

## Role of the human immunodeficiency virus within the lung

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The lung is an important disease site in individuals infected with the human immunodeficiency virus (HIV) as has been discussed in the previous three review articles published in this series. The diseases described in the lung in HIV disease are usually regarded as manifestations of generalised changes, mainly affecting the immune system, which are produced by HIV infection. During the early years of the AIDS epidemic it became clear that certain organ systems, in particular the central nervous system, were directly infected by the virus at an early stage in the natural history of the disease, and that the virus could directly inflict tissue damage without invoking an immune mechanism. It is now known that other organ systems – for example, the gut – may also be directly infected by HIV resulting in disease. Until recently little was known about the role, if any, of HIV in the lung. Over the last few years, using the research tool of bronchoscopic alveolar lavage (BAL), a substantial number of research groups have reported on aspects of this interesting area, and it is the purpose of this article to review what is currently known about the role of HIV in the lung.

Our understanding of HIV, the aetiological agent of AIDS, is rapidly evolving.<sup>1,2</sup> Most research so far has studied HIV-1 obtained from peripheral blood leucocytes (PBL) or peripheral blood mononuclear cells (PBMC) due to ease of availability of these cells. However, the relevance of HIV-1 strains recovered from PBL/PBMC has been questioned when considering pathogenesis of HIV within the human host. Efforts are currently being made to develop sensitive and reliable ways of measuring HIV disease progression, with particular emphasis being placed on the relationship between the quantity of virus at various anatomical sites and prognosis. The extent of virus replication within the host immediately following infection with HIV-1, and during the asymptomatic phase of disease and its relationship to progression to AIDS, is being investigated. For example, whilst HIV-1 is not easily detectable in the peripheral blood of many asymptomatic individuals, it has been shown to be actively replicating in lymph nodes.<sup>3</sup> Active replication

of HIV may well be occurring at other sites including the lung during the asymptomatic phase of infection.

Pulmonary disease is a well established feature of HIV infection, and opportunistic pulmonary disease, most notably pneumocystis pneumonia, is frequently the AIDS-defining diagnosis.<sup>4</sup> Necropsy studies show that the lung is the most common site for AIDS-associated disease processes and is affected in 90% of patients,<sup>5-8</sup> although recently, as a result of widespread use of prophylaxis, there has been a fall in the incidence of pneumocystis pneumonia as an AIDS-defining illness.<sup>9,10</sup> Even so, a recent study reported that the lung, along with lymph nodes and spleen, was an important site for HIV replication in both paediatric and adult AIDS patients.<sup>11</sup> There is now evidence to show that HIV-1 enters the lung early in the natural history of HIV infection.<sup>11</sup> Both HIV-1 and HIV-2 are known to cause AIDS, but HIV-2 is substantially less virulent than HIV-1.<sup>12</sup> Worldwide infection with HIV-2 is important but information on its role in the lung is sparse. A number of recent reviews of HIV-1 and the lung<sup>13-16</sup> have concentrated largely on the immunological and immunopathological mechanisms. This review will analyse recent advances in our understanding of the role of HIV within the lung and its possible contribution to pathogenesis of pulmonary disease.

### Acute HIV infection

Up to 60% of individuals may develop an acute clinical illness 1-3 weeks after infection with HIV.<sup>17,18</sup> Symptoms may include headache, myalgia, lymphadenopathy, and rash.<sup>19,20</sup> In some individuals pneumonitis, diarrhoea, and encephalitis may occur.<sup>18-21</sup> During the acute phase a viraemia is observed, often with high levels of infectious virus detectable in plasma.<sup>22,23</sup> This "early" virus in cell-free peripheral blood is genotypically homogenous<sup>24-26</sup> and may be carried to the lung in the circulation, resulting in early pulmonary infection. A number of cell types in the lung are known to be susceptible to infection with HIV-1 including resident interstitial lymphocytes and alveolar macrophages. HIV-1 also both infects

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and replicates within lung fibroblasts<sup>27,28</sup> and these cells may yet prove to be an important reservoir for HIV in the lung. It is not known yet whether other lung cells such as bronchial epithelial cells or type I or II pneumocytes can support the replication of HIV-1. To establish exactly when HIV-1 reaches the lung following primary infection detailed studies of individuals close to time of infection will be required. An interesting question to be answered relating to acute infection with HIV-1 and lung involvement is whether individuals who do not have acute clinical symptoms relating to primary viraemia subsequently have less severe lung involvement during the course of disease.

#### Cellular receptor for HIV-1 entry into macrophages

It is well established that the CD4 molecule is the primary ligand for the viral envelope glycoprotein gp120 permitting HIV-1 infection of leucocytes.<sup>29,30</sup> Monocytes obtained from peripheral blood express greater numbers of CD4 on their cell surfaces than do tissue macrophages. Even so, macrophages are more susceptible than monocytes to infection by HIV, a higher percentage of macrophages can be productively infected and, following infection, the virus grows to a higher titre.<sup>31</sup> However, HIV-1 entry to macrophages can be competitively blocked by soluble CD4.<sup>32</sup> Some strains of HIV-1, despite being capable of attachment to macrophage surface CD4 and fusion with the cell membrane permitting entry of the virus into the cell, do not productively infect these cells.<sup>32</sup> A recent report has shown that, in some macrophage cell lines, there is a block to productive infection by HIV-1 at the stage between reverse transcription of viral RNA and integration of proviral DNA.<sup>33</sup> This suggests that a host cell-virus interaction may be required between successful virus penetration and integration into the host cell genome.

Several non-primate cell lines that express the human CD4 molecule are also resistant to both penetration by HIV-1 and subsequent syncytium formation.<sup>34-36</sup> The block to infection by HIV-1 in most non-primate cells is at the stage of membrane fusion and this has been attributed to the lack of a necessary second receptor.<sup>37-39</sup> Recently it has been suggested that a type of cell surface protease – dipeptidyl aminopeptidase IV (also called CD26) – is the second receptor for HIV-1.<sup>40</sup> This receptor is a good candidate as many cell types express CD26 on their surface including human activated B and T lymphocytes and macrophages.<sup>41</sup> However, other workers have been unable to show that CD26 is involved in HIV-1 entry into cells.<sup>42-45</sup>

Once inside the macrophage, in order to replicate HIV-1 converts its RNA genome into DNA. This is achieved by reverse transcriptase (RT). Recently RT activity has been shown to be appreciably slower in macrophages than in established lymphocyte cell lines.<sup>46</sup> Whilst HIV viral DNA synthesis was completed by 12–16 hours after infection of a T lymphocyte cell

line H9, the same process required more than 36 hours in macrophages. An important factor in the pathogenesis of HIV infection within the lung could be whether the rate of HIV replication increases when macrophages are activated, and whether productive HIV infection within these cells further abrogates their function in terms of antigen presentation or cytokine production.

#### Detection of HIV-1 in the lung by molecular techniques

An early report of using in situ hybridisation techniques on frozen lung sections indicated that HIV-1 RNA was expressed in lung cells from four out of 11 (36%) patients.<sup>47</sup> In one patient, an infant with lymphocytic interstitial pneumonitis, HIV-1 specific RNA was detected in 0.1% of lung cells, whilst viral RNA was detected in 0–0.002% of lung cells from the adult patients, indicating low levels of viral replication in situ. Hybridisation assays are not as sensitive as the polymerase chain reaction (PCR) for the detection of HIV-1. In one study, HIV-1 proviral DNA was detected by PCR in cells obtained at BAL in all eight adult individuals, even though the virus was not isolated from the cell-free BAL fluid obtained from any of the patients.<sup>48</sup> However, p24 antigen was detected in the BAL fluid of one patient, suggesting active in vivo replication of the virus. A more recent study detected HIV-1 proviral DNA from the alveolar cells of all 15 HIV-1 seropositive individuals who varied in disease stage from CDC II to IV.<sup>49</sup> In another study HIV-1 proviral DNA was detected in the BAL cells of 21 of 44 (47%) individuals with AIDS.<sup>50,51</sup> More extensive investigation by the same group detected HIV-1 proviral DNA in BAL cells of 80 of 124 (65%) AIDS patients using PCR.<sup>52</sup> Other PCR-based studies have reported HIV-1 proviral DNA in BAL cells of between 50% and 85% of adult HIV-1 seropositive individuals tested.<sup>53,54</sup> Interestingly, a recent report detected HIV-1 proviral DNA in the alveolar macrophages of 100% of paediatric AIDS patients but of only 67% of adult AIDS patients.<sup>55</sup> Furthermore, Landay and co-workers, whilst detecting HIV-1 proviral DNA in the alveolar cells (both alveolar macrophages and lymphocytes) of 85% of adult AIDS patients, could detect proviral DNA in the alveolar macrophages of only 57% of them.<sup>54</sup>

In two PCR-based studies the frequency of detection of HIV-1 proviral DNA increased with disease progression. In the first study HIV-1 specific DNA was detected from the BAL cells of 55% of individuals who had been HIV-1 seropositive for less than one year, and from 72% of those seropositive for more than two years.<sup>56</sup> In the other study HIV-1 DNA was detected in the alveolar macrophages of 33% of asymptomatic individuals and 63% of AIDS patients.<sup>53</sup> Peripheral blood CD4 + T lymphocyte cell number was also important, with HIV-1 specific DNA being detected in the BAL cells of 70% of patients with a CD4 + T lymphocyte count of  $<0.2 \times 10^9/l$  in peripheral blood, compared with only 17% with a CD4 cell count of

$>0.2 \times 10^9/l$  in blood.<sup>56</sup> In one study HIV-1 proviral DNA copy number was found in all instances to be higher in alveolar macrophages than in peripheral blood mononuclear cells from the same patients.<sup>53</sup> In contrast, another study found no correlation in proviral DNA copy number between these two cell types.<sup>57</sup> Some groups have also reported higher quantities of proviral DNA in alveolar lymphocytes than alveolar macrophages,<sup>54</sup> but other groups have found no correlation between HIV proviral DNA copy number in these cells.<sup>57</sup>

By the technique of limited cell dilution quantitative PCR, HIV-1 DNA has been detected in between 0.01% and 1% of BAL cells in some adult AIDS patients.<sup>57</sup> A correlation between detection of HIV-1 proviral DNA in BAL cells and progression to death<sup>56</sup> has been observed, suggesting that sequential monitoring of HIV-1 DNA in BAL fluid may have prognostic value; however, this preliminary study awaits verification by others. At present there are no published PCR studies based on detection of HIV-1 RNA from BAL cells. These would provide a sensitive method for determining the number of cells expressing HIV-1 RNA and thus the extent of viral replication in the lung. HIV-1 RNA expression is being studied in peripheral blood quantitatively using reverse transcriptase-based PCR studies.<sup>58-60</sup> It has been shown that abundant expression of HIV-1 RNA in peripheral blood mononuclear cells of infected individuals is predictive of active disease progression.<sup>58</sup> At the point of seroconversion there are no significant differences in HIV RNA copy number in serum samples regardless of whether individuals subsequently remain asymptomatic or rapidly progress to AIDS.<sup>59</sup> However, others have found that HIV-1 proviral DNA copy number is significantly higher early in the course of infection in those with rapid disease progression than in those with slower progression.<sup>60</sup> Interestingly, DNA copy number was found to increase by only 2-5-fold as disease progressed, whereas the number of cells productively infected increased by 21-30-fold. Significant changes in the levels of viral RNA, but not proviral DNA, were observed after the initiation of antiretroviral chemotherapy and also at the point where chemotherapy failed.<sup>61</sup> It will be of interest to undertake similar prospective sequential studies on matched peripheral blood and BAL samples to determine whether the number of cells productively infected increases in both compartments as disease progresses.

#### Significance of the isolation of HIV-1 from BAL cells

Whilst some groups have failed to isolate HIV-1 from BAL cells,<sup>48,49</sup> others have succeeded. The first isolation was from a Haitian woman with lymphocytic interstitial pneumonitis.<sup>62</sup> The differential count of the BAL cells from this woman revealed 57% to be lymphocytes (normal values <10%) of which 87% were of the CD8+ phenotype. Other groups have isolated HIV-1 from BAL fluid in all of four cases;<sup>63</sup> three of 24 (13%);<sup>64</sup> 34 of 75 (45%);<sup>65</sup>

and 37 of 63 (59%)<sup>66</sup> individuals. In one study HIV-1 could be isolated from 38% of purified alveolar macrophages but from 80% of purified alveolar lymphocyte samples.<sup>65</sup> These data suggest that the isolation of HIV from BAL cells may simply reflect the extent of trafficking of lymphocytes into the lung from peripheral blood. Peripheral blood CD4+ T lymphocyte cell number has been shown to have a profound effect on the ability to isolate HIV-1 from BAL cells. Virus is rarely isolated from BAL fluid in individuals with a blood CD4+ count of  $0.3 \times 10^9/l$ .<sup>65-69</sup> This suggests that HIV-1 may be latent in the lung during the asymptomatic phase of disease. Alternatively, there may be fewer HIV-1 infected cells in the lung while the CD4 count remains high, thus increasing the difficulty of recovering infectious virus.

#### Cellular tropism and phenotypic variation of HIV-1

Infection with HIV-1 is not clonal. Genomic diversity among independently obtained HIV-1 strains, to a lesser degree among sequential strains from the same patient, and even within a single isolate from a patient is a well characterised feature of HIV-1.<sup>70</sup> Whilst this diversity is distributed throughout the entire HIV genome, most DNA sequence and amino acid variation is found within the envelope gene and its products. This is particularly so within the external gp120 protein where there are five hypervariable regions referred to as V1 to V5.<sup>70</sup> As HIV disease progresses from the asymptomatic phase to AIDS, amino acid changes in the hypervariable regions are accompanied by the emergence of more virulent strains of HIV-1.<sup>71,72</sup> The biological phenotype of early virus strains is slow growing in culture and is detected in low titre.<sup>71</sup> These viruses fail to induce cytopathic effects in culture and are termed non-syncytium inducing (NSI) strains.<sup>72</sup> A further characteristic of NSI strains is that their growth is restricted to cells of mononuclear phagocyte lineage (macrophage tropic). Later on in the course of HIV infection just before or at the time of progression to AIDS, rapidly growing strains of HIV-1 emerge.<sup>72</sup> These viruses grow to a high titre in culture, cause cytopathic effects, and are termed syncytium inducing (SI) strains.<sup>71,72</sup> A further characteristic of SI strains is that they replicate in a wide range of transformed T cell lines in addition to cells of monocytic lineage. One determinant of cellular tropism is amino acid variation within the third hypervariable region of gp120, the V3 loop. Although this region consists of only 35 amino acids, considerable sequence variability exists between different virus isolates.<sup>70</sup> V3 is important as a major neutralising epitope for antibodies<sup>73</sup> and is also a cellular immune target for HIV-1 restricted cytotoxic T cells.<sup>74</sup> The V3 loop is an important determinant in the cellular tropism of HIV strains, although other regions of gp120 may be involved. SI strains have a basic amino acid at one or more of the following positions: 11, 24, 25, and 32 in the V3 loop, whilst NSI-macrophage tropic viruses have a neutral amino acid at these positions.<sup>70</sup>

Recent reports have failed to find evidence of distinct macrophage tropic strains, although strains of HIV-1 differed in their replication capacity as measured by the production of infectious progeny<sup>75</sup> and in their syncytium forming capacity within macrophages. Rapidly growing SI phenotypes did not differ from slow growing NSI phenotypes in their ability to grow in monocyte derived macrophages.<sup>76</sup> This finding is in contrast to earlier reports which indicated that the phenotype of HIV-1 could be substantially changed by passage through lymphocytes<sup>77</sup> so that these viruses no longer grew in macrophages. The question therefore arises – are the HIV-1 genotypes and phenotypes in the lung the same as those in the peripheral blood? Schuitemaker *et al*<sup>78</sup> found the HIV-1 populations in cells obtained by BAL from one AIDS patient contained mainly NSI-monocytotropic viruses and that the BAL-derived virus grew in culture to a higher titre than the corresponding isolates of peripheral blood leucocytes. This is important as it indicates that HIV-1 strains from the lung may be biologically different from those isolated simultaneously from the peripheral blood. Of note, the replication kinetics of HIV-1 strains obtained from BAL lymphocytes of three HIV-1 seropositive individuals were found to be similar to those obtained from numerous isolates from their peripheral blood leucocytes.<sup>79</sup> It seems likely that, in this instance, lymphocytes obtained from BAL cells included HIV-1 infected CD4 lymphocytes recruited directly from peripheral blood. In this respect, it has been shown that the biological phenotype of HIV-1 isolated from the lung of AIDS patients can be distinguished in all instances from the corresponding strains isolated simultaneously from the peripheral blood.<sup>80</sup> Recently, rapidly changing HIV-1 infection has been described in an AIDS patient who had sequential bronchoscopies where the phenotype of HIV-1 switched from NSI to SI in the lung before a similar change was detected in peripheral blood.<sup>81,82</sup> Genetic analysis of HIV-1 proviral DNA recovered from blood monocytes and alveolar macrophages of eight patients showed that the V3 loop region of HIV-1 strains from both cell types varied.<sup>83</sup> More importantly, as many as eight out of 17 amino acids in the V4 loop were different from the matched blood monocyte and alveolar macrophage viruses. This strongly suggests that these two cell populations are being infected with HIV-1 separately, rather than HIV-1 infected blood monocytes differentiating into alveolar macrophages in the lung. This is of interest as one proposed mechanism for HIV-1 gaining access to the lung is through HIV-1 infected peripheral blood monocytes entering the lung and differentiating into alveolar macrophages.<sup>4,16,68</sup> At post mortem examination it has been shown that the predominant infected cell in the lung is the alveolar macrophage.<sup>84</sup> Even though the amino acid sequences of lung isolates predicted predominantly NSI phenotypes, giant cell syncytia were frequently observed in the lungs of these patients indicating that *in vivo* the distinction between SI and NSI strains may not exist.

### Possible mechanisms of HIV-induced pathogenesis in the lung

Three possible mechanisms exist whereby HIV-1 may deplete alveolar lymphocytes and macrophages. HIV-1 infected alveolar cells may be destroyed by (1) cytotoxic T lymphocyte-mediated cytolysis; (2) virus-mediated cytolysis; and (3) syncytia-mediated cytolysis.<sup>14,16</sup>

A lymphocytic alveolitis which can culminate in lymphocytic interstitial pneumonitis occurs in up to 60% of adult HIV-1 infected patients and can be detected throughout all stages of HIV disease including the asymptomatic phase.<sup>85,86</sup> The lymphocytes are largely HIV specific CD8+ cytotoxic T lymphocytes and the early stages at which these CD8+ cells can be detected in the lung further suggests early infection of the lung by HIV-1.<sup>87,88</sup> Alveolar macrophages from HIV-1 infected individuals at all stages of disease have been shown to express macrophage inflammatory protein-1 $\alpha$  (MIP-1 $\alpha$ ) which exerts a strong chemotactic activity on purified activated blood CD8+ T lymphocytes.<sup>89</sup> HIV-restricted CD8+ T lymphocytes inhibit HIV replication *in vitro* and the extent of inhibition may correlate with disease state.<sup>90</sup> Cytotoxic T lymphocyte activity also occurs in HIV-infected children<sup>91-93</sup> and this may be important in disease progression and the development of lymphocytic interstitial pneumonitis.

An important question is whether HIV-1 can infect and replicate within CD8+ T lymphocytes. HIV-1 has been observed by electron microscopy to be budding from the surface of up to 10% of CD8+ T cells.<sup>94</sup> CD8+ T lymphocytes infected with HIV-1 have recently been reported resulting in the accumulation of unintegrated HIV-1 proviral DNA and the production of progeny virions capable of infecting CD4+ T lymphocytes.<sup>95</sup> Infection by HIV-1 of alveolar CD8+ lymphocytes may therefore offer an alternative mechanism by which HIV-1 might enter the lung and this has implications for pathogenesis.

In virus-mediated cytolysis the accumulation of virus or virus products within an infected cell before the expression of viral antigens on the cell surface could lead to toxic destruction of the cell.<sup>68</sup> In this respect the integration of HIV-1 proviral DNA into the host cell genome is important for productive infection.<sup>96</sup> Integration of HIV-1 DNA appears to be relatively random although there are some privileged sites.<sup>97,98</sup> This integration step could disable the gene into which virus is being integrated or change the function of surrounding open reading frames by either down- or up-regulation at the transcriptional level. This could have a profound effect on the immune function of these cells.<sup>99</sup> Increasing viral burden in peripheral blood CD4+ T lymphocytes is directly proportional to the progressive decline in these cells and to deteriorating clinical course in HIV infected patients.<sup>99,100</sup> Alternatively, cell dysfunction and cell depletion in HIV-infected individuals may be caused by the inappropriate induction of programmed cell death (apoptosis).<sup>101</sup> Induction of apoptosis occurs in both CD4+ and CD8+ T lymphocytes in HIV-1

infected individuals and this process may be important to the pathogenesis of AIDS. Studies involving genetically immunodeficient SCID-hu mice which lack both T and B lymphocytes when reconstituted with adult human T lymphocytes and monocytes showed that infection of these mice with NSI monocytotropic molecular clones of HIV-1 led to a more rapid and profound depletion of CD4+ T lymphocytes than did infection with the more pathogenic lymphocytotropic SI strains.<sup>102</sup> Consequently, similar processes occurring in the lung could lead to depletion of cellular immune function.

The third possible mechanism of virus-mediated pathological damage may result from cytopathology induced by virus replication.<sup>14,68</sup> It has been suggested that HIV-1 infection of alveolar macrophages in vivo is latent due to inadequate stimulation of HIV-1 replication in the alveolar environment.<sup>49</sup> However, this study also detected p24 antigen in the cell-free BAL fluid of the only two patients with CDC stage IV disease, suggesting that HIV was replicating in the lung late in the disease process. Another study detected p24 antigen in the cell-free BAL fluid in 22% of patients with AIDS.<sup>56</sup> Furthermore, HIV-1 RNA has been detected by reverse transcriptase PCR in the cell-free BAL fluid of all HIV seropositive individuals tested, implying that HIV-1 is replicating in the lung in vivo. We have recovered SI HIV-1 strains from BAL cells which are capable of causing cytopathology in both lymphocytes and macrophages in vitro.<sup>67,69,80,81</sup> Others have found HIV-1 infected giant cells in pathological tissue obtained from the lung, suggesting that HIV-1 does contribute to cytopathology in the lung in vivo.<sup>84</sup> What needs to be established is the relationship between productive virus infection in the lung and the manner in which this affects cytokine production.<sup>14,16</sup>

#### Immunological mechanisms in response to HIV within the lung

In addition to CD4+, CD8+ T lymphocytes and alveolar macrophages,<sup>103</sup> lung fibroblasts<sup>28</sup> have also been shown to be infected by HIV both in vitro and in vivo. Other cells such as eosinophils may be infected by HIV via the CD4 molecule,<sup>104</sup> whilst dendritic cells and epithelial cells may become infected through uptake of HIV/antibody complexes.<sup>105</sup>

#### T LYMPHOCYTES

As discussed above, HIV seropositive individuals have a predominantly CD8+ lymphocytic alveolitis in contrast to the profound lymphopenia seen in peripheral blood.<sup>108,109</sup> This is due to an increase in CD8 lymphocytes present in 25% of those with early HIV disease and 50% of AIDS patients.<sup>106</sup> These CD8 cells consist of two populations.<sup>107</sup> Early in HIV infection there is a predominant CD8+ D44+ MHC class I restricted cytotoxic T cell expansion in the BAL cell population with cytolytic activity against HIV infected cells.<sup>87</sup> There is also a population of CD8+ cells which express the CD57 surface marker usually

associated with natural killer cells. However, rather than cytolytic killer function, these cells suppress cytotoxic activity of CD8+ and natural killer cells and inhibit B cell differentiation in vitro. This is dependent on production of a 20–30 kDa glycosylated protein, which is distinct from tumour necrosis factor alpha (TNF $\alpha$ ), TNF $\beta$ , transforming growth factor (TGF)  $\alpha$  and  $\beta$ , interferon (IFN)  $\alpha$  and  $\gamma$ , interleukin 1 (IL-1), and prostaglandins.<sup>110,111</sup> Suppressor CD8+ CD57+ cells have been described in the peripheral blood in response to Epstein-Barr virus infection and have also been demonstrated in the lung in bone marrow recipients. They may thus represent a response to repetitive cytolytic T cell activation generally rather than a specific response to HIV itself.<sup>111</sup> Although the presence of CD8+ CD57+ subset of alveolar T lymphocytes can be detected early in the course of HIV infection, the number of CD8+ D44+ cells appears to decrease as HIV disease progresses, whilst CD8+ CD57+ cells become the predominant T lymphocyte type in BAL fluid. Whether this results from a failure of CD4+ lymphocyte “help” or the emergence of more virulent HIV strains remains to be determined. These findings are compatible with an initial CD8+ cytolytic response to HIV in the lung which may have a role in limiting viral propagation and replication in both CD4+ T lymphocytes and alveolar macrophages.<sup>110,111</sup> Whether this response is protective and why it fails are questions for further research. What is of interest is that CD8+ CD57+ T lymphocytes suppress other effector killer cells in the lung including natural killer and lymphokine activated killer cells.

In contrast to the CD8+ lymphocytosis, CD4+ T lymphocytes are reduced in BAL fluid from subjects with HIV infection, even in early disease. Whether this simply parallels the progressive decline in peripheral blood is unknown. Early loss of reactivity to recall antigens in HIV disease suggests that memory T lymphocytes are selectively affected<sup>112</sup> and lung CD4+ T lymphocytes are almost entirely of memory phenotype.<sup>113</sup> Thus, CD4+ lymphocyte depletion may occur earlier in the lung than in peripheral blood. The lung is an important lymphoid organ with trafficking of memory T lymphocytes through the lung capillary bed, and so is likely to play an important part in surveillance against inhaled antigens. As the lung probably acts as a reservoir for active HIV proliferation as occurs in lymph nodes<sup>114</sup> when HIV is almost undetectable in blood, it is possible that memory CD4+ T lymphocytes become infected during passage through the lung. CD4+ T lymphocytes have been shown to be vital in protection against *Pneumocystis carinii* in animal models,<sup>115</sup> and it is progressive failure of local CD4+ T lymphocyte helper function that probably determines the development of pneumocystis pneumonia in HIV infected individuals.

On the basis of a shift in the cytokine profile produced by phytohaemagglutinin-stimulated peripheral blood mononuclear cells from patients with late HIV disease compared with those with early infection, it has been suggested

that as HIV infection progresses there is a shift from a Th1 pattern of CD4+ T lymphocyte cytokine response with IL-2 and IFN $\gamma$  production to a Th2 pattern with IL-4 and IL-5 production.<sup>116</sup> This is an attractive hypothesis since Th1-type cytokines are associated with cell-mediated immune responses and have been demonstrated to predominate in the lung in non-HIV infected individuals with pulmonary tuberculosis,<sup>117</sup> whereas the Th2 pattern of cytokines is associated with antibody production and atopic allergy and has been demonstrated in the lung in individuals with atopic asthma.<sup>118</sup> In laboratory mice the outcome of infection with *Leishmania* can be shown to depend on the T cell pattern of cytokine response predominant in that particular strain; some strains mount a Th1 response and eliminate the infection, whereas Th2 responders succumb to infection.<sup>119</sup> However, this view that HIV infection is associated with a shift to a Th2 pattern of response has recently been challenged. Analysis of cytokine profiles in lymph nodes from patients with HIV infection did not show a Th2 pattern; indeed, most cytokine production was from non-CD4+ cells.<sup>120</sup> Large numbers of T cell clones from HIV seropositive and normal individuals have been examined and no shift to a Th2 cytokine profile was seen in those with HIV infection.<sup>121</sup> However, there was a reduction in Th1 clones from HIV seropositive individuals, especially in response to PPD, which elicited a predominant Th1 response in normal subjects. Instead, there was a shift to both Th2 and the intermediate Th0 pattern (cells producing both Th1 and Th2 cytokines). Also, in vitro, Th2 and Th0 clones were more susceptible to HIV infection than Th1 clones.<sup>121</sup> This issue is not resolved, but it is of note that individuals with high serum IgE (which accompanies Th2 activation) are reported to progress more rapidly to AIDS than those with normal IgE, and some individuals who mount a cell-mediated response to HIV but no antibody response appear to remain healthy.<sup>122</sup> Whether there are changes in the T cell cytokine profile in the lung either to HIV or to opportunistic infections as disease progresses remains unknown. Different patterns of CD8+ T cell cytokine production are described,<sup>123</sup> and may also have a functional role. It will be important to examine cytokine responses of both CD4+ and CD8+ T cells in the lung if therapeutic manipulation of such cytokines becomes a reality.

#### *Effects of HIV on alveolar macrophage function*

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Increased numbers of alveolar macrophages in BAL fluid in some subjects  
 Change in surface markers  
 Enhanced accessory cell function  
 Spontaneous production of cytokines  
 (TNF $\alpha$ , IL-1, IL-6, GM-CSF, MIP-1 $\alpha$ , TGF $\beta$ )\*  
 Priming for enhanced cytokine response to lipopolysaccharide  
 (TNF $\alpha$ , IL-1, IL-6, GM-CSF)  
 Cytokine production in pneumocystis pneumonia  
 (TNF $\alpha$ , GM-CSF, IL-8)  
 Preserved responsiveness to interferon  $\gamma$  (IFN $\gamma$ )  
 Decreased phagocytosis in AIDS

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\* Reports differ on whether cytokines are produced from alveolar macrophages in HIV spontaneously or after stimulation. This may reflect patients studied or culture conditions used.

Cytokines produced by other cell types profoundly influence T cells, and the interactions with macrophage derived IL-10 (which inhibits both Th1 and Th2 cell cytokine production<sup>124</sup>) or IL-12 (which switches to Th1 IFN $\gamma$  production) remain to be explored in the lung in HIV.

#### ALVEOLAR MACROPHAGES

Alveolar macrophages serve a wide variety of functions in the lung, both as phagocytes and regulators of specific immune responses. Although they express surface HLA DR molecules,<sup>125</sup> these cells are poor antigen-presenting cells (APC) in normal individuals and, indeed, suppress T cell responses.<sup>126,127</sup> However, it has been shown that alveolar macrophages from HIV seropositive subjects demonstrate enhanced APC activity in vitro when compared with cells from normal volunteers.<sup>128</sup> It has also been shown that more IL-1 and IL-6 production occurred from alveolar macrophages from HIV infected donors than normal subjects, and that APC function of alveolar macrophages was less critical on macrophage-T cell adherence in BAL fluid from HIV positive individuals.<sup>129</sup> It has been suggested that the increased interaction between alveolar macrophage and T cells in the lung in HIV infection may be a mechanism for propagation of HIV from alveolar macrophages to non-infected CD4+ T cells. However, the importance of these findings for immune responses is unclear.

Alveolar macrophages may amplify immune responses through production of such pro-inflammatory cytokines as IL-1 and TNF $\alpha$  (which stimulate recruitment of other cells through increased adhesion to vascular endothelium and subsequently activate functions such as neutrophil phagocytosis),<sup>130-132</sup> IL-6 (which stimulates the acute phase response and other inflammatory cells),<sup>133</sup> granulocyte-macrophage colony stimulating factor (GM-CSF) (which recruits neutrophils and monocytes), and chemokines such as IL-8 (which is a neutrophil chemoattractant) and macrophage inflammatory protein 1 $\alpha$  (MIP-1 $\alpha$ ). A number of reports suggest that alveolar macrophages in BAL fluid from HIV infected individuals either release these cytokines spontaneously or are primed to do so in response to lipopolysaccharide from infectious agents such as *Pneumocystis carinii*. These cytokines and chemokines are summarised in the table. The demonstration of MIP-1 $\alpha$  by alveolar macrophage production in HIV is of interest since this agent is chemoattractive to CD8+ cells, and secretion of MIP-1 $\alpha$  by alveolar macrophages in vitro correlates with the intensity of CD8 alveolitis in vivo in HIV positive subjects.<sup>89</sup> As discussed above, this tends to confirm the hypothesis that HIV affects alveolar macrophage function early in disease, since CD8+ alveolitis is seen even in asymptomatic HIV seropositive subjects.

Although enhanced production of pro-inflammatory cytokines by alveolar macrophages in HIV might be beneficial in combating

both HIV and potential opportunists, expression of these cytokines might also have detrimental actions. TNF $\gamma$ , IL-1, and IL-6 can potentially activate HIV replication by actions on the NF $\kappa$ B promoter,<sup>134,135</sup> so might actually propagate HIV within the lung. There may also be protective effects on HIV proliferation from cytokines such as TGF $\beta$ <sup>35</sup> which are also reported to be produced by alveolar macrophages in HIV infection.<sup>128</sup>

Similarly, cytokines may be either directly toxic to pulmonary epithelium or recruit and activate neutrophils producing reactive species that may contribute to the alveolar capillary leak seen in pneumocystis pneumonia in AIDS. Thus, in AIDS, as in other conditions, an exuberant inflammatory response to pathogens may itself contribute to morbidity and mortality. Corticosteroids have been shown to be beneficial as an adjunct in the treatment of pneumocystis pneumonia.<sup>136</sup> It has recently been shown that patients with pneumocystis pneumonia receiving corticosteroids show a reduced alveolar macrophage production of IL-1 $\beta$  and TNF $\alpha$ .<sup>137</sup>

Although alveolar macrophages from HIV infected individuals showed upregulation of IFN $\gamma$ -induced cytotoxicity to *Toxoplasma gondii*,<sup>138</sup> alveolar macrophages isolated from subjects with AIDS and pneumonia had decreased phagocytic capacity.<sup>139</sup> It is possible that different functions of alveolar macrophages are dependent on the cytokine environment present at different states of HIV infection. It is possible that early in HIV infection there is activation of their function leading to cytokine production and paradoxical activation of HIV replication, with a later loss of activation of cytokines such as IFN $\gamma$ .

#### OTHER CELL TYPES

The lung contains a dense network of dendritic cells.<sup>140</sup> It is suggested that dendritic cells may become infected with HIV and this may lead to a loss or alteration in the accessory signals that are vital to co-ordinated T lymphocyte responses. The role of granulocytes in HIV lung pathology is unclear.<sup>141</sup> GM-CSF is chemotactic to neutrophils and eosinophils, as is IL-8 which has been demonstrated in BAL fluid from patients with pneumocystis pneumonia.<sup>142</sup> Raised neutrophil counts in BAL fluid from subjects with pneumocystis pneumonia carries a poor prognosis,<sup>143</sup> and BAL eosinophilia may also accompany pneumocystis pneumonia.<sup>144</sup> The consequences of HIV infection of CD4 + lung fibroblasts are unknown. Whether bronchial and alveolar epithelial cells are directly infected by HIV is unknown, although there is good evidence of alveolar capillary epithelial leak even in asymptomatic HIV disease.

#### Functional consequences of HIV in the lung

Abnormalities of pulmonary function tests, particularly reductions in the carbon monoxide transfer factor (TLCO), have been described in asymptomatic HIV seropositive individuals

without evidence of pulmonary disease,<sup>145</sup> and abnormalities in alveolar capillary permeability, as determined by technetium-99m labelled diethylenetriaminepentaacetic acid (<sup>99m</sup>Tc-DTPA) lung to blood clearance, have also been described in these patients.<sup>146</sup> Attempts to define these abnormalities in physiological terms have not been illuminating and imaging techniques with high resolution computed tomographic scanning have not disclosed any abnormalities such as early interstitial lung disease.<sup>147</sup> However, both of these abnormalities have been correlated with the intensity of CD8 + T cell alveolitis suggesting that they may result from the direct effects of cytotoxic CD8 + T cells on the epithelium.<sup>148</sup> The presence of HIV proviral DNA as detected by PCR does not seem to be related to abnormalities in TLCO as patients with and without detectable HIV in BAL fluid had similar reductions in TLCO.<sup>52</sup> Furthermore, the reductions in TLCO in all categories of HIV disease were not related to treatment with zidovudine, but smoking had an additive effect on decrements in TLCO but could not be invoked to explain all of the abnormality,<sup>145</sup> nor was the ability to isolate HIV by co-culture in vitro related to reductions in TLCO.<sup>52</sup> Again, patients who had HIV recoverable by co-culture in vitro from BAL cells had identical reductions in TLCO when compared with patients where HIV could not be isolated in vitro.<sup>52</sup> Cigarette smoking – in addition to its generally deleterious effects on the lung including abnormalities of lung <sup>99m</sup>Tc-DTPA clearance and reductions in TLCO – seems to have specific adverse effects on the lung in individuals with HIV infection. Firstly, smokers who are infected with HIV progress more rapidly to their first AIDS-defining diagnosis than non-smokers, and have decreased survival.<sup>149</sup> Secondly, HIV is more readily isolated in vitro from BAL fluid from smokers than from non-smokers.<sup>56</sup> It has recently been shown that alveolar macrophages from smokers with HIV infection did not show the upregulation of accessory cell function seen in HIV seropositive non-smokers. This effect appeared to be due to reduced IL-1 and IL-6 production. The lymphocytic alveolitis was also less intense in smokers, suggesting that MIP-1 $\alpha$  might also be reduced.<sup>150</sup>

#### Conclusions

HIV probably enters the lung early during the course of infection and may constitute an important site for virus production in HIV infected individuals. It can be detected in the lung during the asymptomatic phase and the load increases as disease progresses. HIV is present in both alveolar macrophages and alveolar lymphocytes, as well as other cell types within the lung, and may have profound early effects on alveolar macrophage and T lymphocyte function resulting in a CD8 + alveolitis which, although initially may be protective, later appears to be suppressive towards HIV-directed cytotoxicity. The activation of alveolar macrophages by HIV and other infectious agents may, by release of proinflammatory cyto-

kines such as TNF $\alpha$  and GM-CSF, lead to impaired gas exchange but also to further HIV replication. Further refinement of our understanding of the interplay between the virus and the local response within the lung could allow the possibility of therapeutic immunomodulation with local and appropriate cytokine therapy to the lung to reduce the current morbidity and mortality from AIDS.

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