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EDITORIAL

Human retroviruses and their aetiological link to pulmonary diseases

G. Semenzato*, A. de Rossi**, C. Agostini*

During the past decade, there has been an extensive effort to conclusively implicate human retroviruses or their products in the pathogenesis of many inflammatory pulmonary diseases. Progress in this field has been rapid and impressive, fueled primarily by striking conceptual and technical advances in basic immunology and molecular biology. On clinical grounds, a wide clinical spectrum of lung diseases has been reported in patients with human T-leukaemia virus type I (HTLV-I)-related adult T-cell leukaemia/lymphoma (ATL) and tropical spastic paraplegia/HTLV-I associated myelopathy (TSP/HAM). Evaluation of these disorders has raised novel hypotheses concerning the relationship between the retroviral infection and the development of lung involvement. Furthermore, a number of *in situ* complications has been related to the effects of the human immunodeficiency virus (HIV) on the pulmonary immune system.

In this issue of the Journal, on the basis of the observation of patients with HTLV-I infection, uveitis and T-lymphocyte alveolitis, Sugimoto *et al.* [1] suggest that HTLV-I infection can lead to an adaptive increase of pulmonary CD8+ suppressor/cytotoxic cells. The most compelling evidence implicating HTLV-I in the pathogenesis of interstitial lung disease is the presence of a discrete number of cells harbouring HTLV-I within the pulmonary environment [1]. The above observation, coupled to the evidence that HIV-1 enters the lung and infects pulmonary cells [2], supports the hypothesis that the lung parenchyma may serve as an important reservoir for virulent retroviral strains.

Background on human retroviruses

The role of retroviruses in animal diseases was already known early in this century, but it was only at the end of the 1970s that the first human retrovirus (the HTLV-I) was isolated and associated with a human disease, *i.e.* the ATL [3]. HTLV-I is now known to be involved in other human diseases, including HTLV-I-associated TSP/HAM (a neurological disorder similar to multiple sclerosis), uveitis, polymyositis, infective dermatitis of Jamaican children, gastrointestinal malignant lymphoma and type T chronic lymphocytic leukaemia [3]. During the 1980s, at least three new prototypic human retroviruses were identified; of these, two are human immunodeficiency viruses, HIV-1 and HIV-2, which are aetiological agents for the acquired immune deficiency syndrome (AIDS), and one, HTLV-II has occasionally been associated with some haematological malignancies, but its aetiological role in these disorders is far from being completely established [3].

Retroviruses are defined by their ability to reverse the normal flow of genetic information from genomic deoxyribonucleic acid (DNA) to messenger ribonucleic acid (mRNA); this reverse transcription is operated by their own reverse transcriptase enzyme, which converts the single-stranded positive sense viral RNA into a double-stranded linear DNA. According to differences in their biological and pathogenic properties, retroviruses have been classified into three different taxonomic subgroups. *Oncovirinae* include exogenous and endogenous retroviruses that cause neoplastic diseases in the host, but also include some related and non-oncogenic viruses; infectious viruses belonging to this subgroup can be further subdivided into B-, C- and D-types, according to their morphology. HTLV-I and HTLV-II represent the prototypic human type-C exogenous retroviruses. *Lentivirinae* are exogenously transmitted retroviruses that have a unique ability to replicate continuously, but at a restricted rate in host tissues. These viruses are known to be associated with states of immunodeficiency and neurological disorders in several animal species, and may induce pulmonary diseases, particularly diffuse interstitial pneumonias. HIV-1 and HIV-2 are classified in the *Lentivirinae* group. *Spumavirinae* are present in a number of mammaliam species, including man. Their presence causes foamy cytopathic effects in tissue culture, and induces persistent infection without evident pathogenetic effect in their natural hosts.

Genomic organization and replication of human retroviruses

The genomic organization of the human retroviruses is more complex than that seen for most animal oncoviruses [4, 5]. Indeed, HTLVs and HIVs contain a number of accessory genes, in addition to the typical genes, *i.e. gag, pol* and *env,* that encode the nucleocapsid proteins, the replicative enzymes (reverse transcriptase, integrase and protease) and envelope proteins, respectively. At least five additional genes are deputed to the control of viral replication, virion maturation and morphogenesis [4, 5].

*Istituti di Medicina Clinica **di Oncologie dell' Università di Padova, Clinica Medica 1, Via Giustiniani 2, I-35128 Padova, Italy. Supported by Grants from the Minestero della Sanità - Progetto AIDS 1992/1993.

The known human retroviruses replicate in lymphocytes, cells belonging to the monocyte-macrophage lineage and various tumour cell lines. They infect the potential target through an interaction between viral envelope proteins and specific receptors expressed on the host cells surface. In the case of HIVs, CD4 protein is the main host cell surface receptor [6]. The receptor for HTLVs has been mapped to chromosome 17, but the encoded protein has not yet been identified. Once inside the cell, viral RNA is used as a template for the conversion to a double-stranded DNA by reverse transcriptase. For HIV-1, it has been demonstrated that the entire reverse transcription process occurs only in activated cells. The DNA intermediate (which is a complimentary copy of the viral genome) enters the nucleus and covalently integrates into chromosome of the host cell DNA. This integrated DNA, called provirus, is flanked by the characteristic long terminal repeats (LTRs), generated during the process of reverse transcription. LTRs contain promoter and regulatory elements required for the efficient transcription of the retroviral genome, and also contain sequences important for efficient mRNA polyadenylation. The provirus may remain in this form during the entire lifetime of the cell, and may pass on to daughter cells. Transcription of the integrated provirus may result in the synthesis of new RNA progeny particles that, following their assembly in the cytoplasm, may be shed in a process known as budding, thus completing the life cycle of the virus.

The switch from viral latency to productive infection is controlled by complex regulatory mechanisms involving transcriptional as well as post-transcriptional events, determined by interdigitated interactions between viral and cellular factors [4, 5]. In particular, transcriptional regulation exerted by LTRs appears to be strictly related to the establishment of, and escape from, latency. In fact, LTRs contain a large number of *cis*-acting positive and negative regulatory elements that are responsive to viral and cellular *trans*-acting transcriptional factors (*i.e.* the NF-kB binding elements and cyclic adenosine monophosphate (c-AMP) responsive binding proteins), that in turn recognize the *cis*-acting element located within LTR. The inference is that many stimuli that promote transcriptional cellular factors may control viral activation. In fact, *in vitro* systems in which HIV-1 infected cells are maintained in culture and exposed to different stimuli have demonstrated that HIV-1 re-enters the replicative cycle after the exposure of infected cells to activation signals, including phorbol esters, a number of cytokines (interleukin-1 (IL-1), interleukin-6 (IL-6), tumour necrosis factor α and β (TNFα and β) granulocyte-macrophage colony stimulating factor (GM-CSF), and other cellular activation factors (mitogens, exposure to sunlight). Furthermore, it has been shown that herpes viruses are capable of activating HIV-1 expression in HIV-1 infected lymphocytes. Thus, the dissemination of HIV-1 could, theoretically be initiated by a series of cellular and viral factors which are known to be involved in the pathogenesis of AIDS or to be produced during host immune response against HIVs and opportunistic AIDS-associated infections. By using a sensitive technology, retro-transcription polymerase chain reaction (RT-PCR) (see below), it has recently been demonstrated that a true phase of viral latency does not exist in HIV-1 infected subjects, because viral RNA can be found in the plasma of patients at all stages of infection, including the asymptomatic stage, albeit at different levels [7]. Similar studies are needed to verify whether circulating HTLV RNA can be detected in HTLV-infected subjects.

Identification of human retroviruses in the lung

The technique of bronchoalveolar lavage (BAL) deserved full credit for advancing the knowledge of the effects of human retroviruses on the lung microenvironment. Providing access to the different cell populations of the lower respiratory tract, this technique leads us to evaluate the inflammatory events that take place in the pulmonary microenvironment during retroviral infections, as well as the effects of retroviruses on host lung defence mechanisms [2]. In the past years, several techniques have been used to search for HIV-1 in cell populations isolated from the BAL of patients with AIDS, including virus culture of total and fractional BAL cell subpopulations, *in situ* hybridization, p24 HIV protein detection in BAL cells and fluids [8, 9]. By means of these different approaches, HIV has been identified in a variable proportion of the investigated cases, but sensitivity of all these procedures is limited by several biological (each technique enables the identification of specific steps of the viral life cycle) and technical (sample amount) factors. More recently, the introduction of the polymerase chain reaction (PCR), which allows specific amplification of the discrete DNA sequences, has increased the possibility of detecting proviral sequences [10]. Since intermediate DNA is a fundamental step in the retrovirus life cycle, PCR should, theoretically, allow for the identification of all, including latent, infections. PCR analysis of viral RNA following its *in vitro* retrotranscription (RT-PCR) also enables the identification of even low levels of viral RNA, that are undetectable with other procedures. The small amount of sample required for PCR analysis further increases the possibility of finding the virus in particular cell subtypes.

Pulmonary diseases caused by or associated with HTLV-I infection

Pulmonary diseases represent a frequent complication of HTLV-I infection. Over 90% of patients with ATL show involvement of the lower respiratory tract, the incidence rate being significantly higher than in other haemotological malignancies [11]. In about half of ATL patients, diffuse leukaemic infiltrates of the lungs can be demonstrated at the time of the initial diagnosis. Rose *et al.* [12] have reported that malignant cells infiltrating the lung are morphologically identical to circulating leukaemic cells; they express the CD4 phenotype and the IL-2 receptor (CD25), and their T-cell receptor genes are monoclonally rearranged. Another common aspect of most patients with ATL is the development of a variety of

infectious pulmonary complications. The vast majority of episodes of pneumonitis are due to opportunistic infections (*Pneumocystis carinii* pneumonia), thus suggesting that lung involvement during ATL has adverse effects on the local immunocompetence.

Clinical evidence of pulmonary involvement has also frequently been observed in patients with neurological disorders and HTLV-I infection [13, 14] and, as reported in this issue of the Journal by Sugimoto *et al.* [1], in patients with HTLV-I associated uveitis. A T-lymphocyte alveolitis can frequently be demonstrated in both diseases, and phenotypic studies have shown that CD4 and CD8 cells equally account for the increased number of lymphocytes. As a consequence, the pulmonary CD4/CD8 ratio may be normal or, as reported by Sugimoto *et al.* [1], slightly decreased in some patients with HTLV-I associated uveitis. Furthermore, BAL T-cells recovered from the lung of TSP/HAM seem to be preactivated, since they release increased levels of the soluble form of the IL-2 receptor [15].

At least in the southwestern region of Japan it is conceivable that HTLV-I plays a role in the pathogenesis of another interstitial lung disease, which is characterized by a diffuse lymphocyte infiltration, *i.e.* the lymphocytic interstitial pneumonia (LIP) [16]. In fact, the recent finding that a high proportion (84%) of Japanese patients with LIP are seropositive for HTLV-I suggests a direct relationship between the retroviral infection and the appearance of the extensive lymphoid infiltrate. Interestingly, the phenotypic evaluation of BAL cells isolated from these patients has shown that the lymphocytic population is mainly composed of CD8+/CD11- cytotoxic T-lymphocytes. This led to the speculation that cytotoxic subsets are probably involved in the host defence mechanisms against HTLV-I.

In spite of having clearly linked T-lymphocyte alveolitis to the retroviral infection, immunological findings did not establish whether HTLV-I can infect pulmonary cells. An underlying assumption, in the effort to exploit a causative role of HTLV-I in the lung, is represented in the accompanying paper by Sugimoto *et al.* [1]. This study provides new information for understanding the puzzle of interactions occurring in the pulmonary microenvironment as a consequence of HTLV-I infection. In fact, the above authors provide evidence that HTLV-I provirus is present on BAL T-lymphocytes from patients with HTLV-I-associated diseases. In particular, by using a semiquantitative DNA-PCR analysis, they demonstrate that the HTLV-I burden in both peripheral blood mononuclear and BAL cells is higher in patients with uveitis than in HTLV-I infected asymptomatic carriers. Interestingly, in three out of eight subjects studies, the HTLV-I load was higher in the BAL cells than in circulating mononuclear cells. Proviral DNA was detected mainly in alveolar lymphocytes, but PCR analysis of fractionated BAL cell subpopulations was performed in only three cases; in all of these, a low number of macrophages were suspected of carrying the virus.

A number of relevant questions concerning the pathogenesis of HTLV-I infection of the lung still remain unanswered, both from a virological and immunlogical stand-point. It has been demonstrated that HTLV-I can infect several cell types *in vitro*, including CD4+ and CD8+ lymphocytes, B-cells and macrophages [17, 18]. Although the presence of a few HTLV-I infected cells has been demonstrated within a macrophage-enriched population, the issue of whether an alveolar macrophage (AM) infection can occur also *in vitro* remains an open question. Other possibilities should be considered in interpreting the potential infectious role of HTLV-I in the lung. In particular, we do not know whether, in addition to BAL T-cells and AMs, other pulmonary cells may be HTLV-I infected, as occurs in patients with AIDS, where pulmonary fibroblasts, interstitial macrophages and dendritic cells behave as potential HIV-1 target cells [2]. Furthermore, in view of the fact that secondary lymphoid tissues of the lung have recently been claimed to act as a reservoir for the retroviral infections [6], detailed analyses are needed to determine whether a migration of circulating HTLV-I infected CD4+ cells to most of the follicles located throughout the bronchial tree may favour the establishment of HTLV-I infection in the lower respiratory tract. The comprehension of regulatory networks between HTLV-I and the local immune systems represents another key question, which should be addressed in the coming years. In particular, we do not know whether a latent HTLV-I infection of T-cells may affect the defensive ability of the pulmonary immune system, thus favouring the development of opportunistic infections and/or the diffuse infiltration of leukaemia cells in patients with ATL. Moreover, since recent data have demonstrated that activated HTLV-I specific cytotoxic T-lymphocytes may be found in healthy seropositives, as well as in patients with TSP/HAM [19], functional studies for investigating whether T-cells recovered from the BAL of HTLV-I infected patients provide cytotoxic function against HTLV-I infected targets will become mandatory. A third major area of investigation is the pattern of the release of cytokines occurring in the lung of patients with HTLV-I infection. As already mentioned, it is believed that cytokines and other cellular factors that are secreted during the pulmonary immune response against HIV-1 may initiate HIV-1 replication, and its spread within the respiratory tract [2]. HTLV-I is also known to turn on several cellular genes for cytokines, including IL-2, GM-CSF and TNFα. Based on the current knowledge of the pattern of cytokines acting in the lower respiratory tract [20], it is conceivable that several molecules such as IL-2, GM-CSF and TNFα may be actively produced during the immune response against HTLV-I, and their evaluation could determine whether a correlation exists between the initiation and spreading of the retroviral infection of the lung.

Lung involvement in patients with HIV-1 infection

Let us briefly turn to the available knowledge concerning the role of the HIV-1 in the pathogenesis of pulmonary disorders during AIDS and AIDS-related diseases. The mechanisms by which HIV-1 enters the lung and infects pulmonary immunocompetent cells, as well as the

consequence of the retroviral infection on the lung host defence mechanisms, have recently been reviewed [2]. It seems appropriate to mention here the body of evidence supporting the concept that HIV-1 variants, both *in vitro* and *in vivo*, infect AMs and other pulmonary cell types. HIV-1 DNA can be detected in BAL cells obtained from HIV-1 infected patients, and the integration of the HIV-1 genome into AMs and pulmonary fibroblasts of HIV-1 infected patients has recently been demonstrated [21–23]. Entry of infectious HIV-1 requires an interaction with cellular CD4, in view of the fact that soluble CD4 blocks infection of AMs [24]. Following HIV-1 infection with a macrophage tropic strain ($HIV\text{-}1_{JR\text{-}FL}$), AMs show a peak of p24 antigen in supernatants, which is more than 400 fold higher than in peripheral blood monocytes [25]. Freshly recovered AMs from the BAL show an increased expression of CD4 molecules, as detected by OKT4A monoclonal antibodies [26]. Interestingly, HIV-1 clones derived from BAL are highly monocytotropic, and the infection of AMs by monocytotropic HIV-1 variants appears to increase with HIV-1 disease progression [27].

Although the above results clearly demonstrate that HIV-1 infects pulmonary cells, its pattern of infectivity remains a key issue, with the following features being most considered. By employing a DNA-PCR it has been reported that all patients with AIDS harbour high levels of HIV-1 sequences in their AMs [28]. In contrast, the number of pulmonary cells actively expressing HIV-1 RNA is low, as assessed by *in situ* hybridization (no more than 0.002%) [9], and the virus can be isolated only in about 50% of the cases [8]. These data point to the discrepancy between the number of pulmonary cells carrying HIV-1 and the low number of cells expressing HIV-1 RNA. Studies are needed to determine the burden of HIV-1 and the number of HIV-1 initiated copies that complete reverse transcription *in vivo* in AMs. The issue of whether HIV-1 infected AMs display structures containing scores of mature and immature virions represents a topic that deserves further investigation. The above data are crucial to validate the suggestion that AMs serve as a reservoir for monocytotropic HIV-1 strains, from which new viral variants may be generated. In turn, this event could cause a slow but progressive decrease of local immune surveillance, and in time the emergence of the virulent variants that are associated with disease progression [2].

Another topic that should attract the attention of researchers concerns the possibility that CD4- pulmonary cells may be involved in the pathogenesis of the *in situ* spreading of HIV-1 infection. In fact, evidence has been provided that HIV-1 can enter cells lacking the CD4 molecule (such as some neural cells, liver cells, epithelia) suggesting that every human cell could, theoretically, be infected by this retrovirus. To explain this finding, it has been hypothesized that target cells susceptible to HIV-1 express a fusion receptor on their cell surface that, with or without virus attachment to the CD4 molecule, may interact with the putative gp41 fusion domain of HIV-1. Another hypothesis postulates that HIV-1 can infect target cells through the binding of the antiviral antibodies with HIV-1 components. The immunocomplexes generated during this process could be brought into the cell *via* Fc or complement receptors expressed on the cell surface of susceptible targets. Because AMs and other pulmonary cells express weak positivity for CD16 (type III Fc receptor), the possibility cannot be ruled out that the complex HIV-1/anti-HIV-1 antibody may also enter pulmonary cells *via* the Fc receptor.

One line of enquiry, that is creating much interest, has to do with the comprehension of the regulatory network between HIV-1 infection of pulmonary cells, opportunists superinfecting pulmonary interstitium and the *in situ* release of cytokines. It is known that opportunistic viral pathogens may interact with HIV-1 encoding transactivator proteins, which in turn are capable of increasing the expression of the virus. In this respect, it has recently been demonstrated that AMs from HIV-1 infected individuals spontaneously release a series of biological mediators of the immune response, including TNFα, IL-6, and GM-CSF [2]. In addition to functional activation of pulmonary lymphocytes and macrophages, these cytokines are thought to have deleterious side-effects on the local immunocompetence, as a consequence of their ability to increase HIV-1 expression in primary mononuclear phagocytes (see above). Studies at the pulmonary level are needed to determine whether these cytokines might possibly synergize with pulmonary opportunists in the induction of HIV-1, thus accounting for the broad range of lung cells that are susceptible to HIV-1.

In conclusion, the results of a number of recent studies confirm the role of the lung as a reservoir for retroviral infections, but the immunopathogenetic mechanisms of the HTLV and HIV infections of the lung are complex and not fully elucidated. It is certain that the comprehension of the role of the pulmonary immune system in the control of viral infection will ultimately provide important clues for elucidating the pathogenesis of the lung diseases. Furthermore, with the advances that have marked the field of immunology and virology in recent years, we can expect to see an accelerated pace in the translation of experimental findings to clinical applications.

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