Clinical SCIENCE

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Clinical Science (2008) 114, 533–541 (Printed in Great Britain) doi:10.1042/CS20070356

REVIEW

Killer cells in chronic obstructive pulmonary disease

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ABSTRACT

COPD (chronic obstructive pulmonary disease) is a treatable and preventable disease state, characterized by progressive airflow limitation that is not fully reversible. It is a current and growing cause of mortality and morbidity worldwide, with the WHO (World Health Organization) projecting that total deaths attributed to COPD will increase by more than 30% in the next 10 years. The pathological hallmarks of COPD are destruction of the lung parenchyma (pulmonary emphysema), inflammation of the central airways (chronic bronchitis) and inflammation of the peripheral airways (respiratory bronchiolitis). The destructive changes and tissue remodelling observed in COPD are a result of complex interactions between cells of the innate and adaptive immune systems. The focus of the present review is directed towards the role of $CD8^+$ T-lymphocytes, NK (natural killer) cells and NKT cells (NK T-cells). These three classes of killer cell could all play an important part in the pathogenesis of COPD. The observed damage to the pulmonary tissue could be caused in three ways: (i) direct cytotoxic effect against the lung epithelium mediated by the activities of perforin and granzymes, (ii) FasL (Fas ligand)-induced apoptosis and/or (iii) cytokine and chemokine release. The present review considers the role of these killer cells in COPD.

COPD (CHRONIC OBSTRUCTIVE PULMONARY DISEASE): THE CLINICAL PROBLEM

COPD is a treatable and preventable disease state, characterized by progressive airflow limitation that is not fully reversible [1]. It is a current and growing cause of mortality and morbidity worldwide, with the WHO (World Health Organization) projecting that total deaths attributed to COPD will increase by more than 30% in the next 10 years [2].

Active smoking, as the predominant risk factor, is well established, although less than a quarter of smokers develop COPD and more than 15% of COPD occurs in never smokers, suggesting an important contribution of other factors, such as air pollution and other airborne irritants [3].

At present, the severity of the disease is assessed using spirometry, with additional information given by the BODE index [BMI (body mass index), dyspnoea and exercise tolerance; Table 1] [4]. The pathological hallmarks of COPD are destruction of the lung

Key words: chronic obstructive pulmonary disease (COPD), cytokine, lung, natural killer cell, T-cell receptor, T-lymphoycyte.

Abbreviations: BAL, bronchoalveolar lavage; BMI, body mass index; BODE, BMI, dyspnoea and exercise tolerance; COPD, chronic obstructive pulmonary disease; DN, double-negative; DP, double-positive; α-GalCer, α-galactosylceramide; IFN-γ, interferon-γ; IL, interleukin; MAIT cell, mucosal-associated invariant T-cell; NK cell, natural killer cell; NKT cell, NK T-cell; iNKT cell, invariant NKT cell; SP single-positive; Tc, T-cytotoxic; TCR, T-cell receptor; Th, T-helper.

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Table 1 COPD severity can be assessed using spirometry, BMI, dyspnoea and exercise tolerance, the BODE index score

FEV_1, forced expiratory volume in 1 s

	BODE index score			
	0	1	2	3
FEV_1 (% predicted)	$\geqslant 65$	50–64	36–49	$\leqslant 35$
Distance walked in 6 min (m)	$\geqslant 350$	250–349	150–249	$\leqslant 149$
MRC dyspnoea questionnaire	0–1	2	3	4
BMI	> 21	$\leqslant 21$		

parenchyma (pulmonary emphysema), inflammation of the central airways (chronic bronchitis) and inflammation of the peripheral airways (respiratory bronchiolitis) [5–7] (Figure 1). Histopathological studies reveal that most inflammation in COPD occurs in the bronchioles and lung parenchyma. The bronchioles are obstructed by peribronchiolar fibrosis and there is destruction of lung parenchyma [5], which results in pulmonary emphysema. Although primarily a disease that affects the airways, COPD is increasingly recognized as a systemic disease and body mass alterations and endocrine disturbances have all been reported [8–10].

The destructive changes and tissue remodelling observed in COPD are a result of complex interactions between cells of the innate and adaptive immune systems. However, the focus in the present review is directed towards the role of $CD8^+$ T-lymphocytes, NK cells (natural killer cells) and NKT cells (NK T-cells).

Several studies have shown an increase in $CD8^+$ T-lymphocytes within both the peripheral airways [5] and lower respiratory tract in patients with COPD [11,12], but this effect is less conclusive in peripheral blood, with some investigators reporting a decrease [12] and others no change [11,13–16].

Circulating NK cells are decreased in smokers with COPD and have reduced phagocytic activity [17], and parallel changes in NK cells have been reported in normal smokers [18]. No difference in NK cell numbers or functional activity has been found in lung parenchyma of patients with COPD [19], although a decrease has been seen in the BAL (bronchoalveolar lavage) of patients with chronic bronchitis [20].

To date, no in-depth study of NKT cells in patients with COPD has been performed, although an increased number of Vα24-Vβ11 iNKT cells (invariant NTK cells) have been reported in asthma [21,22].

KILLER CELLS: PHENOTYPE AND FUNCTION

Many cells of both the innate and adaptive immune systems have the potential to kill. However, the present review will focus on the three main classes of human killer cells, which are heterogeneous and functionally distinct; namely, $CD8^+$ T-lymphocytes, NK cells and NKT cells.

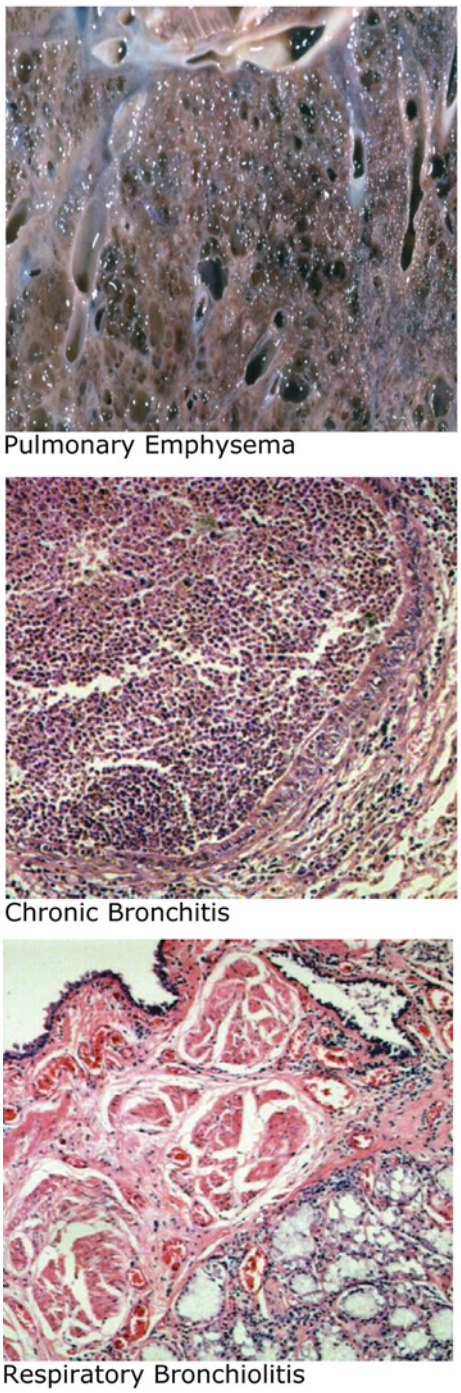

Figure 1 Representative images of the pathological hallmarks of COPD

$CD8^+$ T-lymphocytes originate from thymocytes, multipotent DN (double-negative) precursor cells for both $\alpha\beta$ $CD4^+$ and $CD8^+$ T-cells, and $\gamma\delta$ T-cells. The latter of which, although interesting due to their distinctive antigen recognition properties, are outside of the scope of this review. The predominant forms of these thymocytes express $\alpha\beta$ TCRs (T-cell receptors) and, as they develop, they progress through three main

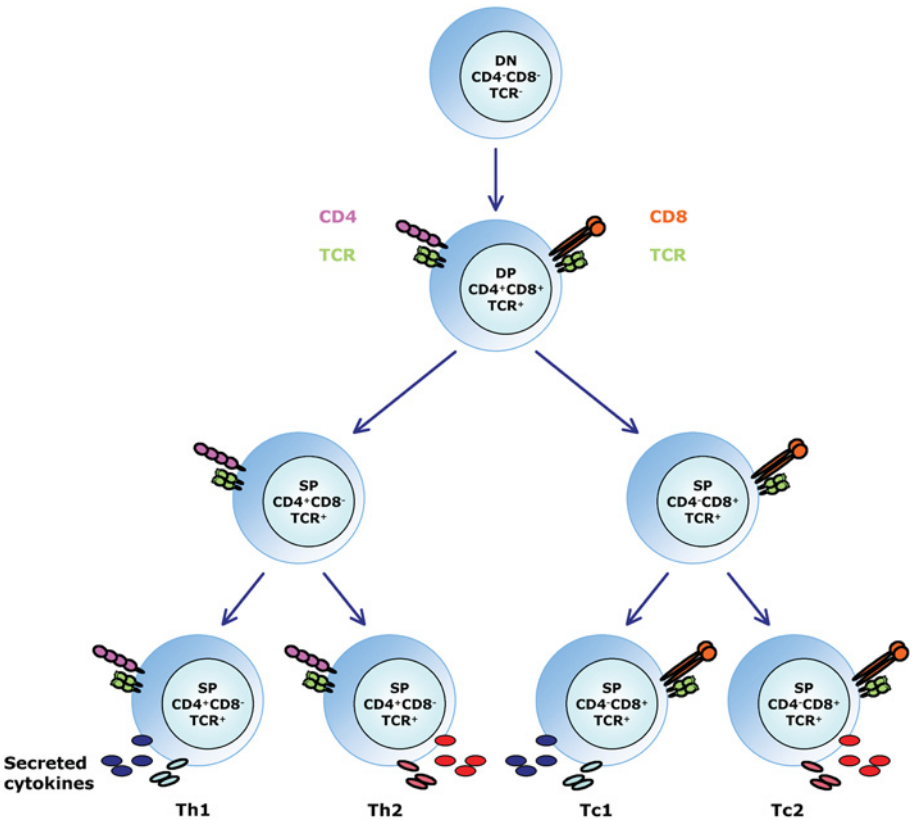

Figure 2 **Development of SP T-cells in the thymus**

Following positive and negative selection, both SP $CD4^+$ Th and $CD8^+$ Tc cells differentiate further into either a type 1 or type 2 cell.

stages defined by the differential expression of the CD4 and CD8 co-receptor molecules: $CD4^-CD8^-$ (DN), $CD4^+CD8^+$ [DP (double-positive)] and $CD4^+CD8^-$ or $CD4^-CD8^+$ [SP (single-positive)] (Figure 2). DP thymocytes first express a complete $\alpha\beta$ TCR complex and undergo the processes of positive and negative selection based on their relative ability to interact with thymic-selecting ligands. Occurring in parallel with positive selection, and therefore difficult to disentangle experimentally, is the process of alternative commitment to cytotoxic or helper T-cell lineages, characterized by the respective down-modulation of CD4 or CD8 co-receptors. Commitment involves passage through a series of transitional stages with intermediate CD4 and CD8 expression, most notably the $CD4^+$CD8lo stage, which includes progenitors of both the CD4 and CD8 lineages [23–26]. Once committed, the resulting $CD4^+$ Th (T-helper) cells and the $CD8^+$ Tc (T-cytotoxic) cells differentiate further into either a type 1 (Th1 and Tc1) or type 2 (Th2 or Tc2) cell.

One of the main immunological paradigms is that the pattern of cytokines, produced by activated T-lymphocytes, governs the qualitative and quantitative nature of immune responses. Type 1 T-cells secrete cytokines, such as IFN-γ (interferon-γ), crucial in the activation of macrophages and in the response to viral and bacterial infections [27,28], whereas type 2 T-cells secrete cytokines, such as IL (interleukin)-4, IL-5 and IL-13, involved in IgE-mediated responses and eosinophilia, and are characteristic of allergic diseases [29]. These cytokines can also modulate fibroblast proliferation and matrix production, which may result in tissue remodelling [30]. Several *in vitro* studies have demonstrated that type 1 and type 2 T-cells express distinct sets of chemokine receptors, which regulate the recruitment of these T-lymphocyte subsets to inflammatory sites [31,32]. In particular, it has been shown that the chemokine receptor CXCR3 is preferentially expressed on type 1 cells, which then selectively migrate towards its ligand CXCL10 [IP-10 (inducing protein-10)], expressed by lung epithelial cells [29,33,34]. This ligand has been shown to be up-regulated on lung epithelial cells when infected with rhinovirus, a common upper respiratory tract pathogen [34].

$CD8^+$ T-lymphocytes cause lysis of target cells by two mechanisms: membranolysis, in which secreted molecules such as perforin and granzymes form pores in the membrane of target cells [35]; and apoptosis, mediated through the triggering of apoptosis-inducing (Fas-like) surface molecules on the target cell [36]. Both

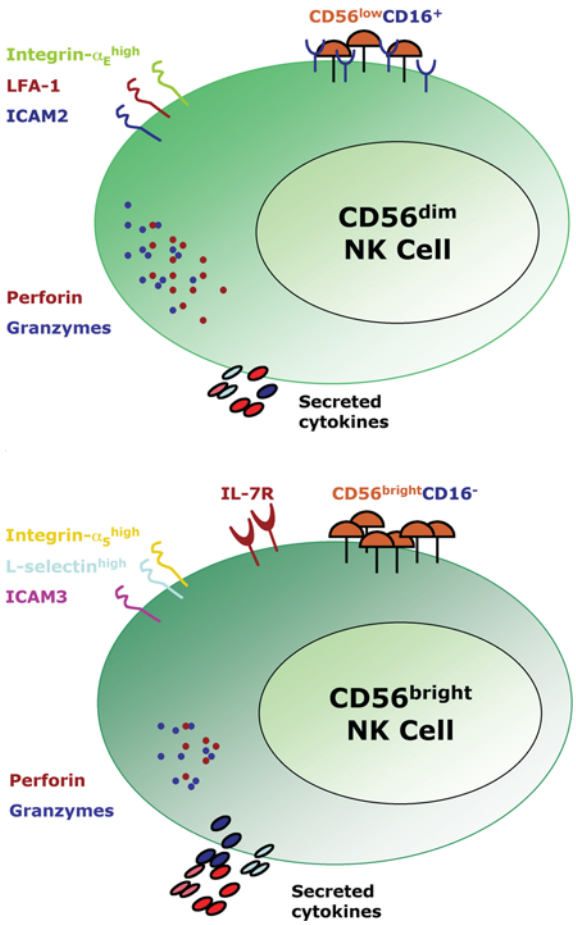

Figure 3 Schematic representation of human NK CD56^{dim} and CD56^{bright} subsets, showing representative key differences

are dependent on ligation of the TCR by MHC class I–peptide complexes.

NK and NKT cells participate in innate immune responses [37,38] and contribute to combating intracellular pathogens [39]. Immune cells with NK cell markers can influence acquired immunity [40]. There are two distinct subsets of human NK cells identified by the cell-surface density of CD56 [NCAM (neural cell adhesion molecule)]. The majority (approx. 90%) of circulating NK cells are CD56^{dim} and express high levels of FcγRIII (CD16), whereas a minority (approx. 10%) are CD56^{bright} and CD16$^{dim/neg}$ (Figure 3). At sites of inflammation and in lymph nodes, however, CD56^{bright} NK cells predominate. NK cells can activate Th1 cells by secreting IFN-γ or Th2 cells by secreting IL-4. The CD56^{bright} subset has a lower cytotoxic potential, but are poised to secrete cytokines and are, therefore, regarded as immunoregulatory [41–43]. Furthermore, the CD8$^+$ subset of these CD56^{bright}CD16$^-$ NK cells are thought to secrete Th2-type cytokines [44], which could have particular significance within the lung by skewing the microenvironment towards Th2/Tc2, as has been reported previously in COPD [16]. CD56^{dim} plays a key role in natural and antibody-mediated cell cytotoxicity. The phylogeny and ontogeny of human NK cells are poorly understood, with comparative murine studies being greatly hindered by a lack of a CD56 homologue. Subset development models have therefore relied on *in vitro* studies, which have given rise to three possible models. First, a common precursor differentiates into either a CD56^{bright} or CD56^{dim} cell. Secondly, the subsets are capable of switching between forms and are governed by the microenvironment. Thirdly, one subset may be a precursor of the other. Supporting evidence has been documented for all three models [41,45,46].

NKT cells have been classified as innate-like lymphocytes, which share features of both innate and adaptive immune cells [47]. NKT cells are a subset of T-cells that co-express an $\alpha\beta$ TCR, of which most recognize lipid antigens presented on the MHC class I-like molecule CD1d [48–51]. NKT cells share receptor structure and function with both NK cells and T-cells [52,53]. They can express T-cell membrane proteins, such as CD3, CD4 and CD8, and NK cell markers, including CD56, CD161 and inhibitory NK cell receptors [KIRs (killer Ig-like receptors)] [52–55]. The immune regulatory role of NKT cells remains poorly defined, both for the overall NKT cell population and for the phenotypically different subtypes [56]. The most widely studied subset of NKT cells is that which recognizes the exogenous glycolipid antigen α-GalCer (α-galactosylceramide) through CD1d, a member of the family of non-polymorphic class I-like antigen-presenting molecules [57]. In humans, CD1d-dependent NKT cells generally express invariant Vα24 TCRs. Considerably less is known about the CD1d-independent NKT cells, but this subset expresses a non-biased TCR repertoire. When activated, NKT cells promptly secrete large amounts of cytokines [58], including IL-4 and IFN-γ. They can regulate immune responses in diverse situations, such as autoimmune diseases [59], tumour rejection [60] and different types of infections [61]. NKT cells are abundant among T-lymphocytes in the liver and bone marrow, are also found in the thymus and spleen, and are more frequent in pancreatic and mesenteric than other lymph nodes [62], although they are present in extremely low numbers in the peripheral blood [55].

Recent studies have highlighted the distinct Th1- and Th2-type cytokine profiles of NKT cell subpopulations [63–67]. The CD4$^+$CD8$^-$ NKT cells (CD4$^+$ NKT cells) produce both Th1- and Th2-type cytokines [63,65–67], and the CD4$^-$CD8$^+$ (CD8$^+$ NKT cells) and CD4$^-$CD8$^-$ NKT cells (DN NKT cells) produce predominantly Th1-type cytokines [64–67]. The different cytokine profiles of NKT cell subpopulations suggest that these cells are likely to have different actions on different immune effector cells important in Th1- or Th2-type

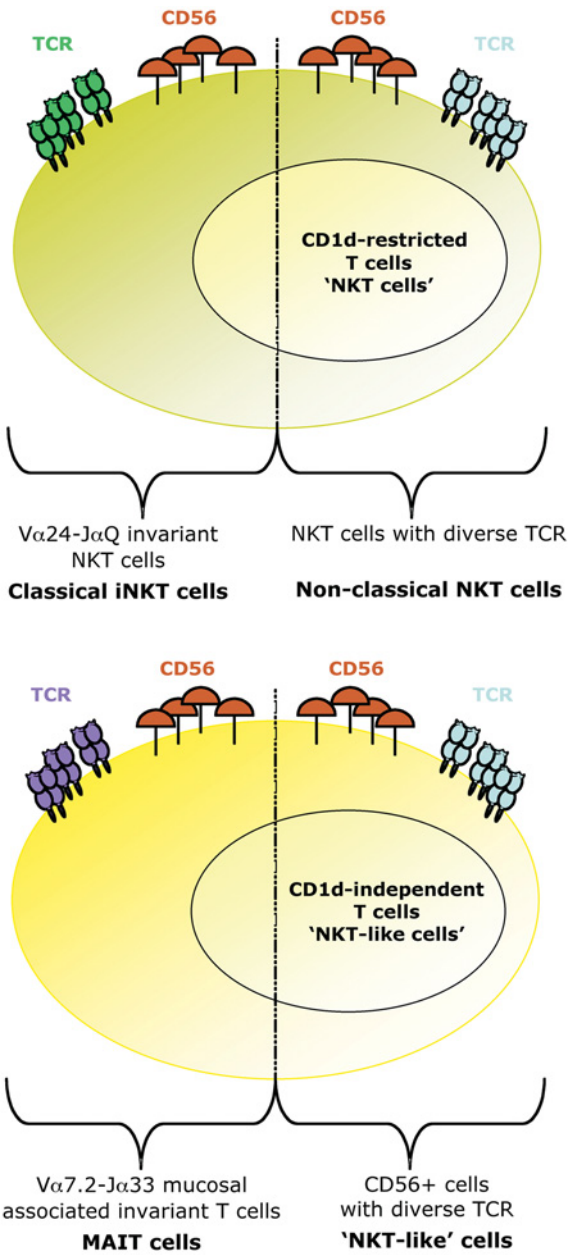

Figure 4 Schematic representation of the subsets of human NKT cells

immune activities. This may contribute to the reported paradoxical functions of NKT cells.

The definition 'NKT cell' is not consistent, although recent reviews have tried to unify the terminology [50,68–70]. As shown in Figure 4, $CD56^+CD3^+$ cells in humans are either CD1d-restricted or CD1d-independent. On the basis of the TCR expressed, CD1d-restricted T-cells, in turn, can be divided into subgroups of classical and non-classical NKT cells. CD1d-independent $CD56^+CD3^+$ T-cells may be called 'NKT-like' [50], with a final group named MAIT cells (mucosal-associated invariant T-cells) that are MR1-restricted [70,71]. MR1 is a β_2m (β_2-microglobulin)-dependent non-polymorphic MHC class I-like molecule encoded by a gene linked to the *CD1d* gene [70,71]. CD1d-restricted T-cells comprise a heterogeneous and functionally divergent population. A proportion of the cells in human express TCRs consisting of an invariant Vα24-JαQ TCRα chain combined with a Vβ11 TCRβ chain [48,57]. This subset is often referred to as iNKT cells or classical NKT cells (Figure 4). Cells with this family of TCRs can be activated by the synthetic ligand α-GalCer presented on CD1d-loaded tetramers [72]. A lysosomal glycosphingolipid was identified as an endogenous ligand for iNKT cells [73]. The CD1d-restricted cells not using the Vα24 invariant TCR [74–77] appear to share the main characteristic features of NKT cells, such as the expression of NK cell markers, rapid cytokine secretion upon activation and a memory surface phenotype [77–80]. They are thought to express a relatively diverse TCR repertoire; however, this subset may also contain sets of cells with invariant receptors different from the Vα24 type [81]. CD1d-restricted cells, which do not use the Vα24 invariant TCR, are referred to as non-classical NKT cells (Figure 4). Non-classical NKT cells are not activated by α-GalCer [82,83], but other CD1d-restricted ligands have been identified for this group of NKT cells [84]. CD1d-restricted T-cells with diverse TCRs may be more frequent than the Vα24 iNKT subset in humans [85–87], emphasizing the need for further analysis of this subset. MR1-restricted MAIT cells have been researched less thoroughly than classical iNKT cells, although, due to their presence in mucosal sites of the gut and lung [71,88–90], they could be of great interest in COPD. These cells express TCRs consisting of an invariant Vα7.2-Jα33 TCRα chain, which is predominantly combined with either a Vβ13 or a Vβ2 TCRβ chain [70,91] (Figure 4). Findings have postulated that α-ManCer (α-mannosylceramide) can activate these cells [92–94].

KILLER CELLS IN COPD: THE CURRENT STATUS

The three main classes of killer cell discussed in the present review could all play an important part in the pathogenesis of COPD, although their specific contributions have yet to be established. The observed damage to the pulmonary tissue could be caused in three ways. A direct cytotoxic effect against the lung epithelium mediated by the activities of perforin and granzymes [95–98] and/or FasL (Fas ligand)-induced apoptosis [27,99]. Cytokine and chemokine release could either induce changes within the lung epithelial cells or activate and recruit other inflammatory cells to the lung.

A type 1 profile has been hypothesized in COPD, particularly because viral and/or bacterial infections are associated with the development of the disease [27,28].

Table 2 Representative peripheral results suggesting a decrease in the proportion and cytolytic ability of both NK and NKT cells in COPD

Findings from R. A. Urbanowicz, J. R. Lamb, H. F. Sewell, I. Todd, J. Corne and L. Fairclough, unpublished results.

	Healthy participants		Healthy smokers		Smokers with COPD	
Killer cell	Proportion of cells (%)	Cytotoxic ability (%)	Proportion of cells (%)	Cytotoxic ability (%)	Proportion of cells (%)	Cytotoxic ability (%)
NK cells ($CD3^{-}CD56^{+}$)	9	60–75	7	45–60	5	0–30
NKT cells ($CD3^{+}CD56^{+}$)	3	60–75	2.5	45–60	0.5	0–30

This phenotype has been demonstrated in the peripheral blood of subjects with COPD [15], and others have also described it in bronchial biopsies of patients with COPD [100]. However, two recent studies have shown a type 2 profile in BAL [16,101], suggesting that there is intra-compartment variability within the disease.

As described previously, an increased number of $CD8^{+}$ T-lymphocytes have been consistently identified in both the lower and upper respiratory tract of patients with COPD, although results have been less conclusive in peripheral blood. In-depth studies investigating the presence or absence of NK and NKT cells in either the lung or peripheral blood have not been completed, although our own findings suggest that both are reduced in number in the peripheral blood of smokers with COPD (R. A. Urbanowicz, J. R. Lamb, H. F. Sewell, I. Todd, J. Corne and L. Fairclough, unpublished results). In a further analysis of their potential effector functions, we failed to detect any differences in cytokine production; however, the cytolytic activity of the cells in an *in vitro* assay was significantly reduced (Table 2). This outcome may be the result of either selective recruitment into the lung or a systemic suppression of number and cytotoxic function.

The abilities of all three killer cells to cause direct damage to the lung epithelium and to recruit other cells of the immune system into the lung indicate their crucial role in driving the pathogenesis of COPD and should, therefore, be studied in much greater detail.

ACKNOWLEDGMENTS

R.A.U. was funded by a GlaxoSmithKline PhD studentship.

REFERENCES

1 Celli, B. R. and MacNee, W. (2004) Standards for the diagnosis and treatment of patients with COPD: a summary of the ATS/ERS position paper. Eur. Respir. J. **23**, 932–946
2 World Health Organization (2007) COPD Factsheet No, 315, World Health Organization (ttp://www.who.int/mediacentre/factsheets/fs315/en/index.html)
3 Brown, C., Crombie, I. and Tunstall-Pedoe, H. (1994) Failure of cigarette smoking to explain international differences in mortality from chronic obstructive pulmonary disease. J. Epidemiol. Community Health **48**, 134–139
4 Celli, B. R., Cote, C. G., Marin, J. M. et al. (2004) The body-mass index, airflow obstruction, dyspnea, and exercise capacity index in chronic obstructive pulmonary disease. N. Engl. J. Med. **350**, 1005–1012
5 Saetta, M., Di Stefano, A., Turato, G. et al. (1998) CD8+ T-lymphocytes in peripheral airways of smokers with chronic obstructive pulmonary disease. Am. J. Respir. Crit. Care Med. **157**, 822–826
6 Finkelstein, R., Fraser, R. S., Ghezzo, H. and Cosio, M. G. (1995) Alveolar inflammation and its relation to emphysema in smokers. Am. J. Respir. Crit. Care Med. **152**, 1666–1672
7 Finkelstein, R., Ma, H. D., Ghezzo, H., Whittaker, K., Fraser, R. S. and Cosio, M. G. (1995) Morphometry of small airways in smokers and its relationship to emphysema type and hyperresponsiveness. Am. J. Respir. Crit. Care Med. **152**, 267–276
8 Bernard, S., LeBlanc, P., Whittom, F. et al. (1998) Peripheral muscle weakness in patients with chronic obstructive pulmonary disease. Am. J. Respir. Crit. Care Med. **158**, 629–634
9 Rahman, I., Morrison, D., Donaldson, K. and MacNee, W. (1996) Systemic oxidative stress in asthma, COPD, and smokers. Am. J. Respir. Crit. Care Med. **154**, 1055–1060
10 Kamischke, A., Kemper, D. E., Castel, M. A. et al. (1998) Testosterone levels in men with chronic obstructive pulmonary disease with or without glucocorticoid therapy. Eur. Respir. J. **11**, 41–45
11 Ekberg-Jansson, A., Andersson, B., Avra, E., Nilsson, O. and Lofdahl, C. G. (2000) The expression of lymphocyte surface antigens in bronchial biopsies, bronchoalveolar lavage cells and blood cells in healthy smoking and never-smoking men, 60 years old. Respir. Med. **94**, 264–272
12 Kim, W. D., Kim, W. S., Koh, Y. et al. (2002) Abnormal peripheral blood T-lymphocyte subsets in a subgroup of patients with COPD. Chest **122**, 437–444
13 Leckie, M. J., Jenkins, G. R., Khan, J. et al. (2003) Sputum T lymphocytes in asthma, COPD and healthy subjects have the phenotype of activated intraepithelial T cells (CD69+ CD103+). Thorax **58**, 23–29
14 Lewis, S. A., Pavord, I. D., Stringer, J. R., Knox, A. J., Weiss, S. T. and Britton, J. R. (2001) The relation between peripheral blood leukocyte counts and respiratory symptoms, atopy, lung function, and airway responsiveness in adults. Chest **119**, 105–114
15 Majori, M., Corradi, M., Caminati, A., Cacciani, G., Bertacco, S. and Pesci, A. (1999) Predominant TH1 cytokine pattern in peripheral blood from subjects with chronic obstructive pulmonary disease. J. Allergy Clin. Immunol. **103**, 458–462
16 Barcelo, B., Pons, J., Fuster, A. et al. (2006) Intracellular cytokine profile of T lymphocytes in patients with chronic obstructive pulmonary disease. Clin. Exp. Immunol. **145**, 474–479

17 Prieto, A., Reyes, E., Bernstein, E. et al. (2001) Defective natural killer and phagocytic activities in chronic obstructive pulmonary disease are restored by glycophosphopeptical (inmunoferon). Am. J. Respir. Crit. Care Med. **163**, 1578–1583
18 Zeidel, A., Beilin, B., Yardeni, I., Mayburd, E., Smirnov, G. and Bessler, H. (2002) Immune response in asymptomatic smokers. Acta Anaesthesiol. Scand. **46**, 959–964
19 Majo, J., Ghezzo, H. and Cosio, M. (2001) Lymphocyte population and apoptosis in the lungs of smokers and their relation to emphysema. Eur. Respir. J. **17**, 946–953
20 Costabel, U., Maier, K., Teschler, H. and Wang, Y. M. (1992) Local immune components in chronic obstructive pulmonary disease. Respiration **59** (Suppl. 1), 17–19
21 Akbari, O., Faul, J., Hoyte, E. et al. (2006) CD4+ invariant T-cell-receptor+natural killer T cells in bronchial asthma. N. Engl. J. Med. **354**, 1117–1129
22 Vijayanand, P., Seumois, G., Pickard, C. et al. (2007) Invariant natural killer T cells in asthma and chronic obstructive pulmonary disease. N. Engl. J. Med. **356**, 1410–1422
23 Suzuki, H., Punt, J. A., Granger, L. G. and Singer, A. (1995) Asymmetric signaling requirements for thymocyte commitment to the CD4+ versus CD8+ T cell lineages: a new perspective on thymic commitment and selection. Immunity **2**, 413–425
24 Lucas, B. and and Germain, R. N. (1996) Unexpectedly complex regulation of CD4/CD8 coreceptor expression supports a revised model for CD4+CD8+ thymocyte differentiation. Immunity **5**, 461–477
25 Lundberg, K., Heath, W., Kontgen, F., Carbone, F. R. and Shortman, K. (1995) Intermediate steps in positive selection: differentiation of CD4+8^{int}t TCR^{int} thymocytes into CD4-CD8+TCR^{hi} thymocytes. J. Exp. Med. **181**, 1643–1651
26 Kydd, R., Lundberg, K., Vremec, D., Harris, A. W. and Shortman, K. (1995) Intermediate steps in thymic positive selection. Generation of CD4-CD8+ T cells in culture from CD4+8+, CD4^{int}8+, and CD4+8^{int} thymocytes with up-regulated levels of TCR-CD3. J. Immunol. **155**, 3806–3814
27 Saetta, M., Turato, G., Maestrelli, P., Mapp, C. E. and Fabbri, L. M. (2001) Cellular and structural bases of chronic obstructive pulmonary disease. Am. J. Respir. Crit. Care Med. **163**, 1304–1309
28 Retamales, I., Elliott, W. M., Meshi, B. et al. (2001) Amplification of inflammation in emphysema and its association with latent adenoviral infection. Am. J. Respir. Crit. Care Med. **164**, 469–473
29 Saetta, M., Mariani, M., Panina-Bordignon, P. et al. (2002) Increased expression of the chemokine receptor CXCR3 and its ligand CXCL10 in peripheral airways of smokers with chronic obstructive pulmonary disease. Am. J. Respir. Crit. Care Med. **165**, 1404–1409
30 Sempowski, G. D., Derdak, S. and Phipps, R. P. (1996) Interleukin-4 and interferon-γ discordantly regulate collagen biosynthesis by functionally distinct lung fibroblast subsets. J. Cell. Physiol. **167**, 290–296
31 Baggiolini, M. (1998) Chemokines and leukocyte traffic. Nature **392**, 565–568
32 Luster, M. I. and Simeonova, P. P. (1998) Asbestos induces inflammatory cytokines in the lung through redox sensitive transcription factors. Toxicol. Lett. **102–103**, 271–275
33 D'Ambrosio, D., Mariani, M., Panina-Bordignon, P. and Sinigaglia, F. (2001) Chemokines and their receptors guiding T lymphocyte recruitment in lung inflammation. Am. J. Respir. Crit. Care Med. **164**, 1266–1275
34 Spurrell, J. C., Wiehler, S., Zaheer, R. S., Sanders, S. P. and Proud, D. (2005) Human airway epithelial cells produce IP-10 (CXCL10) *in vitro* and *in vivo* upon rhinovirus infection. Am. J. Physiol. Lung Cell. Mol. Physiol. **289**, L85–L95
35 Berke, G. (1994) The binding and lysis of target cells by cytotoxic lymphocytes: molecular and cellular aspects. Annu. Rev. Immunol. **12**, 735–773
36 Hashimoto, S., Kobayashi, A., Kooguchi, K., Kitamura, Y., Onodera, H. and Nakajima, H. (2000) Upregulation of two death pathways of perforin/granzyme and FasL/Fas in septic acute respiratory distress syndrome. Am. J. Respir. Crit. Care Med. **161**, 237–243
37 Cooper, M. A., Fehniger, T. A. and Turner, S. C. et al. (2001) Human natural killer cells: a unique innate immunoregulatory role for the CD56^{bright} subset. Blood **97**, 3146–3151
38 Cooper, M. A., Fehniger, T. A., Fuchs, A., Colonna, M. and Caligiuri, M. A. (2004) NK cell and DC interactions. Trends Immunol. **25**, 47–52
39 Andoniou, C. E., van Dommelen, S. L., Voigt, V. et al. (2005) Interaction between conventional dendritic cells and natural killer cells is integral to the activation of effective antiviral immunity. Nat. Immunol. **6**, 1011–1019
40 Smyth, M. J., Cretney, E., Kelly, J. M. et al. (2005) Activation of NK cell cytotoxicity. Mol. Immunol. **42**, 501–510
41 Nagler, A., Lanier, L. L., Cwirla, S. and Phillips, J. H. (1989) Comparative studies of human FcRIII-positive and negative natural killer cells. J. Immunol. **143**, 3183–3191
42 Jacobs, R., Stoll, M., Stratmann, G., Leo, R., Link, H. and Schmidt, R. E. (1992) CD16− CD56+ natural killer cells after bone marrow transplantation. Blood **79**, 3239–3244
43 Dalbeth, N., Gundle, R., Davies, R. J., Lee, Y. C., McMichael, A. J. and Callan, M. F. (2004) CD56^{bright} NK cells are enriched at inflammatory sites and can engage with monocytes in a reciprocal program of activation. J. Immunol. **173**, 6418–6426
44 Smyth, M. J. and Nutt, S. L. (2006) IL-7 and the thymus dictate the NK cell 'labor market'. Nat. Immunol. **7**, 1134–1136
45 Farag, S. S. and and Caligiuri, M. A. (2006) Human natural killer cell development and biology. Blood Rev. **20**, 123–137
46 Chan, A., Hong, D. L., Atzberger, A. et al. (2007) CD56^{bright} human NK cells differentiate into CD56^{dim} cells: role of contact with peripheral fibroblasts. J. Immunol. **179**, 89–94
47 Bendelac, A., Bonneville, M. and Kearney, J. F. (2001) Autoreactivity by design: innate B and T lymphocytes. Nat. Rev. Immunol. **1**, 177–186
48 Bendelac, A., Rivera, M. N., Park, S. H. and Roark, J. H. (1997) Mouse CD1-specific NK1 T cells: development, specificity, and function. Annu. Rev. Immunol. **15**, 535–562
49 Porcelli, S. A. and Modlin, R. L. (1999) The CD1 system: antigen-presenting molecules for T cell recognition of lipids and glycolipids. Annu. Rev. Immunol. **17**, 297–329
50 Godfrey, D. I., MacDonald, H. R., Kronenberg, M., Smyth, M. J. and Van Kaer, L. (2004) NKT cells: what's in a name? Nat. Rev. Immunol. **4**, 231–237
51 Brigl, M. and Brenner, M. B. (2004) CD1: antigen presentation and T cell function. Annu. Rev. Immunol. **22**, 817–890
52 Kronenberg, M. and Gapin, L. (2002) The unconventional lifestyle of NKT cells. Nat. Rev. Immunol. 2 **8**, 557–568
53 Emoto, M. and Kaufmann, S. H. (2003) Liver NKT cells: an account of heterogeneity. Trends Immunol. **24**, 364–369
54 Norris, S., Doherty, D. G., Collins, C. et al. (1999) Natural T cells in the human liver: cytotoxic lymphocytes with dual T cell and natural killer cell phenotype and function are phenotypically heterogenous and include Vα24-JαQ and $\gamma\delta$ T cell receptor bearing cells. Hum. Immunol. **60**, 20–31
55 Kenna, T., Golden-Mason, L., Porcelli, S. A. et al. (2003) NKT cells from normal and tumor-bearing human livers are phenotypically and functionally distinct from murine NKT cells. J. Immunol. **171**, 1775–1779
56 Oren, A., Husebo, C., Iversen, A. C. and Austgulen, R. (2005) A comparative study of immunomagnetic methods used for separation of human natural killer cells from peripheral blood. J. Immunol. Methods **303**, 1–10

57 Bendelac, A., Lantz, O., Quimby, M. E., Yewdell, J. W., Bennink, J. R. and Brutkiewicz, R. R. (1995) CD1 recognition by mouse NK1+ T lymphocytes. Science **268**, 863–865

58 Yoshimoto, T. and Paul, W. E. (1994) CD4^{pos}, NK1.1^{pos} T cells promptly produce interleukin 4 in response to *in vivo* challenge with anti-CD3. J. Exp. Med. **179**, 1285–1295

59 Hammond, K. J. and Kronenberg, M. (2003) Natural killer T cells: natural or unnatural regulators of autoimmunity? Curr. Opin. Immunol. **15**, 683–689

60 Smyth, M. J., Crowe, N. Y., Hayakawa, Y., Takeda, K., Yagita, H. and Godfrey, D. I. (2002) NKT cells: conductors of tumor immunity? Curr. Opin. Immunol. **14**, 165–171

61 Skold, M. and Behar, S. M. (2003) Role of CD1d-restricted NKT cells in microbial immunity. Infect. Immun. **71**, 5447–5455

62 Laloux, V., Beaudoin, L., Ronet, C. and Lehuen, A. (2002) Phenotypic and functional differences between NKT cells colonizing splanchnic and peripheral lymph nodes. J. Immunol. **168**, 3251–3258

63 Takahashi, T., Nieda, M., Koezuka, Y. et al. (2000) Analysis of human Vα 24+ CD4+ NKT cells activated by α-glycosylceramide-pulsed monocyte-derived dendritic cells. J. Immunol. **164**, 4458–4464

64 Takahashi, T., Chiba, S., Nieda, M. et al. (2002) Cutting edge: analysis of human Vα 24+CD8+ NK T cells activated by α-galactosylceramide-pulsed monocyte-derived dendritic cells. J. Immunol. **168**, 3140–3144

65 Gumperz, J. E., Miyake, S., Yamamura, T. and Brenner, M. B. (2002) Functionally distinct subsets of CD1d-restricted natural killer T cells revealed by CD1d tetramer staining. J. Exp. Med. **195**, 625–636

66 Kim, C. H., Butcher, E. C. and Johnston, B. (2002) Distinct subsets of human Vα24-invariant NKT cells: cytokine responses and chemokine receptor expression. Trends Immunol. **23**, 516–519

67 Lee, P. T., Benlagha, K., Teyton, L. and Bendelac, A. (2002) Distinct functional lineages of human Vα24 natural killer T cells. J. Exp. Med. **195**, 637–641

68 Cardell, S. L. (2006) The natural killer T lymphocyte: a player in the complex regulation of autoimmune diabetes in non-obese diabetic mice. Clin. Exp. Immunol. **143**, 194–202

69 Bendelac, A., Savage, P. B. and Teyton, L. (2007) The biology of NKT cells. Annu. Rev. Immunol. **25**, 297–336

70 Wingender, G. and Kronenberg, M. (2008) The role of canonical natural killer T cells in mucosal immunity and inflammation. Am. J. Physiol. Gastrointest. Liver Physiol. **294**, G1–G8

71 Treiner, É., Duban, L., Bahram, S. et al. (2003) Selection of evolutionarily conserved mucosal-associated invariant T cells by MR1. Nature **422**, 164–169

72 Kawano, T., Cui, J., Koezuka, Y. et al. (1997) CD1d-restricted and TCR-mediated activation of vα14 NKT cells by glycosylceramides. Science **278**, 1626–1629

73 Zhou, D., Mattner, J., Cantu, III, C. et al. (2004) Lysosomal glycosphingolipid recognition by NKT cells. Science **306**, 1786–1789

74 Cardell, S., Tangri, S., Chan, S., Kronenberg, M., Benoist, C. and Mathis, D. (1995) CD1-restricted CD4+ T cells in major histocompatibility complex class II-deficient mice. J. Exp. Med. **182**, 993–1004

75 Zeng, D., Dick, M., Cheng, L. et al. (1998) Subsets of transgenic T cells that recognize CD1 induce or prevent murine lupus: role of cytokines. J. Exp. Med. **187**, 525–536

76 Roark, J. H., Park, S. H., Jayawardena, J., Kavita, U., Shannon, M. and Bendelac, A. (1998) CD1.1 expression by mouse antigen-presenting cells and marginal zone B cells. J. Immunol. **160**, 3121–3127

77 Behar, S. M., Podrebarac, T. A., Roy, C. J., Wang, C. R. and Brenner, M. B. (1999) Diverse TCRs recognize murine CD1. J. Immunol. **162**, 161–167

78 Skold, M., Faizunnessa, N. N., Wang, C. R. and Cardell, S. (2000) CD1d-specific NK1.1+ T cells with a transgenic variant TCR. J. Immunol. **165**, 168–174

79 Stenstrom, M., Skold, M., Ericsson, A. et al. (2004) Surface receptors identify mouse NK1.1+ T cell subsets distinguished by function and T cell receptor type. Eur. J. Immunol. **34**, 56–65

80 Duarte, N., Stenstrom, M., Campino, S. et al. (2004) Prevention of diabetes in nonobese diabetic mice mediated by CD1d-restricted nonclassical NKT cells. J. Immunol. **173**, 3112–3118

81 Park, S. H., Weiss, A., Benlagha, K., Kyin, T., Teyton, L. and Bendelac, A. (2001) The mouse CD1d-restricted repertoire is dominated by a few autoreactive T cell receptor families. J. Exp. Med. **193**, 893–904

82 Gumperz, J. E., Roy, C., Makowska, A. et al. (2000) Murine CD1d-restricted T cell recognition of cellular lipids. Immunity **12**, 211–221

83 Makowska, A., Kawano, T., Taniguchi, M. and Cardell, S. (2000) Differences in the ligand specificity between CD1d-restricted T cells with limited and diverse T-cell receptor repertoire. Scand. J. Immunol. **52**, 71–79

84 Jahng, A., Maricic, I., Aguilera, C., Cardell, S., Halder, R. C. and Kumar, V. (2004) Prevention of autoimmunity by targeting a distinct, noninvariant CD1d-reactive T cell population reactive to sulfatide. J. Exp. Med. **199**, 947–957

85 Exley, M. A., Tahir, S. M., Cheng, O. et al. (2001) A major fraction of human bone marrow lymphocytes are Th2-like CD1d-reactive T cells that can suppress mixed lymphocyte responses. J. Immunol. **167**, 5531–5534

86 Exley, M. A., He, Q., Cheng, O. et al. (2002) Cutting edge: compartmentalization of Th1-like noninvariant CD1d-reactive T cells in hepatitis C virus-infected liver. J. Immunol. **168**, 1519–1523

87 Fuss, I. J., Heller, F., Boirivant, M. et al. (2004) Nonclassical CD1d-restricted NK T cells that produce IL-13 characterize an atypical Th2 response in ulcerative colitis. J. Clin. Invest. **113**, 1490–1497

88 Kawachi, I., Maldonado, J., Strader, C. and Gilfillan, S. (2006) MR1-restricted Vα19i mucosal-associated invariant T cells are innate T cells in the gut lamina propria that provide a rapid and diverse cytokine response. J. Immunol. **176**, 1618–1627

89 Treiner, E., Duban, L., Moura, I. C., Hansen, T., Gilfillan, S. and Lantz, O. (2005) Mucosal-associated invariant T (MAIT) cells: an evolutionarily conserved T cell subset. Microbes Infect. **7**, 552–559

90 Treiner, E. and Lantz, O. (2006) CD1d- and MR1-restricted invariant T cells: of mice and men. Curr. Opin. Immunol. **18**, 519–526

91 Shimamura, M. and and Huang, Y. Y. (2002) Presence of a novel subset of NKT cells bearing an invariant V(α)19.1-J(α)26 TCRα chain. FEBS Lett. **516**, 97–100

92 Okamoto, N., Kanie, O., Huang, Y. Y., Fujii, R., Watanabe, H. and Shimamura, M. (2005) Synthetic α-mannosyl ceramide as a potent stimulant for an NKT cell repertoire bearing the invariant Vα19-Jα26 TCR α chain. Chem. Biol. **12**, 677–683

93 Shimamura, M., Huang, Y. Y., Okamoto, N. et al. (2007) Modulation of Vα19 NKT cell immune responses by α-mannosyl ceramide derivatives consisting of a series of modified sphingosines. Eur. J. Immunol. **37**, 1836–1844

94 Shimamura, M., Huang, Y. Y., Okamoto, N. et al. (2007) Glycolipids with nonreducing end α-mannosyl residues that have the potential to activate invariant Vα19 NKT cells. FEBS J. **274**, 2921–2932

95 Nikos, S. (2007) 'In the beginning' of COPD: is evolution important? Am. J. Respir. Crit. Care Med. **175**, 423–424

96 Chrysofakis, G., Tzanakis, N., Kyriakoy, D. et al. (2004) Perforin expression and cytotoxic activity of sputum CD8+ lymphocytes in patients with COPD. Chest **125**, 71–76
97 Vernooy, J. H., Moller, G. M., van Suylen, R. J. et al. (2007) Increased granzyme A expression in type II pneumocytes of patients with severe chronic obstructive pulmonary disease. Am. J. Respir. Crit. Care Med. **175**, 464–472
98 Hodge, S., Hodge, G., Nairn, J., Holmes, M. and Reynolds, P. (2006) Increased airway granzyme b and perforin in current and ex-smoking COPD subjects. COPD **3**, 179–187
99 Domagała-Kulawik, J., Hoser, G., Dąbrowska, M. and Chazan, R. (2006) Increased proportion of Fas positive CD8+ cells in peripheral blood of patients with COPD. Respir. Med. **101**, 1338–1343
100 Panina-Bordignon, P., Papi, A., Mariani, M. et al. (2001) The C-C chemokine receptors CCR4 and CCR8 identify airway T cells of allergen-challenged atopic asthmatics. J. Clin. Invest. **107**, 1357–1364
101 Barczyk, A., Pierzchała, W., Kon, O., Cosio, B., Adcock, I. and Barnes, P. (2006) Cytokine production by bronchoalveolar lavage T lymphocytes in chronic obstructive pulmonary disease. J. Allergy Clin. Immunol. **117**, 1484–1492

Received 9 October 2007/28 November 2007; accepted 13 December 2007
Published on the Internet 13 March 2008, doi:10.1042/CS20070356