8^+$ T-cells was increased in COPD patients in comparison to smokers (92). This, and a negative correlation between FEV_1 and numbers of $CD3^+$ ($r= -0.47$, $p= 0.029$) and $CD8^+$ ($r= -0.62$, $p= 0.005$) T-cells in smokers and COPD patients in the same study favours the hypothesis that T-cells are involved in disease development rather than just a phenomenon of cigarette smoking. In addition to the correlative data between T-cell numbers and decline in lung function, a positive correlation between

$CD8^+$ T-cells and number of pack years ($r= 0.61$, $p= 0.006$) was found in smokers and COPD patients, highlighting the difficulties in dissociating effects of cigarette smoking from COPD driven inflammation.

Although the previous studies provide evidence associating $CD8^+$ T-cells in the bronchial mucosa with COPD, contradictory studies have also been published. Di Stefano et al. compared COPD to CB patients in one study and COPD patients (with varying severity of the disease) to smokers in an other study and found no differences in numbers of $CD8^+$ cells between the study groups (93, 94). The T-cell occurrence in COPD may however vary with severity of the disease. Another study by Di Stefano et al. showed lower $CD3^+$ and $CD8^+$ cell counts in severe COPD patients than in mild COPD patients and smokers and a trend towards lower numbers of $CD8^+$ T-cells in mild COPD patients in comparison to smokers (86). The number of $CD3^+$ and $CD8^+$ T-cells was positively correlated with FEV_1 % of predicted.

Studies of peripheral airways have shown increased numbers of $CD8^+$ T-cells in COPD patients compared to smokers (95, 96). In one of the studies the number of $CD4^+$ T-cells was also increased (96). The pattern of increased numbers of $CD8^+$ T-cells continues deeper into the lungs, in the parenchyma (97, 98), as well as around bronchial arteries (97, 99, 98) and glands (100). In relation to emphysema, studies have shown increased numbers of $CD4^+$ T-cells in more severe emphysematic lesions and also high numbers of $CD8^+$ T-cells in emphysematous lungs (101-103). Results from different studies made on T-cell occurrence in lung tissue from COPD and CB patients, smokers and non-smokers are summarized in Table 1.

Table 1
Statistically significant differences in T-cell numbers in lung tissue from COPD, CB, smokers and non-smokers

Study	CD3	CD4	CD8	Groups studied	Inv. corr. with FEV_1
Large airways					
Saetta, 1993 (43)	CB > NS			10CB, 6 NS	
O'Shaughnessy, 1997 (38)	COPD > NS		COPD > NS	13 COPD, 11 CB, 5 NS	CD8
Lams, 2000 (92)			COPD > S	11 COPD, 8 S, 11 NS	CD3, CD8
Di Stefano, 1996 (94)	COPD > CB			14 COPD, 10 CB	CD3
Di Stefano, 1998 (93)				10 sCOPD, 8 mCOPD, 12 S	
Di Stefano, 2001 (86)	mCOPD > sCOPD		mCOPD > sCOPD	9 sCOPD, 9 mCOPD, 14 S	
Rutgers, 2000 (37)				18 eS-COPD, 11eS	
Ekberg-Jansson, 2000 (104)					CD8
Peripheral airways					
Saetta, 1998 (95)			COPD > S	9 COPD, 7 S	
Lams, 1998 (105)				22 S, 22NS	
Turato, 2002 (96)		COPD > S	COPD > S	9 COPD, 9 S	
Parenchyma					
Majo, 2001 (97)	eS > S and NS		eS > S and NS	10e S, 6 S, 6 NS	
Saetta, 1999 (98)			COPD > NS	10 COPD, 6 S, 8 NS	
Smooth muscle					
Barabaldo, 2004 (35)			COPD > NS	8 COPD, 10 S, 8 NS	
Bronchial arteries					
Peinando, 1999 (99)	COPD > NS		COPD > NS	20 COPD, 12 S, 7 NS	
Turato, 2002 (96)				9 COPD, 9 S	
Saetta, 1999 (98)			COPD > NS	10 COPD, 6 S, 8 NS	CD8
Glands					
Saetta, 1997 (100)			CB > S	9 CB, 9 S	

Severe COPD (sCOPD), moderate COPD (mCOPD), smokers (S), ex-smoker (eS), ex-smoker with COPD (eS-COPD), non-smoker (NS). Empty table cell means that no difference in cell numbers between the study groups was found.

T-cells in sputum and lavage from COPD patients

All of the above mentioned studies were performed using immunohistochemistry on bronchial biopsies or peripheral resected lung tissue. As such they reflect the cell populations within the tissue. To evaluate cells in the airway- and alveolar lumen, sampling techniques such as sputum induction and bronchoalveolar lavage (BAL) can be used. Sputum sampling from COPD patients has shown increased numbers of $CD8^+$ T-cells when compared to smokers and non-smokers in studies made by Tzanakis and Chrysofakis et al. (106, 107) while Leckie at al. did not find any differences in T-cell counts in a study comparing COPD patients and non-smokers (108). It is sometimes argued that sputum sampling reflects the luminal content of the more central airways while bronchoalveolar lavage reflects more distal parts of the lung. Therefore results from studies on BAL and sputum may vary, making comparisons between the two interesting (109).

Very few studies have been published on $CD4^+$ and $CD8^+$ T-cell counts in BAL from COPD patients. Two studies, looking at the percentage of lymphocytes and $CD3^+$ T-cells respectively, found no differences between COPD patients and non-smokers (110, 111), while a third study found lower lymphocyte percentages in COPD patients and smokers when compared to non-smokers (112). A study by Pons et al. comparing

COPD patients, smokers and non-smokers found no significant differences between the groups concerning $CD4^+$ and $CD8^+$ T-cell percentages in the T-cell ($CD3^+$) population (113). However, trends towards higher percentages of $CD8^+$ and lower percentage of $CD4^+$ T-cells were seen in COPD patients and smokers when compared to non-smokers. An increase in $CD8^+$ T-cells in BAL from smokers has previously been published in studies comparing smokers and non-smokers (114, 115, 104, 116) and recently an ATS conference abstract showed higher percentage of $CD8^+$ T-cells in COPD patients than in healthy controls (117). In addition, a correlation between percent $CD8^+$ T-cells and airway obstruction was found in a study on CB patients (118) although no differences in percentages of $CD4^+$ and $CD8^+$ T-cells were found between CB patients, smokers and non-smokers in that study. Results from different studies made on T-cell occurrence in airway lumen in COPD and CB patients, smokers and non-smokers are summarized in Table 2.

Table 2
Statistically significant differences in T-cell numbers in airway lumen in COPD, CB, smokers and non-smokers

Study	CD3	CD4	CD8	Groups studied	Inv. corr. with FEV_1
Sputum					
Tzanakis, 2004 (107)			COPD > NS	36 COPD, 25 S, 10NS	
Leckie, 2003 (108)				7 COPD, 8 A, 6 NS	
BAL					
Costabel, 1992 (36)				11 CB, 15 S, 15 NS	CD8
Hoser, 1999 (115)	S > NS		S > NS	9 S, 12 NS	
Ekberg-Jansson, 2000 (104)			S > NS	30 S, 18 NS	
Pons, 2005 (113)				20 COPD, 20 S, 10 NS	

Smokers (S), non-smoker (NS), asthma (A). Empty table cell means that no difference in cell numbers between the study groups was found.

All of the above studies concern the evaluation of αβ T-cells. Concerning γδ T-cells in COPD, a study made by Pons et al. showed increased numbers of γδ T-cells in BAL and blood from smokers when compared to both COPD patients and non-smokers and hypothesized that a blunted γδ-response in smokers could be involved in declining lung function (113).

To summarize, the balance of published studies indicates an increase of $CD8^+$ T-cells in the lungs of COPD patients. Since this increase has also been correlated with a decline in lung function, $CD8^+$ T-cells are likely to be involved in the progression of the disease rather than just being there as a response to cigarette smoke.

Intraepithelial T-cells in lungs of COPD patients

Since intraepithelial T-cells are localised close to the airway lumen they may be one of the earliest cell types to be exposed to components from cigarette smoke. As such they are interesting to study in regard to a smoking related disease like COPD. In addition, intraepithelial T-cells are involved in other inflammatory diseases like Crohn's disease and ulcerative colitis (119). Studies have shown that most of the T-cells

located in the airway epithelium, in both healthy subjects and patients, are $CD8^+$ (82, 120, 96) in contrast to T-cells in the subepithelial zone, which constitute a more equal mix of $CD4^+$, and $CD8^+$ T-cells (95, 96). Moreover, increased numbers of intraepithelial $CD8^+$ T-cell have been found in COPD patients when compared to non-smokers (121). Cells from blood, sputum and BAL can be stained with antibodies against CD103 to distinguish their intraepithelial T-cell phenotype. A study on sputum cells from COPD patients and healthy non-smokers showed that the percentage of cells expressing CD103 was higher in $CD8^+$ T-cells (app. 70 %) than in $CD4^+$ T-cells (app. 30 %) in both COPD patients and non-smokers, and thus reflecting the proportions of $CD4^+$ and $CD8^+$ T-cells in the epithelium. In COPD patients the percentage of $CD8^+$ T-cells expressing CD103 was lower than in non-smokers (108). Since no studies, apart from Study I presented in this thesis, have been published on CD103 expression on BAL cells from COPD patients, no comparisons between sputum and BAL findings in COPD patients have been made. Studies on healthy subjects though, have showed that 70-80% of $CD8^+$ T-cells and 10-30 % of $CD4^+$ T-cells in BAL expressed CD103 (122-124).

T-cell occurrence in blood of COPD patients

The majority of studies on T-cell occurrence in COPD have been made on lung tissue specimens. However, since COPD has systemic components (125) and T-cell recruitment involves trafficking from the blood to the tissues, studies on T-cell populations in peripheral blood may add valuable information about their role in COPD pathogenesis. The increased occurrence of $CD8^+$ T-cells seen in lungs of COPD patients is not as apparent in the blood. A higher percentage of blood $CD8^+$ T-cells and a lower CD4/CD8 ratio were found in a study by Hodge et al. when comparing COPD patients with healthy non-smokers (126). This is however contradictory to other studies that have failed to show any differences in percentages of $CD4^+$ and $CD8^+$ T-cell populations in COPD patients when compared to smokers and non-smokers (127, 108, 128). As discussed above, cigarette smoking might lower the CD4/CD8 ratio in the lungs; in the blood however; cigarette smoking instead increases the number of $CD4^+$ T-cells (129, 130, 118, 131-133). This opens up for the possibility that if increased numbers of $CD8^+$ T-cells are present in the blood of COPD patients, the difference in percentages of cells or the CD4/CD8 ratio is blunted in patients that are still smoking. Such a hypothesis is supported by a study in which the percentage of $CD8^+$ T-cells was increased in non-smoking COPD patients when compared to healthy non-smokers while no difference between COPD patients and healthy non-smokers was seen when smoking COPD patients were added to the analysis (134).

T-cell activity in COPD

The recruitment and presence of T-cells per se in the lungs of COPD patients is not enough to explain the T-cell involvement in COPD. Their modes of action also have to be elucidated. A number of theories have arisen regarding the role of T-cells in COPD. For example, cytotoxic T-cells could drive structural cells into apoptosis resulting in destruction of lung tissue. Supporting this hypothesis is the finding of increased T-cell cytotoxic capacity and perforin expression in sputum samples from COPD patients (106). In concordance with this, an increase in apoptotic epithelial cells have been found in the airways of COPD patients (135). Another hypothesis is that T-cells contribute to the recruitment and overload of inflammatory cells seen in COPD, by producing proinflammatory cytokines involved in activation of other T-cells and inflammatory cells. Both the above hypothesis are affected by the type of T-cells present and active in COPD and by the different cytokines produced by these cells. The production of INF-γ, for example, not only drives T-cell responses into cell based cytotoxic killing of structural cells, but also acts as a strong activator of macrophages (136), which have the potential to drive the inflammation forward.

As described earlier, T-cells can crudely be divided into Th1/Tc1 or Th2/Tc2 categories depending on their secretion of cytokines. A few studies on COPD patients have been made to elucidate which categories of T-cells that dominate in COPD. The results are in favour of the INF-γ producing cells, with Tc1 cells being increased in sputum (107) and Th1 cells in blood (137). A study made on peripheral lung tissue showed increased numbers of CXCR3 (a chemokine receptor involved in the recruitment of Th1/Tc1 cells responsive to INF-γ inducible protein 10, CXCL-10) positive T-cells in COPD patients (87), which points towards a Th1/Tc1 category inflammation with production of INF-γ present. Additional evidence towards Th1/Tc1 involvement was found in a study on bronchial biopsies and BAL from COPD patients, smokers and non-smokers, in which the number of cells expressing phosphorylated STAT-4 and INF-γ were increased in COPD patients compared to smokers and non-smokers respectively (138). The picture of INF-γ producing T-cell phenotypes in COPD can however be questioned by a study of BAL and blood $CD8^+$ T-cells from chronic bronchitis patients with airway obstruction, asymptomatic smokers and non-smokers. In that study IL-4 was the dominant cytokine produced in the patients and INF-γ was produced only in smokers and non-smokers (137). In the latter study, emphysematic patients were excluded, which could explain the differences seen in the cytokine pattern. More comprehensive studies are needed to give a more reliable answer as to whether Th1/Tc1 or Th2/Tc2 categories of T-cells dominates the inflammation in COPD, and perhaps patients ought to be divided into groups of emphysema and chronic bronchitis in order to understand the cause and effect of T-cell cytokine production in COPD. The classic Th1/Tc1 and Th2/Tc2 cytokines are not the only cytokines produced by T-cells likely to be involved in COPD pathogenesis. The neutrophil chemoattractant IL-17 for example is produced by T-

cells and could be responsible for T-cell driven neutrophil recruitment in the lungs of COPD patients (139).

Besides driving inflammation and tissue destruction forward, T-cells could influence the course of the disease by being silent rather than over productive and thereby impairing the immune defence of the lung. Nicotine has in several studies been shown to suppress T-cell receptor induced activity (140-142) and bacterial clearance is delayed in mice exposed to cigarette smoke as shown by Drannik et al. (143). Airway infections are common in COPD patients and often contribute to the onset of exacerbations, which has been shown to negatively affect the course of the disease (144). If T-cell activity and responsiveness are suppressed in smokers with COPD this could affect the defence against airway infections and occurrence of exacerbations and in the longer run influence the outcome of the disease.

A few studies have determined the degree of T-cell activation in COPD patients. Studies on blood T-cells have shown a mixed T-cell phenotype with both $CD45RA^+$ naïve cells and $CD45R0^+$ memory cells (128) with low expression of CD25 and CD69 and with no significant differences between patients and controls. Studies have also been made on T-cells in BAL from smokers (115, 104), sputum from COPD patients (108) and in tissue from patients with chronic bronchitis and COPD (38, 99, 43). In these studies T-cells resident in the airway lumen and tissue were predominantly (over 90%) memory T-cells expressing CD45R0, while only 10-20% of the cells expressed the IL-2 receptor CD25. CD69 on the other hand was present on 80% of BAL T-cells and 60 % of sputum T-cells. Few differences in expression of activation markers were found between smokers and non-smokers and between COPD patients and control groups. However, CD69 expression on $CD8^+$ T-cells in sputum from COPD patients was significantly lower than in non-smokers (108) and CD25 expression on T-cells in the bronchial mucosa of chronic bronchitis patients was higher than in healthy non-smokers although the expression in the patients was only around eight percent (43).

Dual roles of T-cells in COPD?

As discussed above T-cells may have several roles in the pathogenesis of COPD. If they are over productive they could drive the inflammatory process forward and their cytotoxic proteins could add to the destruction of lung tissue. However, the responsiveness of the same cells is needed in the defence against infections, since infections also contribute to the progression of the disease. In order to understand the duality of the T-cell role, further studies on distribution of T-cell subsets in different compartments of the lung, T-cell activation in relation to COPD progression, and T-cells response to exogenous and endogenous factors involved in COPD are needed.

AIMS OF THIS THESIS

The aim of this thesis was to study the involvement of T-cells in COPD pathogenesis, through examination of patient material and *in vitro* cell cultures. Four studies are included in this thesis with the following specific aims:

I To study $CD8^+$ T-cells in BAL from COPD patients and healthy subjects with focus on their expression of αEβ7 and activation state and to study the differentiation of peripheral T-cells into an αEβ7 expressing phenotype *in vitro*

II To study potential effects of cigarette smoke on T-cell defence mechanisms using an *in vitro* system in which T-cells are exposed to cigarette smoke extract and analysed with regard to T-cell activation, proliferation and cytotoxic potential

III To study the expression of T-cell activation markers in peripheral blood $CD4^+$ and $CD8^+$ T-cells in relation to lung function in COPD patients, smokers and never-smokers

IV To study lymphocyte ($CD3^+$, $CD4^+$, $CD8^+$ T-cells and $CD20^+$ B-cells) distribution in intra- and subepithelial compartments of small airways and in lymphoid aggregates in peripheral lung tissue from COPD patients, smokers and never smokers

METHODS

Collection of bronchoalveolar lavage, BAL, fluid (Study I)

Bronchoalveolar lavage, BAL, samples are often used to look for alterations in cell populations or microorganism flora in different lung diseases. During bronchoscopy, saline is instilled in to the lung in portions of approximately 50 ml, which are immediately withdrawn using suction and collected in a flask. When the total amount of saline has been instilled and withdrawn, the saline, now containing both proteins, cells and in case of infection also microorganisms, is referred to as BAL fluid and can be further analysed in the lab with regard to protein concentrations and cell counts. In Study I bronchoscopy with BAL was performed on five patients with mild to moderate COPD, according to GOLD guidelines (1), and five age-matched non-smoking healthy subjects recruited at the Lund University Hospital. All patients were in a stable state of the disease and all of the subjects were without signs of airway infections at the time of the bronchoscopy. They had all given written informed consent to participate in the study, which was approved by Lund University ethics committee. Under local anaesthesia saline was instilled into the middle lobe in 3 x 50 ml portions to a maximum of 150 ml and each portion was gently aspirated and collected in a polypropylene tube. The BAL was immediately placed on ice and kept cold while transported to the laboratory. The BAL was then centrifuged to separate BAL fluid from BAL cells. Total cell count of BAL cells was determined by counting the cells in Bürker chamber.

Preparation of PBMCs from buffy coats (Study I, II)

Buffy coats from healthy blood donors were purchased from the blood centre at Lund University Hospital. The buffy coat, containing mainly white blood cells but also some red blood cells, plasma and thrombocytes, was diluted 1:1 with phosphate buffered saline. The diluted buffy coat was then carefully layered on top of the density gradient Ficoll Paque Plus and tubes were centrifuged 400g for 40 minutes. The top layer, plasma, was aspirated and the second layer containing the peripheral blood mononuclear cells (PBMC) was carefully collected using a Pasteur pipette (Figure 4A). The cells were washed in phosphate buffered saline, PBS, and stored on ice until further analysis.

Magnetic cell sorting, MACS (Study I, II)

PBMC were stained with antibodies coupled to magnetic micro beads. Antigen specificities were CD3 (T-cells), CD8 (cytotoxic T-cells) and CD14 (monocytes). The different cell types were then extracted from the PBMCs using an AutoMACS instrument where cells stained with the antibody were trapped in a magnetic field and

separated from the rest of the cells through positive selection. Trapped cells were released from the magnetic field, washed and used in cell culture (Figure 4B).

Cell culturing (Study I, II)

T-cell mono-cultures

T-cells were cultured in cell culture medium (RPMI 1640 with glutamax-1 and HEPES) supplemented with 10% foetal bovine serum and antibiotics (penicillin-streptomycin). They were kept in tissue culture treated polystyrene plates at 37°C for up to 8 days. The cells were stimulated in various ways. T-cell receptor stimulation, provided *in vivo* by T-cell binding to antigen presenting cells, was accomplished using an anti-CD3 antibody bound to the plastic well and as a second signal a soluble anti-CD28 antibody was used. Interleukin-2 (IL-2) was added to cells cultured more than three days. In Study I cells were stimulated with transforming growth factor-beta, TGF-β, and in additional experiments also cultured on plates coated with ICAM-1. In Study II cells were stimulated with cigarette smoke extract (CSE).

T-cell and monocyte co-cultures

Culturing of T-cells in the presence of antigen presenting cells like monocytes leads to activation of T-cells as the antigen presenting cell provides the T-cells with costimulatory signals, for instance through binding of CD80/CD86 to CD28 and ICAM to LFA-1. In Study I and II co-cultures of autologous $CD3^+$ or $CD8^+$ T-cells and monocytes were cultured under the same conditions and with the same stimuli as described for the T-cell mono-cultures. The two cell types were cultured together from day zero to day three. T-cells were then removed from the plate-bound monocytes and culture of the T-cells continued in new plates without monocytes. In Study I a blocking anti-LFA-1 antibody was added to some of the co-cultures.

Co-cultures in Transwell inserts

In co-cultures where cell-to-cell contact between T-cells and monocytes was to be avoided, culture plates with transwell inserts were used. This is a system often used in co-culturing of cells when two cell types are supposed to share soluble mediators without being able of cell-to-cell contact. T-cells were cultured in the lower compartment and monocytes were kept in the inserts, with a pore size of 0.4 μm (Figure 4D).

Preparation of cigarette smoke extract, CSE (Study II)

Exposing cells cultured in suspension to components in cigarette smoke can be accomplished by adding cigarette smoke extract, CSE, to the cells. In Study II CSE was prepared similar to what has previously been published (84). Smoke from two Kentucky research cigarettes, 1R4F containing 11 mg tar and 0.8 mg nicotine, were bubbled through 50 ml of cell culture medium at a speed of 5 minutes per cigarette using a vacuum pump (Figure 4C). The cigarette smoke extract was then filtered through a 0.2 μm sterile filter and added to the cell cultures within 20 minutes of preparation. CSE prepared in this way could correspond to cigarette smoke dissolved in the epithelium lining fluid *in vivo*.

Flowcytometry

Cells from BAL, blood and *in vitro* cultures were subjected to flowcytometric analysis. Using this technique cells can be identified based on size, granularity and expression of proteins targeted by fluorescent labelled antibodies. BAL cells (that had been strained through a 100 μm nylon mesh) as well as *in vitro* cultured cells were suspended in PBS containing 1% bovine serum albumin and then incubated with antibodies for 30 min on ice. Blood cells were labelled by adding antibodies directly to whole blood and incubating 15 min in room temperature. Specific antibodies and isotype controls, coupled to three different fluorochromes, FITC, phycoerythrine (PE) and phycoerythrine-cychrome5 (PE-Cy5) were used for labelling. After incubation cells were fixed in commercial fixing solutions based on 4% paraformaldehyde and red blood cells were lysed using a commercial lysing reagent. After washing, cells were analysed on a FACS Calibur. In samples from blood and *in vitro* cultured cells, data from a total of 10000 cells were collected and in samples from BAL cells data was collected until data from 10000 cells in a lymphocyte gate had been collected. Data analysis was performed using the software CellQuestPro and based on a live gate set up for each sample.

Bromodeoxyuridine, BrdU, incorporation (Study I, II)

The replication of DNA in dividing cells can be used to measure proliferation of cells (145). A modified nucleotide, bromodeoxyuridine (BrdU) was added to the cell cultures to be incorporated in the newly replicated DNA during mitosis. The degree of incorporated BrdU was then measured by flowcytometry using an antibody against BrdU. In Study I and II BrdU was added to the *in vitro* cultured cells two hours prior to harvest.

Annexin-V staining

When cells are driven into programmed cell death, apoptosis, the cell membrane lipid bilayer flip-flops, resulting in phosphatidylserine being exposed on the cell surface. In Study I and II apoptosis of *in vitro* cultured cells was measured by binding of FITC-labelled annexin-V to inverted phosphatidylserine on the cellular membrane surface. Annexin-V positive apoptotic cells were then detected by flowcytometry, as previously described (146), and excluded from the live gate and further analysis.

Luminex – multiplex cytokine analysis (Study I)

Cytokine production in T-cell cultures in Study I was measured using a multiplex assay based on the Luminex system. In this assay several cytokines can be analysed at the same time in a 96 well format. Cell supernatants were incubated in a filter-bottom 96 well plate together with antibodies specific for different cytokines and coupled to specific beads, each with a unique mix of red and infrared fluorophores. During incubation, antibodies on the specific beads bound to cytokines present in the cell culture supernatant. A biotin-conjugated second antibody mix with specificities to the same cytokines, but with epitopes different from the ones of the antibodies coupled to the beads, was added to the wells. After incubation and washing the biotin-conjugated antibodies were detected using streptavidin-phycoerythrin. The plate was read in a Luminex plate reader, where a red laser excited the fluorochromes in the beads and the specificity of the bead was determined. At the same time a green laser excited the phycoerythrin coupled antibodies to determine the amount of cytokine associated with the specific bead (Figure 4E).

Collection of human blood and lung biopsies (Study III and IV)

Blood and lung biopsies were collected from patients visiting Lund University Hospital for surgery of suspected lung tumour. Blood from seventeen patients with mild to moderate COPD, eight smokers and six never-smokers was collected in EDTA tubes at a hospital visit prior to surgery. The blood was kept at room temperature until cell count analysis in an automated differential cell counter (Sysmex K-4500) and flowcytometry was performed as described above. Lung biopsies were taken from fifteen of the COPD patients, five of the smokers and the six never-smokers at the time of the lung resection. Biopsies were taken distant to the tumour, fixed in 4% paraformaldehyde and embedded in paraffin. All patients were free of bronchial hyper responsiveness and had been free of signs of airway infections for a month prior to participating in the study. None of the patients were treated with oral or inhaled corticosteroids. Age did not differ between the patient groups and neither did the number of pack years between the COPD patients and the smokers. Due to the definition of COPD, the COPD patients had significantly lower lung function than

did smokers and never-smokers. All of the patients gave written and informed consent to participate in the study, which was approved by the Ethics committee in Lund.

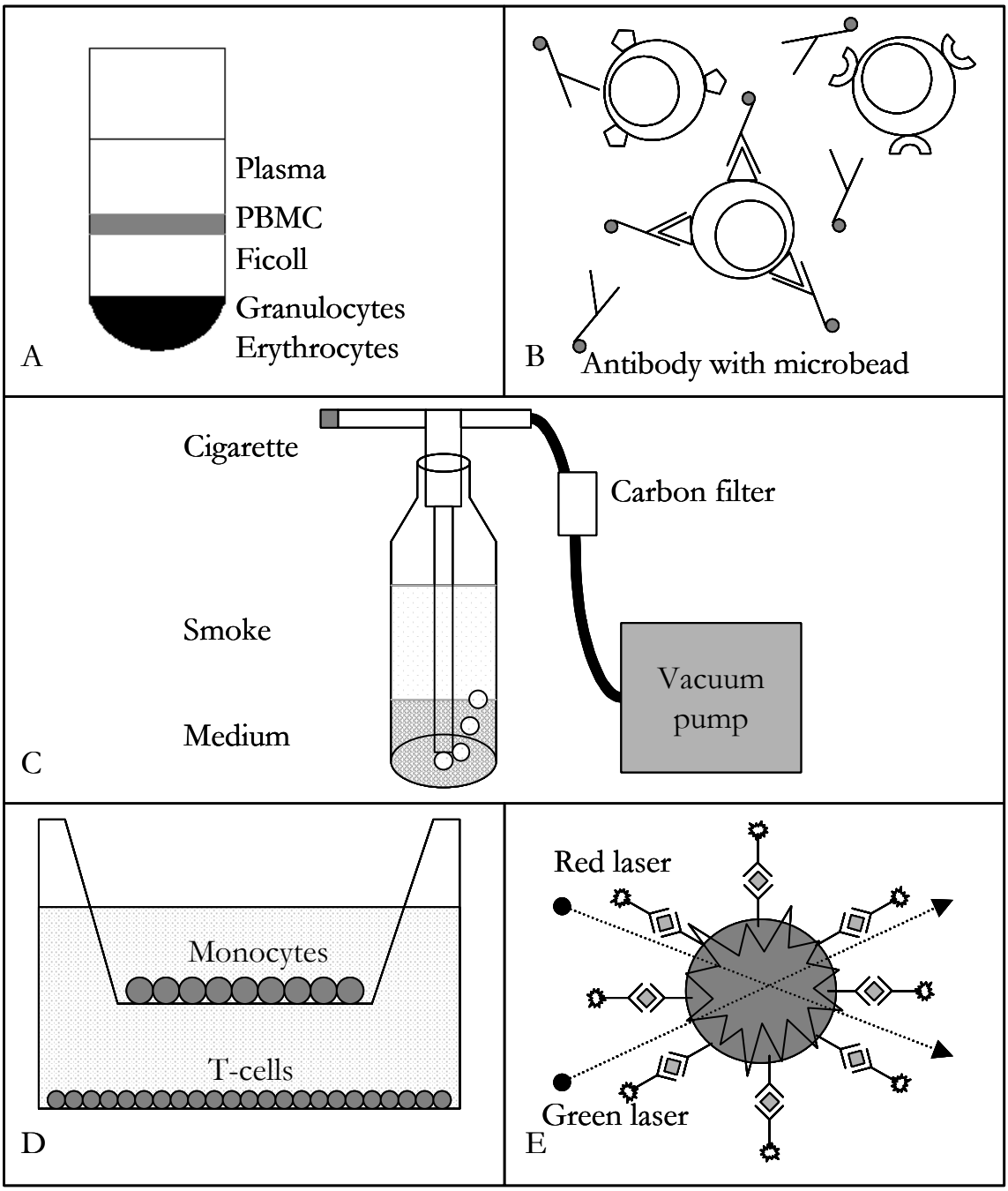

Figure 4 PBMC separation was performed using gradient centrifugation on Ficoll Paque (A). Monocytes and T-cells were separated from PBMC using magnetic cell sorting (B). Cigarette smoke was bubbled through cell culture medium to yield cigarette smoke extract (C). Transwell systems enable cell communication via soluble mediators but prevent cell-to-cell interactions between cells in the upper and lower compartment (D). The Luminex multiple protein analysis detects light emitted from an array of fluorescent beads and antibodies excited by a green and a read laser (E).

Immunohistochemistry (Study IV)

Immunohistochemistry was performed on human lung biopsies to evaluate the occurrence of lymphocytes in different structures in the tissue. Paraffin embedded tissue was cut into 3 μm sections and stained with antibodies against lymphocyte markers after appropriate antigen retrieval methods (microwaving in EDTA or citrate buffer) had been used to expose the antigens. Secondary antibodies coupled to horseradish peroxidase (HRP) recognized primary antibodies directed against the different lymphocyte markers. Endogenous peroxidase had been blocked with hydrogen peroxide before staining was performed, and when diaminobenzidin, DAB, was added to the tissue brown staining precipitated near the antibody labelled cells (Figure 5). The slides were counter stained with haematoxylin (HTX) and additional slides were stained with HTX-eosin for evaluation of morphological structures within the tissue.

Histological examinations (Study IV)

The tissue slides stained with HTX-eosin were examined with regard to bronchioles, blood vessels and lymphoid aggregates. The structures found in the HTX-eosin slides were then identified in the antibody stained slides and cells belonging to the lymphocyte subgroups were counted. Intraepithelial lymphocytes, situated within the epithelium, subepithelial lymphocytes, situated below the epithelial basement membrane, and cells within the lymphoid aggregates were counted separately (Figure 5). An Olympus microscope was used during the examinations and bronchiole perimeters, subepithelial areas and areas of lymphoid aggregates were measured using the image analysis software Image J 1.34s. from National Institute of Health, USA.

Statistics

In all the studies non-parametric tests were used, as the assumption of normal distribution could not be made. Kruskal-Wallis test was used to discover possible differences between more than two study groups; Wilcoxon-Mann Whitney to detect differences between two groups of data and Wilcoxon-Signed-Ranks was used to test differences between paired data. Spearman rank correlation was used to calculate correlations between two sets of data. Differences were considered statistically significant when p-values were equal to or lower than 0.05.

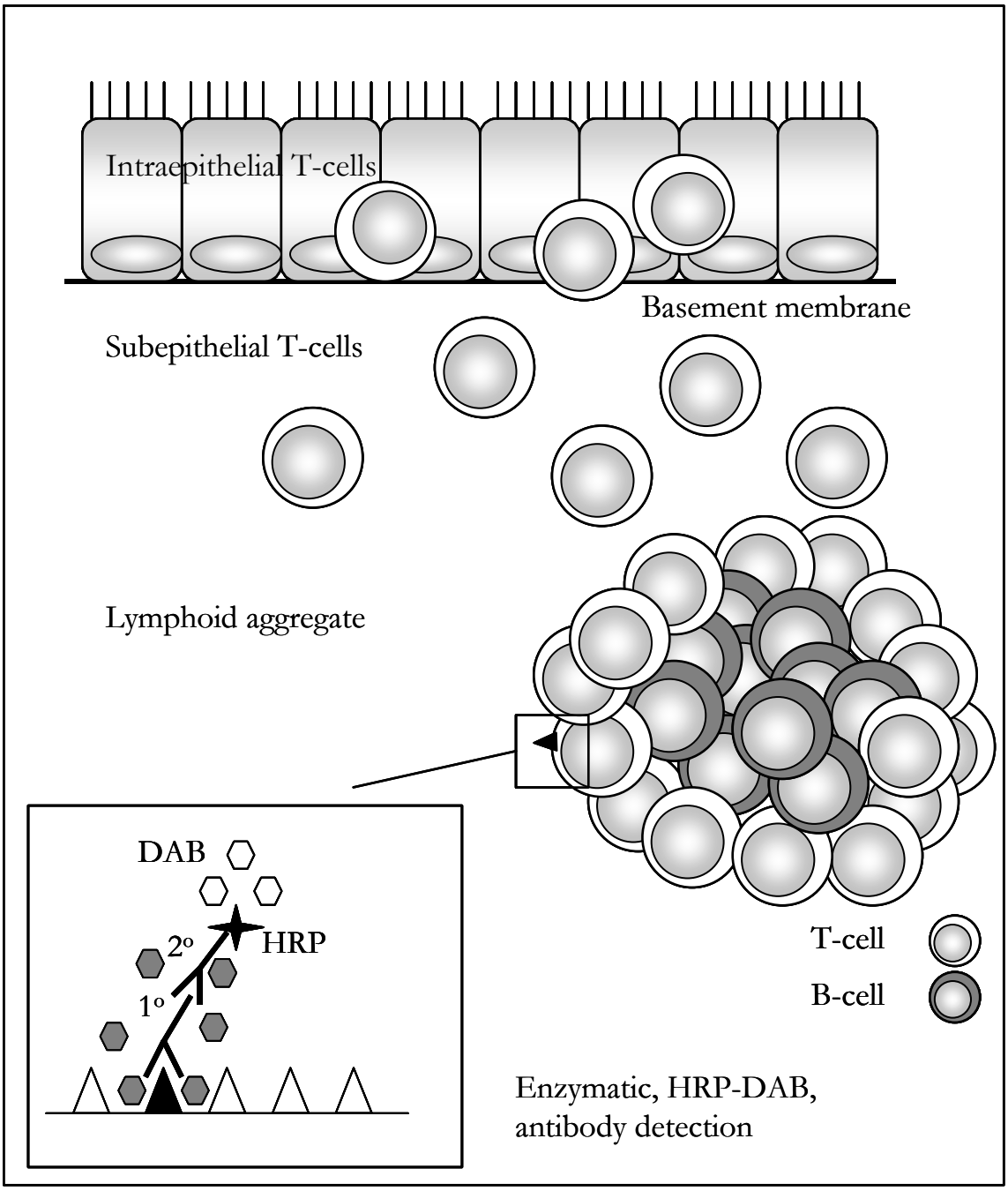

Figure 5 Immunohistological staining was performed using primary antibodies directed against lymphocyte markers and detected with horseradish peroxidase (HRP) coupled secondary antibodies reacting with diaminobenzidine (DAB) and forming brown precipitation. Stained intraepithelial and subepithelial cells were counted in addition to cells in lymphoid aggregates.

RESULTS AND DISCUSSION

T-cell subpopulations in BAL (Study I)

A few studies have prvided insights to the cellular picture in BAL from patients with chronic bronchitis (36) and COPD (113) as well as in smokers (115, 104). However, none of those studies looked at αEβ7 expression on BAL T-cells from COPD patients. Since the expression of αEβ7 have been shown to be varying in some other respiratory diseases (124) we wanted to study the expression of this integrin in COPD using flowcytometry. In addition we looked at $CD4^+$ and $CD8^+$ T-cell populations, as well as expression of activation markers on the T-cells in BAL from five COPD patients and five healthy non-smokers.

There was a trend towards lower BAL fluid recovery but higher total cell counts in BAL from COPD patients compared to healthy subjects. The flowcytometry analysis of the cell populations showed more auto fluorescence and larger populations of granulocytes and macrophages and a smaller lymphocyte population in the COPD BAL, making the interpretation of T-cell data more difficult (Figure 6). The percentages of $CD4^+$ and $CD8^+$ T-cell populations did not differ between the two groups. A trend towards a lower CD4/CD8 ratio was seen but was not statistically significant. Both COPD patients and healthy subjects had a substantial part of their $CD8^+$ T-cells expressing CD103 and almost all expressed CD45R0, which is in line with previous publications on T-cells from BAL fluid in healthy subjects (122). Few or none of the cells expressed CD45RA or CD25 and the expression of these markers did not differ between the groups. Table 3 shows some data additional to what was published in Paper I.

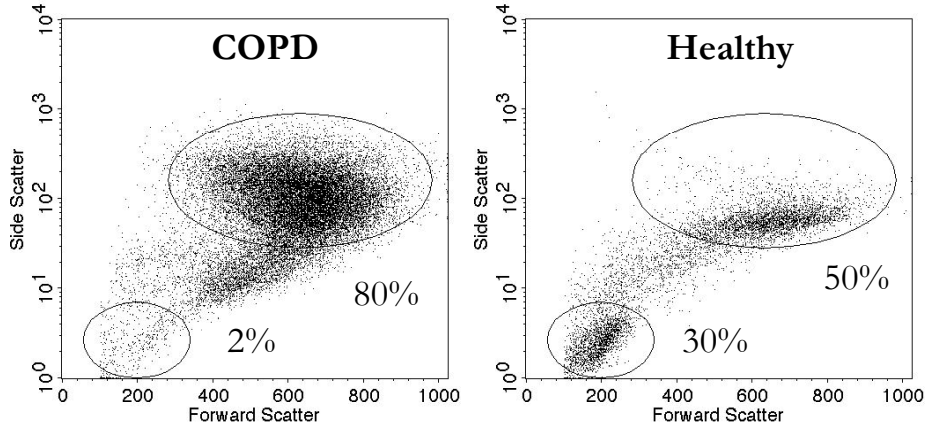

Figure 6 Flowcytometry analysis showing size (forward scatter, x-axis) and granularity (side scatter, y-axis) of BAL cells from COPD patients to the left and healthy subjects to the right.

The lack of differences in $CD4^+$ and $CD8^+$ T-cell populations between COPD patients and healthy subjects in this study is in line with what has previously been published on T-cells in COPD BAL (113), but is contradictory to studies on BAL T-cells from smokers where a decreased $CD4^+/CD8^+$ ratio in smokers has been found when compared to non-smokers (115, 104). The phenotype of the T-cells in our study, with high expression of CD45R0 and low expression of CD45RA and CD25, indicate that these cells are memory T-cells that are not currently activated. Since the cells also express αEβ7 they have most probably been situated within the epithelium as intraepithelial lymphocytes.

Table 3
T-cell populations in BAL

	COPD median	range	Healthy median	range
$CD4^+$ (% of $CD3^+$)	53	22-71	62	54-81
$CD8^+$ (% of $CD3^+$)	38	24-55	31	13-41
$CD4^+/CD8^+$ ratio	1.4	0.5-3	2	1.3-5
$CD103^+$ (% of $CD8^+$)	70	64-89	59	37-85
$CD25^+$ (% of $CD8^+$)	2	0-11	2	1-2
$CD45R0^+$ (% of $CD8^+$)	88	80-98	93	91-98
$CD45RA^+$ (% of $CD8^+$)	4	2-13	2	0-4

Peripheral blood T-cells are to a large extent negative for αEβ7, while many of the T-cells within the epithelium and in BAL, as shown in the present study, are positive for this integrin. This implies that circulating cells develop their intraepithelial phenotype on their way to the tissue epithelium. We were interested in examining the mechanisms involved in differentiation of T-cells into an αEβ7 expressing phenotype and did so using an *in vitro* based system.

T-cell expression of αEβ7 *in vitro* (Study I)

Based on a study by Rihs et al., where T-cells in PBMC cultures stimulated with TGF-β started to express αEβ7 (124), we cultured pure peripheral T-cells on anti-CD3 coated plates in the presence of anti-CD28, IL-2 and TGF-β. The expression of αEβ7 increased in response to TGF-β, but was lower than the expression seen in the original study by Rihs et al. Co-cultures of T-cells and different fractions of the PBMC population were then set up to reveal any costimulatory factors in the PBMC population. Monocytes appeared to be the cell type responsible for the costimulatory effect (Figure 7). To examine whether the costimulatory effect was due to soluble factors or cell-to-cell interactions, T-cells and monocytes were co-cultured in a transwell system, which allowed communication via soluble mediators without cell-to-

cell contact. In such cultures the monocytes exhibited no costimulatory effect, and the interaction between the T-cells and monocytes was concluded to be due to binding between cell bound molecules. The common signalling pair ICAM-1 and LFA-1 proved to be involved in this interaction. Blocking of LFA-1 markedly reduced the αEβ7 expression while culturing of T-cells on ICAM-1 coated plates, in the absence of monocytes, increased the αEβ7 expression on the T-cells to similar levels as seen in co-cultures.

In addition to αEβ7 expression, status of general activation and proliferation was measured, by expression of CD25 and BrdU incorporation respectively. In co-cultures of T-cells and monocytes the kinetics of activation was faster than in mono-cultures containing only T-cells. At day eight in culture, T-cells from both co-cultures and mono-cultures had the same level of CD25 expression while T-cells in mono-cultures were lower in αEβ7 expression. This would suggest that expression of αEβ7 was not directly dependent on the degree of activation. The kinetics of proliferation was also faster in T-cells grown in the presence of monocytes, but from day six in culture cells in both co-cultures and mono-cultures proliferated equally well and TGF-β did not have any over all effect on the proliferation in any of the cultures.

T-cell derived cytokine secretion in response to TGF-β was measured in monocyte stimulated T-cells. The two cell types were grown together, in the presence or absence of TGF-β, until day three when T-cells were harvested and the plate bound monocytes discarded. T-cells were grown, in the presence or absence of TGF-β, for an additional five days and thereafter multiplex cytokine analysis was performed on the supernatants. Under these conditions the secretion of IL-10, TNF-α, INF-γ and GM-CSF was decreased while IL-8 secretion was sustained.

Our *in vitro* study suggests that presence of TGF-β and interaction between T-cells and cells expressing ligands to LFA-1 are two important factors in the differentiation of T-cells into αEβ7 expressing cells. High numbers of macrophages and elevated levels of TGF-β have been found in the lungs of COPD patients (42, 47). This would supply the recruited circulating T-cells with the factors needed for up-regulation of αEβ7. Another source of both ICAM-1 and TGF-β could be inflamed epithelium, also present in the lungs of after cigarette smoke exposure (147, 148). The sustained IL-8 secretion is interesting since IL-8 is known as a strong chemoattractant for neutrophils, an important cell type in COPD.

Our BAL study showed that T-cells are present in the airway lumen and that they are also likely to be present as intraepithelial cells in the lungs of COPD patients, two sites that both are exposed to air pollutants. We next wanted to study T-cell function in response to one such pollutant, which is also the most common inducer of COPD, cigarette smoke.

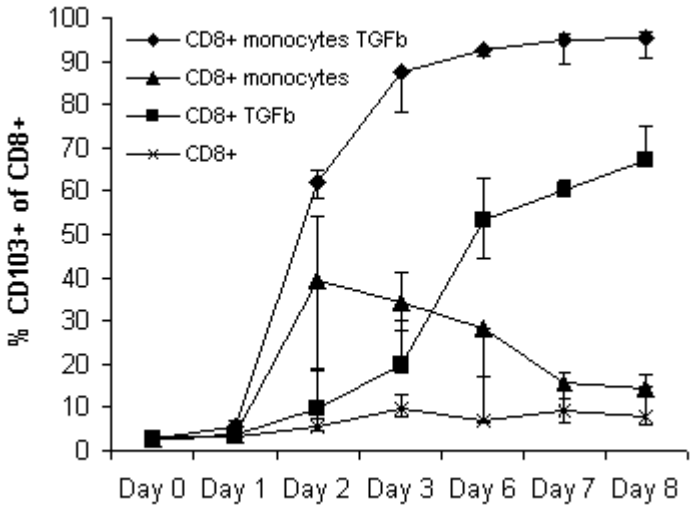

Figure 7 The graph shows the effect of TGF-β stimulation on αEβ7 expression of $CD8^+$ T-cells grown in the presence or absence of monocytes.

T-cell responses to cigarette smoke extract (Study II)

Studies have shown increased numbers of T-cells in the lungs of COPD patients and smokers (90). Despite this potential of boosting T-cell defence, many COPD patients suffer from exacerbations triggered by infections (7, 9, 10) and smokers often show colonization with bacteria in areas of the lung that are normally sterile (149, 150, 29, 151). We hypothesized that this may reflect impaired respiratory defence mechanisms due to cigarette smoking and decided to examine effects of cigarette smoke on T-cell and epithelial cell defence mechanisms. Degree of activation, proliferation and production of cytotoxic granule proteins (granzyme-b and TIA-1) was studied in T-cell cultures. In air liquid interface cultures of bronchial epithelial cells the integrity of the epithelium and production of IL-8 and MUC5AC was studied. The epithelial cell studies will not be further discussed here, as epithelial function is not the primary focus of this thesis and readers are instead referred to Paper II.

T-cells were exposed to cigarette smoke extract, CSE, at concentrations of 0-1%. Both mono-cultures of $CD3^+$ T-cells as well as co-cultures of $CD8^+$ T-cells and monocytes were evaluated. In addition to what is published in Paper II, co-cultures stimulated with TGF-β, to gain T-cells expressing αEβ7, were also included. The effect of CSE was similar in all cultures. In a dose dependent manner, CSE reduced activation (CD25 expression), proliferation (BrdU incorporation) and expression of the cytotoxic granule proteins TIA-1 and granzyme-b, while the expression of αEβ7 (CD103) was unaffected (Figure 8).

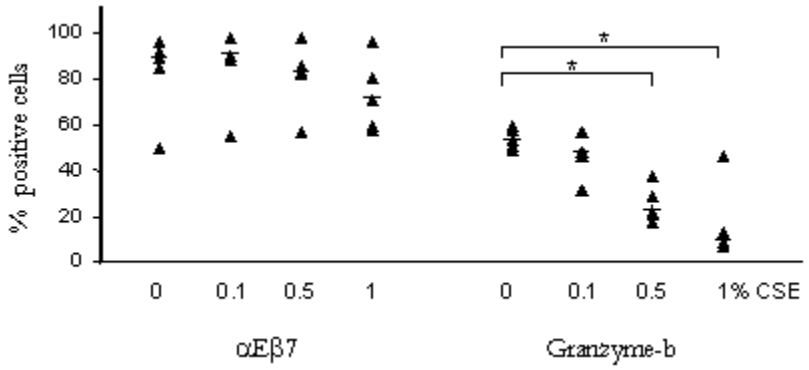

Figure 8 CSE did not alter the expression of αEβ7 (CD103) on $CD8^+$ T-cells grown in the presence of monocytes and TGF-β while in the same cell population granzyme-b was significantly reduced.

These results indicate that T-cells exposed to components from cigarette smoke have an impaired ability to function as effector cells, although they are still able to express αEβ7 allowing them to reside within the epithelium *in vivo*. The hypothesis of T-cell impairment by components of cigarette smoke has been confirmed in studies on tar, nicotine and tobacco smoke, showing reduced T-cell proliferation and activation in response to these components (152-154, 140). Further more, studies on BAL T-cells from smokers have showed reduced proliferation and cytokine secretion, in response to mitogens, when compared to non-smokers (155, 156).

Since COPD exacerbations, which have been shown to influence the long-term fall in lung function (144, 157) and to increase mortality in these patients(5), often are triggered by viral or bacterial infections, a well functioning defence against these infections is of great importance. Current smokers have shown to be more susceptible to lower respiratory infections than sustained quitters, with decline in lung function being pronounced in current smokers after respiratory illness (158). This could reflect an impaired immune response due to cigarette smoking. As intraepithelial T-cells most probably constitutes parts of the first line of defence, the exposure of these cells to cigarette smoke could enhance the development of COPD.

In addition to the lung, other organs may suffer secondarily to cigarette smoking. Immunosuppressive substances, like nicotine, are taken up in the lung and delivered to the rest of the body through the blood. Therefore, we next sought to explore whether cigarette smoking affects the degree of T-cell activation and COPD pathogenesis at a systemic level.

T-cell subpopulations in blood (Study III)

COPD inflammation has mainly been studied locally in the lungs. However, systemic components of the disease are beginning to gain attention (159-161) and substances in cigarette smoke can affect parts of the body outside of the lungs. While

studies on T-cell populations in COPD lung tissue have shown increased numbers of $CD8^+$ T-cells, studies made on lymphocyte populations in blood from smokers and patients with chronic bronchitis and COPD have shown less CD4/CD8 alterations (127, 108). However, other T-cell alterations, such as degree of T-cell activation, might be present systemically and could influence the pathogenesis of the disease. Using flowcytometric analysis we examined the expression of five activation markers (CD25, CD28, CD29, CD69, CD45R0) to look at systemic T-cell activation in COPD patients, matched smokers and never-smokers and to correlate the expression of these activation markers to lung function in smokers with or without COPD.

Proportions of the lymphocyte populations B-cells ($CD19^+$), NK cells ($CD16^+CD56^+$) and T-cell ($CD3^+$) did not differ between the three groups in the study. Neither did T-cell subpopulations of $CD4^+$ and $CD8^+$ T-cells. However, cell counts (cells/ml blood) differed between the groups with smokers having higher numbers of lymphocytes, $CD3^+$ T-cells and $CD4^+$ T-cells than never-smokers. In addition, the number of $CD4^+$ T-cells was also higher in smokers than in COPD patients. These data are in line with previously published studies showing increased numbers of $CD4^+$ T-cells in smokers (129, 130, 118, 131-133). The analysis of expression of T-cell activation markers was separated into analysis of $CD4^+$ and $CD8^+$ T-cells. No differences in percent of $CD4^+$ or $CD8^+$ T-cells expressing the different activation markers were found between the groups, confirming previous studies (134, 108, 128). However, when correlating T-cell activation to lung function in smokers with or without COPD, CD69 expression on $CD4^+$ T-cells correlated to FEV_1 % of predicted. When the group was subdivided into ex-smokers and current smokers the correlation grew stronger in the group of current smokers while it was missing in the group of ex-smokers (Figure 9).

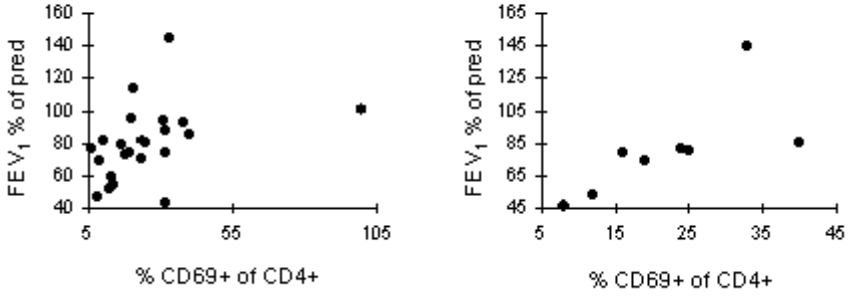

Figure 9 Percent of blood $CD4^+$ T-cells expressing CD69 correlated with lungfunction in a group of smokers with and without airway obstruction (left graph, rs=0.55 p= 0.0065). The correlation was even stronger when only current smokers were analysed (right graph, rs=0.93, p<0.0009).

The positive correlation between CD69 and FEV_1 in current smokers suggests that systemic CD69 expression on $CD4^+$ T-cells may indicate a more active immune system with ability to protect smokers from airway obstruction while exposure to

cigarette smoke is still ongoing. Perhaps individuals less sensitive to the inhibitory effects of cigarette smoke, discussed in Study II, would retain their capability to activate their T-cells and that this in turn could protect against infections that could trigger exacerbations. Another suggestion is that the activation of $CD4^+$ T-cells in blood could be related to a sustained $CD4^+/CD8^+$ ratio in lung tissue. The latter hypothesis was tested in Study IV where we examined lymphocyte subpopulations in peripheral lung tissue from COPD patients.

T-cells in peripheral lung tissue (Study IV)

T-cells have been studied in tissue from COPD patients and smokers in several studies. Most of those studies have focused on T-cells in central airways and on the occurrence of T-cells located intra- and subepithelially to the bronchi. Few studies have been published upon T-cells in peripheral lung tissue (95, 121, 96) and rarely such studies have examined BALT-like lymphoid structures in the peripheral tissue (82, 83). In Study IV we evaluated T- and B-cell occurrence in peripheral lung tissue resected from COPD patients, smokers and never-smokers undergoing surgery for suspected lung tumour. The tissue was stained with antibodies against the T-cell markers CD3, CD4, CD8 and with the B-cell marker CD20. In accordance to other studies (82, 120) most of the intraepithelial T-cells were of $CD8^+$ phenotype (see picture on the cover of this thesis) and these were higher in number in a joint group of smokers with and without COPD than in never-smokers ($p=0.02$). The hypothesis formed in the previous study, that high numbers of $CD69^+$ $CD4^+$ T-cells in blood affects $CD4^+$ and $CD8^+$ T-cell numbers in the tissue, was tested in this tissue but no correlations between $CD4^+$ or $CD8^+$ T-cell counts and CD69 expression on $CD4^+$ T-cells in blood was found, and hence the hypothesis could not be supported in this material.

While $CD8^+$ T-cells dominated the epithelium, the $CD4^+$ T-cells together with B-cells dominated the lymphoid aggregates found in this tissue. The aggregates had some resemblance to BALT, with a core of $CD20^+$ cells surrounded by $CD4^+$ T-cells and scattered $CD8^+$ T-cells (Figure 10). The aggregates were in approximately half of the cases found in the near vicinity a blood vessel but only occasionally near bronchioles and were thus often surrounded by parenchyma. While the structure and size of the aggregates was the same in COPD patients, smokers and never-smokers, the number of aggregates per tissue cm^2 tended to be higher in COPD patients than in the two other groups. This observation is in line with results from a study by Hogg et al., where the percentage of bronchioles containing lymphoid follicles was higher in more severe stages of the disease (GOLD COPD stage 2-4) than in less severe stages (stage 0-1) (83). As discussed earlier, in the present study aggregates were not only found adjacent to bronchioles but also situated in the parenchyma, which resembles the data on lymphoid nodules in smokers with and without obstruction published by Bosken et al (82).

The different lymphocyte populations thus reside in different structures within the peripheral lung tissue, which encourages further studies on COPD pathogenesis with hypothesises specifically directed against each structure.

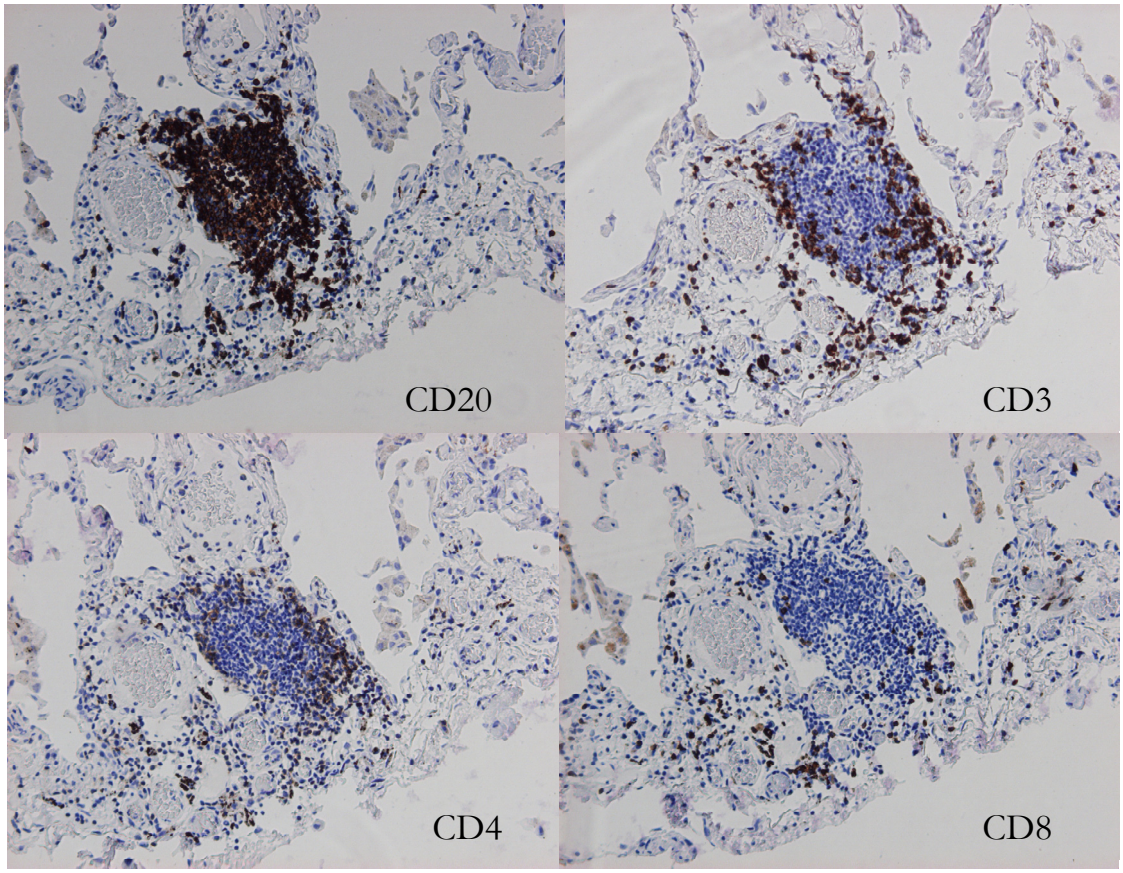

Figure 10 Lymphoid aggregates often found adjacent to a blood vessel, as seen in this picture, constituted of a B-cell ($CD20^+$) core surrounded by T-cells ($CD3^+$) of mainly CD4+ phenotype and scattered $CD8^+$ T-cells.

SUMMARY OF FINDINGS

$CD8^+$ T-cells in BAL from both healthy non-smokers and COPD patients are to a large extent memory T-cells expressing CD45R0. The majority of these cells are expressing αEβ7, which suggests that they have been residing in the airway epithelium before migrating into the airway lumen. Most T-cells in blood from the same subjects are on the other hand αEβ7 negative. The differentiation of αEβ7 negative T-cells into a αEβ7 positive phenotype is enhanced by exposure to TGF-β and stimulation via LFA-1.

When exposed to components in cigarette smoke, T-cell activity and proliferation declines. Production of the cytotoxic granule proteins granzyme-b and TIA-1, providing an indirect measurement of T-cell cytotoxic capacity, also decreases. This points towards an impaired T-cell defence in smokers.

In blood from smokers and COPD patients the number of $CD4^+$ T-cells is increased while the numbers of $CD8^+$ T-cells, B-cells and NK-cells do not differ from never-smokers. CD69 expression on blood $CD4^+$ T-cells in current smokers correlates with FEV_1 percent of predicted. These data indicate that the number of $CD69^+$ $CD4^+$ T-cells protects against COPD development while cigarette smoking is still present.

In peripheral tissue from the same subjects, lymphoid aggregates, consisting of a B-cell core surrounded by mainly $CD4^+$ T-cells and scattered $CD8^+$ T-cells, can be found both in COPD patients, smokers and never-smokers. T-cells harbouring the small airway epithelium are almost exclusively of $CD8^+$ phenotype and are more abundant in a joint group of smokers and COPD patients than in never-smokers.

CONCLUDING REMARKS AND FUTURE PERSPECTIVES

Although quite a few studies on T-cell occurrence in the lungs of COPD patients have been published, many questions remain unanswered. T-cell antigen specificity in COPD is one of those. T-cell responses in COPD are probably not driven by one single pathogen derived antigen, but rather reflects a range of antigens in the lung. However, a few suggestions to different antigens leading to recruitment of T-cells in COPD have been made. These include autoantigens (88) and adenoviral peptides (103) and other antigens arising from respiratory infections may also be of importance. So far studies on TCR repertoires in lungs of smokers and COPD patients have been very scarce but oligoclonal expansion of lung T-cells from patients with emphysema have been shown in a study by Sullivan et al. (162) implying T-cell recruitment in response to specific antigens. Additional studies of TCR repertoire at different sites within the lung need to be performed to evaluate T-cell antigen specificities of T-cell subgroups. Such analysises may answer questions such as; what are the functions of viable T-cells in the airway lumen, and which are the roles of intraepithelial T-cells in host defence or tolerance towards antigens encountering the airway mucosa?

The effector functions of recruited T-cells also need further attention. Histological staining for cytotoxic proteins such as perforin, granzymes and TIA-1, as well as cytokine expression could give answers to the function of different T-cell subsets. It could also tell whether the T-cell suppressive effects of cigarette smoke seen *in vitro* in Study II are evident also *in vivo*. Again different compartments within the lung tissue or airways need to be studied separately. Whereas cytotoxic effector functions may be important at sites exposed to airborne viruses, T-cells at sites like the lymphoid aggregates may be involved mainly in facilitating B-cell antibody production.

Apart from heterogeneity of T-cell populations within different compartments in the lung, there is also heterogeneity within COPD patients as a group. This is something that could affect the picture of T-cell involvement in this disease and that has to be taken into account when designing studies or interpreting already published studies. First, COPD patients are most often either ex-smokers or current smokers. When studying COPD and CB patients, as well as smokers, the smoking pattern of these individuals is important to clarify. Parameters such as number of pack years and time since smoking cessation are important information when interpreting data such as T-cell occurrence and activity. Study II in this thesis shows that acute exposure to components in cigarette smoke can directly affect T-cell activity and in Study III it is exemplified how stratification of patients based on smoking status can influence the outcome of the analysis. When possible, adding both current, ex- and non-smokers to studies of COPD patients contributes to the possibility of separating inflammatory responses to cigarette smoke from responses more specifically involved in COPD pathogenesis.

Secondly, the different pathological features of COPD ranging from bronchitis to bronchiolitis and emphysema make it, in some respects, difficult to study COPD as a single disease entity. Chronic bronchitis with airflow obstruction and emphysema are commonly present in the same patient and this makes the diagnosis of COPD relevant in the clinic. However, improved characterisation of COPD patients with regard to the different features of COPD pathology would be helpful from a research perspective. Instead of hypothesising about the role of T-cells in COPD, questions could then be more focused to answer e.g. the T-cell involvement in host defence during infections in obstructed chronic bronchitis patients or possible roles of T-cells in the destruction of lung tissue in patients with emphysema.

The third kind of heterogeneity is perhaps the most troublesome to study and involves stable phases vs. exacerbations in COPD patients. Invasive examinations of exacerbating patients are difficult due to severity of the disease. Therefore, the cellular involvement during exacerbations in these patients is so far inadequately studied. Part of the pathogenesis of COPD may be driven by exacerbations, provoked by infections. Patchy histological features with inflammation and emphysema seen to a varying degree in different areas of the lung tissue could be indirect evidence of this. As patients cannot always be subjected to bronchoscopy or surgery during infection, the histological picture seen in studies is often a snapshot of the remains of the battlefield, in which we may then look for traces of the enemy.

Considering all of the above-mentioned concerns, the answer to the question raised in the title of this thesis is not easily given. Data included in this thesis as well as studies published by other groups indicate that T-cells are present in the lungs of COPD patients in quite high numbers and that many of them are memory T-cells waiting to once again encounter their specific antigen. The degree and nature of the T-cell response will then affect the outcome of the inflammation and the healing after infection or injury. A fast T-cell response is important and very helpful in case of infection, however, if the T-cell response is too harsh and not properly controlled, the T-cells may be harmful and drive both inflammation and tissue destruction forward.

SVENSK POPULÄRVETENSKAPLIG SAMMANFATTNING (SUMMARY IN SWEDISH)

Patienter med kroniskt obstruktiv lungsjukdom, KOL, har en försämrad lungfunktion och lider av bronkit och andnöd. Dessa symptom beror troligtvis på en ständigt pågående inflammation i lungan som dels leder till ökad slemproduktion och förträngning av luftrören, dels till emfysem dvs. nedbrytning av lungvävnaden vilket försämrar gasutbytet i lungan. Cigarrettrökning är den största riskfaktorn vid KOL och rökavvänjning är i dag det bästa sättet för KOL patienter att stoppa utvecklingen av sjukdomen. Forskning pågår för att förstå orsakerna till inflammationen vid KOL. Flera celler i immunförsvaret tros vara inblandade i den inflammatoriska processen däribland T-celler. Detta är celler som normalt skyddar kroppen mot infektioner och oönskade cellförändringar. De har förmågan att signalera till andra celler i immunförsvaret och kan även själva motarbeta skador och infektioner.

Denna avhandling syftar till att studera närvaron av olika typer av T-celler i lungan och blodet hos KOL patienter, rökare och icke-rökare och till att vidare studera T-cellers respons då de utsätts för cigarettrök. Resultaten från studierna i lunga visade att aggregat av T-celler, med ännu okänd funktion, är vanliga hos KOL patienter. Dessa aggregat kan vara ett resultat av deposition av partiklar från cigarettrök eller ett sätt att försvara lungan mot infektioner som är vanligt förekommande hos KOL patienter. Ett ökat antal s.k. cytotoxiska ($CD8^+$) T-celler påvisades i luftvägsslemhinnan hos rökare och KOL patienter. Denna typ av T-celler har förmågan att driva virusinfekterade värdceller i apoptos, självinducerad celldöd, och har därför föreslagits vara involverade i nedbrytning av lungvävnad. Resultat från studien på blod visade att T-cellers aktivitet i blodet eventuellt kan påverka utvecklingen av KOL. Rökare med många aktiverade s.k. hjälpar ($CD4^+$) T-celler i blodet hade en bättre lungfunktion än rökare med färre celler av denna typ. Resultat från T-cellskulturer odlade i laboratorium visade att ämnen från cigarettrök försämrade T-cellers förmåga att aktiveras, tillväxa och producera de proteiner som T-cellerna använder i försvaret mot virus. Dessa resultat tyder på att cigarrettrökning kan försämra försvaret mot infektioner, vilket kan få stora konsekvenser för KOL patienter som ofta drabbas av akuta försämringsperioder i samband med luftvägsinfektioner.

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