Immunology of interstitial lung diseases: cellular events taking place in the lung of sarcoidosis, hypersensitivity pneumonitis and HIV infection

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ABSTRACT: This paper summarizes our research and the results obtained on the topic of Immunology of interstitial lung disorders. Areas of investigation mainly included sarcoidosis, hypersensitivity pneumonitis (HP), and more recently the pulmonary involvement in acquired immunodeficiency syndrome (AIDS). In sarcoidosis patients two major mechanisms account for the alveolitis, i.e., an in situ cellular proliferation and a cellular redistribution from the peripheral blood to the sites of disease activity, including the lung. These findings involve both lymphocytes (CD4 helper-related cells) and macrophages, which lead to the formation and provide maintenance of sarcoid granuloma. In patients with hypersensitivity pneumonitis the lung infiltrates are characterized by cells bearing suppressor/cytotoxic phenotype. The expansion of cells with these characteristics in the lung of these patients is likely to be related to a local immune response to the antigenic stimulus. In the lung of patients with AIDS we also found a discrete lymphocytic alveolitis bearing the CD8 cytotoxic-related phenotype. The role of cytotoxic events, related to the lymphocytes and macrophages, which are operative in the lung of AIDS patients, is being evaluated. The analysis of cells recovered from the lavage, mainly lymphocytes and macrophages, in terms of surface phenotype, functional in vitro evaluations and molecular analysis, has provided new insights into the pathogenesis of the above quoted interstitial lung disorders.


Recent immunological studies have greatly improved our understanding of several pathogenetic mechanisms in interstitial lung disorders and full credit for advances in this field must be given to the bronchoalveolar lavage (BAL) technique. Although the role of lavage as a routine diagnostic procedure is still under discussion, it has been extremely useful in providing access to cell populations which line the surface of the lower respiratory tract. This technique, associated with newly generated technologies of monoclonal antibodies (MoAb) production, immunohistology, cell culture facilities, the possibility to quantitate many mediators of immune responses and more recently, molecular biological techniques have led to the possibility of improved investigation of the immunological events taking place in the lung of patients with interstitial lung disease.

Firstly, we attempted to establish the usefulness of BAL in sampling effector cell populations in the lung. The concept that lavage recovers cell populations which are representative of those present in the interstitium was further substantiated by the data obtained when we tried to discover whether bronchoalveolar lavage cellularity reflects lung histology. Combined immunological and immunohistochemical analyses have been used to evaluate lymphocytes and macrophages, respectively. Results obtained demonstrated that, at least in sarcoidosis and hypersensitivity pneumonitis (HP) cells retrieved from BAL do reflect the cell populations observed in the lung parenchyma [1].

BAL has brought immunological studies closer to the focus of inflammatory events. In particular, it allows us to study alveolitis, which is referred to as an infiltration of the lung parenchyma by immunoinflammatory cells, and which represents the crucial step in the evolution towards granuloma and fibrosis. More importantly, alveolitis is the last step during this cascade of events which is reversible, before processes take place...
which lead to phenomena that imply a destruction of the pulmonary parenchyma. The sequential, immunohistological studies that we have performed on lung biopsies of sarcoid patients have demonstrated that the process of alveolitis is likely to represent the first step leading to the granuloma formation [2].

We tried to transpose our research in a prospective clinical view, always bearing in mind the concept that the clinical management of patients should benefit from new advances in basic research [3].

Sarcoidosis

The lung of patients with sarcoidosis is a representative model for analysing the key events that lead to alveolitis. In this regard, in recent years we have produced some information which has proved useful for the comprehension of the pathogenesis of sarcoidosis and this information can be applied to the general concepts of alveolitis and granuloma formation.

From a pathogenetic point of view, two major mechanisms account for the accumulation of immuno-inflammatory cells in the lung: i.e. cellular redistribution from the peripheral blood to the lung and in situ proliferation (table 1). Although these two mechanisms are strictly correlated, they will be considered independently.

Table 1. - Pathogenesis of alveolitis in sarcoidosis

<table>
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<tr>
<th>Cellular redistribution</th>
<th>Lymphocytes</th>
<th>Monocytes</th>
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| In situ proliferation   | Lymphocytes | Macrophages |

Concerning the redistribution of lymphocytes, only indirect proof is presently available to substantiate the concept of T-cell redistribution. This proof is based on the evaluation of T-cell subset distribution in the peripheral blood and in the lavage of sarcoid patients that has revealed a completely different pattern. Whilst the number of CD4+ cells is diminished in the blood, CD4+ lymphocytes are dramatically increased when evaluated on cell suspensions recovered from BAL. The opposite behaviour has been observed for the CD8 population. Further confirmation of this data comes from the study of lung tissue sections using immunohistological techniques. The discrepancy of data obtained in the peripheral blood and in the lung suggests the hypothesis that a migratory process takes place from the blood to the pulmonary parenchyma [4].

It is worth mentioning that sarcoidosis is a multisystem disease and the pattern described for the lung can be observed at all sites of disease activity, including lymph nodes, liver, spleen, conjunctiva, skin, etc. [5, 6]. At all of these sites of granuloma formation, the CD4/CD8 ratio is extremely high (usually greater than 10) due to the increase of CD4+ cells. Combining this with all of the other information available on sarcoidosis, we proposed a model of T-cell redistribution suggesting that CD4+ cells migrate from the blood vessels to different sites of disease activity, thus leading to a compartmentalization of the CD4 lymphocytic subset [7]. The process is thought to be the consequence of an enhanced release of soluble factors at sites of granuloma formation. Although the precise molecules involved in these mechanisms are still unknown, for the time being the most reasonable candidates are likely to be interleukin-1 (IL-1), and another recently cloned lymphokine, interleukin-8.

Evaluation of the degree of CD4 alveolitis can be tentatively utilized to define patients in different phases of the disease. In particular, since the CD4 population can be subdivided into different subsets [8] expressing discrete in vitro and possibly in vivo functions, we found it useful to detect the frequency of CD4+/HLA-DR+ cells in the lung of sarcoid patients. Since CD4+/HLA-DR+ lymphocytes have been demonstrated to release interleukin-2 (IL-2) in vitro spontaneously, we quantitated these cells in the lavage of sarcoid patients in different phases of the disease. In fact, we observed that CD4+/HLA-DR+ cells are increased in the lung of sarcoid patients and this increase is highly statistically significant in the active phase of the disease, with the significance being less evident or even lacking in the split and inactive phases of the disease, respectively [9]. This type of evaluation could also tentatively give some idea of the state of activation of the IL-2 system in the lung of these patients, thus avoiding direct quantitation of the spontaneous production of IL-2, a test which is not easily available in every centre.

Lymphocytes are not the only cells involved in the redistribution. We reported that an enhanced number of alveolar macrophages express surface markers related to peripheral blood monocytes, such as those defined by CD11b, CD13, CD14, and CB12 monoclonal antibodies, thus providing indirect evidence of the redistribution of monocytes [10, 11].

More recently, we addressed the problem of the redistribution of monocytes directly by considering the production of type IV collagenase as a model of the mechanisms that are responsible for the migration of immunocompetent cells from the blood to the lung of sarcoid patients. Some events that accomplish the recruitment of adherent cells from the blood stream to the sites of ongoing inflammation have recently been identified. The migration of monocytes out of the blood stream is mediated by the release of type IV collagenase, an enzyme which is capable of binding and degrading the major structural component of the basement membrane of vessel walls, notably type IV collagen. By modifying the macromolecular organization of this structure, type IV collagenase causes discrete discontinuities through which peripheral blood monocytes may enter the inflammed tissues [12].
Since type IV collagenase plays a functional role only during the actual time of basement membrane traversal, young macrophages newly differentiated from recently recruited monocytes express this enzymatic property only for a limited period of time. In fact, freshly isolated peripheral monocytes degrade significant amounts of type IV collagen during the first 24 hours. After that period the activity decreases significantly over the subsequent hours of culture and is undetectable after 4 days, when the majority of monocytes have differentiated into mature macrophages [12]. Accordingly, already mature alveolar macrophages obtained from controls no longer release this enzyme. In other words, the transient expression of type IV collagenolytic activity by cells cultured in vitro would be in line with the hypothesis of the presence of monocytes among pulmonary phagocytes.

To verify the hypothesis that newly recruited mononuclear phagocytes are present in the alveolar space of sarcoid lung, in a series of patients with sarcoidosis, we evaluated adherent cells freshly recovered from the BAL for their capacity to produce the type IV collagenase. We demonstrated that sarcoid alveolar macrophages from patients with active sarcoidosis release significantly increased levels of type IV collagenase in vitro with respect to controls [13]. The kinetic production of type IV collageanase by alveolar macrophages is similar to that of peripheral blood monocytes. Boosting of alveolar macrophages both with IL-2 and gamma-interferon (gamma-IFN) did not influence their release of the enzyme, suggesting that these two lymphokines are not responsible for the modulation of the production of the type IV collagenase.

As further support for the concept that type IV collagenase is produced by freshly recruited cells, we performed additional experiments showing that a relationship exists between CD11 and CD14 positive cells and the production of type IV collagenase, that is the phenotype related to young cells belonging to the monocytic lineage. In addition, removal of these CD11 and CD14 positive cells from the BAL suspensions before the test abolishes the type IV collagenolytic activity. Biochemical characterization has shown that the enzyme released by pulmonary macrophages and the degradative enzyme of blood monocytes are virtually identical [13]. These findings, taken together, suggest the hypothesis that a type IV collagenase-mediated influx of adherent cells from the peripheral blood takes place in the lung of sarcoid patients.

On clinical grounds we were able to demonstrate that the property of alveolar macrophages to release type IV collagenase is related to the active phase of the disease. This finding stimulated more extensive follow-up studies and correlations with other parameters commonly used for determining the activity of the disease, with the aim of verifying the utility of this test in monitoring the macrophage component of alveolitis. Hence, in a series of sarcoid patients, we studied the relationship between gallium scan positivity and the capacity of alveolar macrophages to release the enzyme. We found that persistent gallium positivity was still associated with a consistently increased expression of enzymatic activity. By contrast, in cases of disappearance of gallium positivity following 6 mth's therapy, a parallel decrease of type IV collagenolytic activity was found. This finding further emphasizes the potential clinical use of this marker.

The second mechanism responsible for the accumulation of immunoinflammatory cells (both lymphocytes and macrophages) in sarcoid pulmonary parenchyma is related to the ability of cells in the lung to proliferate at that site. As for lymphocytes, current concepts on T-cell activation indicate that following mitogen or antigen activation, a subset of T-cells (IL-2 producer cell) co-operates with macrophages (in turn release IL-1) and synthesizes IL-2. At the same time, another T-cell subset (IL-2 responder cell) acquires the capacity to react to IL-2, expressing specific surface receptors (IL-2R). By the combination of these two mechanisms the cells are committed to proliferate. It is important to note that anti-Tac monoclonal antibody (CD25) recognizes these IL-2R (p55, low affinity).

In a series of sarcoid patients, we studied both peripheral blood and cells recovered from the BAL. We demonstrated that increased numbers of BAL T-cells expressing Tac antigen are present in the lavage but not in the blood [14]. The same pattern has been observed on tissue sections from transbronchial lung biopsies and from lymph nodes, using both the immunofluorescence and immunoperoxidase methods [2, 14]. In terms of mechanisms which regulate the T-cell activation, confirming previous data provided by another group, we observed that lung T-cells spontaneously release high amounts of IL-2 [2]. Taking all these considerations together, we can conclude that an in situ proliferation definitively contributes to the development of the lymphocytic alveolitis in these patients.

Unfortunately, the immunological methods we deal with each day seem to become more difficult and sophisticated, and often they are not available in every laboratory. It is therefore not easy to convert basic research into a clinical prospective. To overcome these difficulties in the area of IL-2 mediated T-cell activation, we made use of a relatively simple test, i.e. the soluble IL-2R assay. The specific interaction between a T-lymphocyte and the antigen leads to the transcription and translation of IL-2 and IL-2R genes followed by IL-2 interaction with its high affinity receptor and then by cellular proliferation. Under specific in vivo and in vitro conditions, the IL-2R may be released from the cell surface in a soluble form and it may be measured using a simple immunoenzymatic assay. We found increased levels of soluble IL-2R in the serum of sarcoid patients [15]. More importantly, a relationship is being elaborated between the levels of soluble IL-2R and the activity of the disease [16]. Whilst other markers of cell activation found in the peripheral blood of sarcoid patients have not proved to be useful for detecting discrete disease phases [17–19], the evaluation of serum soluble IL-2R may be an effective, non-invasive method for estimating different phases of sarcoidosis. In terms of pathobiology, since the soluble
IL-2R, like its cellular counterpart, is capable of binding IL-2 efficiently, it could remove the available IL-2 present in the milieu, thus down-modulating the immune responses. This starvation of IL-2 could explain the finding of the reduced in vitro proliferative response to mitogens and the impaired helper activity in the blood of sarcoid patients, all of these functions being basically mediated by IL-2. Thus, the blocking activity of soluble IL-2R could represent the major source of the still undefined inhibitory serum factors that we described in the peripheral blood of sarcoid patients some years ago [20–22].

To provide direct evidence that alveolar macrophages are actively proliferating in the lung of sarcoid patients, we used an immunostaining technique with the Ki67 monoclonal antibody which binds to nuclear antigens expressed by cells in the G1, G2, M and S proliferative phases, whilst cells at resting conditions are always negative. Phenotypic characterization of Ki67+ cells was performed by double marker analysis, using both differentiation antigens and enzymes specific for different T-cell subsets and macrophages. Variable numbers of Ki67+ macrophages were found in all BAL samples from patients with sarcoidosis [23]. The evidence that we provided that some alveolar macrophages are positive with the cell-cycle-related Ki67 monoclonal antibody reveals that these cells are committed to proliferate. This finding indicates that in situ replication of macrophages could represent an additional mechanism accounting for the development of the macrophage component of alveolitis in these patients.

The examples reported here relate to data obtained in an attempt to provide evidence for the two major mechanisms accounting for alveolitis in sarcoidosis. Of course, this information can be applied to the general concepts of alveolitis in other interstitial lung disorders. With this background, we proposed [3] a pathogenetic model for the alveolitis and granuloma formation in sarcoidosis, suggesting that an unknown antigen activates T-cells and macrophages, which are able to maintain each other in a state of activation. Following co-operation with macrophages, activated helper T-lymphocytes release a series of mediators, including IL-2, gamma-IFN, chemotactic factors, etc. Together, these lymphokines contribute to maintaining the granuloma formation by promoting an exaggerated cell proliferation and by the accumulation of newly recruited lymphocytes and macrophages from the blood stream.

As far as functional properties of lung cells recovered from BAL are concerned, I mentioned previously that lung T-cells from these patients are able to spontaneously release IL-2 and gamma-IFN [2]. In addition, using a pokeweed mitogen (PWM) driven B-cell differentiation assay we demonstrated that CD4 positive cells in vitro provide helper function [2]. In line with this latter property is the evidence that plasma cells can be observed in lung tissue sections [6]. This finding might explain the presence of the hypergammaglobulinemia usually detectable in the serum of these patients.

From a functional point of view, alveolar macrophages from sarcoid patients release increased quantities of superoxide anion [24]. Interestingly, gamma-INF, which usually increases the production of superoxide anion in patients with inactive disease (or in controls), in patients with active sarcoidosis is unable to trigger these cells in vitro. This feature suggests that alveolar macrophages in active sarcoid are already activated in vivo and are no longer susceptible to further activation in vitro [24].

The fundamental question to be answered deals with defining the stimulus that triggers cells to proliferate at the lung level. To address this point, a strict relationship between antigen presenting cells and helper T-lymphocytes was observed using immunohistological techniques [2]. Furthermore, in the autologous mixed lymphocyte reaction, alveolar macrophages (as target cells) provide a strong stimulus for sarcoid T-cells (effector cells), much higher than control macrophages [2]. Studies in this field are limited, however, by the fact that the antigen in this disorder is not known.

To further investigate the model that governs the growth at lung level we made use of molecular biological techniques, and studied the configuration of the T-cell receptor (TCR) beta gene of lymphocytes recovered from BAL. Using the BamHI enzyme restriction, we found the presence of an extra band (at 13.5 kb) in the lung lymphocytes of sarcoid patients [25]. Data are still preliminary, but this behaviour does not suggest the presence of a polyclonal population, nor that we are dealing with a monoclonal cell expansion. It rather supports the presence of an oligoclonal model of growth, which is likely to be consistent with the cell proliferation of a limited number of clones possibly expanding as a consequence of a chronic stimulation. Further studies at the molecular level on cells cloned from BAL suspensions are needed to clarify the issue. Unfortunately, the pattern that we have shown is not specific for sarcoidosis but is similar to the pattern that we observed in other interstitial lung diseases (see below).

Hypersensitivity pneumonitis

In the mid 1980’s we started to study lung lymphocytes from patients with HP. Initially the rationale for studying HP lung lymphocytes was related to the possibility of comparing cells recovered from sarcoid patients with other interstitial lung conditions. We immediately realized that these cells represented an ideal model for studying the lung environment during specific, immunologically mediated reactions and in particular for investigating the cytotoxic mechanisms taking place in this organ. For this reason, in the last few years we have focused our attention on the phenotypic, functional, and molecular evaluation of cells retrieved from the lung of HP patients.

Cellular recovery from the BAL of patients with HP is fivefold that observed in controls and the cells accounting for this increase are mostly represented by lymphocytes [26]. With immunological marker
evaluation we demonstrated that only a few BAL lymphocytes express B-cell related markers, the majority of them being represented by T-lymphocytes [26]. The analysis of T-cell subsets showed that CD8+ lymphocytes are the predominant cells retrieved from the BAL of these patients. As a result, the CD4/CD8 ratio is extremely low (around 0.5 vs 1.8 controls). Although the number of cells bearing the proliferation associated markers (CD71 and CD25 antigens) is quite low in terms of percentage, a statistically significant difference with respect to controls exists when the absolute number of cells is considered. An increase of lymphocytes bearing HLA-DR determinants has also been demonstrated.

To further characterize the structures involved in the recognition of putative antigens, and considering the emerging importance of gamma/delta cells in pulmonary responses, we recently evaluated the distribution of BAL T-cells expressing the membrane products of the, α/β or γ/δ chains products of the TCR. We found a slight increase in the percentage of γ/δ positive cells; however, if we consider the absolute number of these cells in BAL fluid, it appears that there is a marked increase in the lung of HP patients [27]. It has been proposed that γ/δ TCR lymphocytes display non-major histocompatibility complex (non-MHC) restricted cytotoxicity, and this finding fits well with other phenotypic markers and functional studies performed on BAL HP cells reported below.

In the BAL of HP patients, we also demonstrated an increase, with respect to BAL control lymphocytes, of Very Late Activation (VLA-1) antigen positive cells [28]. In view of the fact that VLA-1 antigen is shared late during T-cell activation, the observation that a high percentage of HP lung lymphocytes bear VLA-1 antigen permits the hypothesis that CD8+ cells represent a long-term activated and homing population in the lung of these patients, possibly as the result of a continuous stimulation. Since VLA-1 MoAb defines one of the molecules belonging to the integrin family, this structure could also be involved as an adhesion protein in cell-cell interactions. These molecules might have a relevant role in the interaction with other cells in the lung microenvironment, possibly contributing to the cytotoxic mechanisms taking place in HP patients.

With regard to the frequency of cells bearing cytotoxic related markers [28, 29], notably the pattern of reactivity with monoclonal antibodies defining cells with cytotoxic phenotype (including natural killer, specific and non-specific cytotoxic T-lymphocytes), a statistically significantly increased number of cells positive for HNK-1 (CD57) and NKH-1 (CD56) reagents has been observed in the lavage of HP patients with respect to controls [28, 29]. The number of CD56 and CD57 cells co-expressing T-cell markers is predominant over the number of cells that lack these determinants. By contrast, other markers strictly defining natural killer cells are lacking on the surface membrane of BAL cells [26]. Thus, the alveolitis in HP patients is mostly represented by CD3+, CD8+, CD57+, CD56+, CD16- non-major histocompatibility complex (MHC) restricted cytotoxic lymphocytes.

To evaluate the pattern of growth of lung T-cells in HP patients, attempts have been made to assess the clonality of expanded populations in the lung [28, 30] by studying the molecular organization of the TCR. Although the analysis of the configuration of the TCR and genes showed that BAL lymphocytes are polyclonally expanded in vivo in the majority of HP patients, following digestion with BamHI and EcoRI restriction enzymes in a few cases we observed novel fragments. This finding makes it compelling to hypothesize that such polyclonal recruitment, in at least a few cases, seems to be biased toward cells which have rearranged and possibly expressed particular Vβ or Vγ genes. The pattern that we have found is consistent with the suggestion that the events taking place in the lung of HP patients might sometimes induce an oligoclonal proliferation of BAL lymphocytes in these patients. The difference in the rearrangement patterns observed in our patients might be viewed as the consequence of a selection and/or a preferential usage of Vβ or Vγ gene segments for the recognition of a given antigen or, alternatively, to the fact that different antigen specificities might use the same V gene segment. We could perhaps be dealing with a preferential usage in the lung of particular V region segments as what has been observed in other organs. For instance, the usage of Vγ6 segment predominates among TCR γ/δ+ lymphocytes detectable in the oesophageal mucosa, whereas Vγ7 usage is characteristic of the small intestine and Vγ5 cells are predominant in the skin. The molecular characterization of αβ and γ/δ V segments expressed on cell clones generated from HP BAL lymphocytes could possibly clarify this issue.

In terms of functional activities, using a PWM induced B-cell differentiation assay we were able to demonstrate that lung T-cells from HP patients display a suppressor in vitro activity [26, 31]. The finding that lung T-cells in these patients express, in addition to their suppressor-related phenotype, a suppressive function in vitro offers major clues to the pathological pattern of HP. In fact, compelling evidence has been accumulating both in experimental and human pathology that mechanisms leading to granuloma formation are modulated by the presence of regulatory T-cells. A mononuclear cell infiltration precedes the development of granuloma, and in particular the presence of different T-cell subsets is crucial in regulating the appearance and maintenance of granulomas, perhaps by the release of a number of lymphokines. In particular, it has been demonstrated that helper T-cells are correlated with an active granuloma formation, whereas suppressor/cytotoxic T-cells and NK cells are associated with the regression of this phenomenon [32]. Thus, suppressor cells may slow down the granuloma formation in our patients and that could explain why granulomas are not as prominent in HP as in other granulomatous disorders, for instance in sarcoidosis where the alveolitis is characterized by the accumulation of CD4+ lymphocytes.
With regard to the cytotoxic in vitro function, a statistically significant increase of spontaneous cytotoxicity was observed in HP patients [29]. By contrast, BAL lymphocytes from asymptomatic farmers display a cytotoxic in vitro function superimposable on that of controls [26]. The differences between HP patients and asymptomatic farmers observed in functional studies, notably those dealing with cytotoxic systems, seem to be promising in explaining the different pathogenetic mechanisms in the two groups of subjects but must be substantiated by additional analyses. In this regard, attempts have been made to characterize the nature of cytotoxic cells accounting for the alveolitis in patients with HP. These experiments demonstrated that different types of cytotoxic mechanisms are provided by lung cells from HP patients including natural killer (NK) cells, non-MHC restricted T-cytotoxic cells, and lymphokine activated killer (LAK) cells.

To evaluate the evolution of alveolitis in HP patients, we performed a follow-up study in which we subdivided patients into two groups, according to their exposure to the relevant antigens, i.e., patients who continued to be regularly exposed to the aetiopathological antigens at work from those individuals that, after the first acute episode, were no longer directly exposed to the specific antigens [33]. At the time of the first evaluation, a high number of CD8+ cells with a reversal of the CD4/CD8 ratio was demonstrated in all patients with HP. Consecutive BAL evaluations demonstrated a persistent increase of CD8+ cells and a persistent reversal of the CD4/CD8 ratio in patients who continued to be regularly exposed to aetiopathological antigens at work.

In terms of functional in vitro activities, cytotoxic cells showed a persistently enhanced in vitro lytic function during the entire follow-up in patients who continued to be regularly exposed to the aetiopathological antigens at work, even though there appeared to be a trend toward the normal range. Patients who continued to live in agricultural environments but were not further exposed to specific antigens exhibited a recovery of CD4+ cells, a decrease of CD8+ cells, and an increase of CD4/CD8 ratio to the normal range 6 mths after the first observation, thus suggesting that the immunological abnormalities in these patients progress towards normality [33].

Immunohistological analysis, performed at the time of the first evaluation, demonstrated a diffuse infiltration of lung parenchyma by CD8+ cells [1, 33]. The subsequent immunohistological observations revealed a persistent CD8+ infiltrate in the group of patients who continued to be regularly exposed to the aetiopathological antigens at work, whereas an increase of CD4+ cells had been observed after 6 mths in the lung of subjects that were not further directly exposed to specific antigens [33].

The expansion of cells with suppressor and cytotoxic characteristics in the lung of these patients is likely to be related to a local immunological response to the antigenic stimulus. The intensity of this alveolitis may be modulated by the exposure to inciting antigens and by the frequency of sensitization. These mechanisms may be relevant to further specify the pathogenesis of this disorder [32].

HIV infection

Since most studies in our laboratory are nowadays devoted to the evaluation of cytotoxic events taking place in the lung in different interstitial lung disorders, one condition that obviously represents the best candidate for providing an in-depth characterization of lung effector cells is the human immunodeficiency virus (HIV) infection. Life-threatening complications of the respiratory tract are quite common during the clinical course of HIV infection and a tentative explanation for the abnormally high susceptibility of patients with acquired immune deficiency syndrome (AIDS) to develop pulmonary complications rests on the fact that HIV affects the defensive ability of immune-competent cells of the lung [34]. For these reasons, the role of cytotoxic events which operate in the lung of AIDS patients is being evaluated.

We are facing this problem in a series of AIDS patients in different stages of the disease by studying the cells recovered from the lavage. We found a discrete lymphocytic alveolitis bearing the CD8 phenotype. However, despite the presence of an increased number of cells with cytotoxic phenotype in the lung of these patients (including CD8, CD56 and CD57 related lymphocytes) these cells do not lyse the appropriate targets in vitro. The majority of cells from these patients are unable to provide cytotoxic in vitro function [35].

We are now specifying the nature of the above defect. Experiments designed to solve this problem included the evaluation of the production of natural killer cytotoxic factor (NKCF) and the single cell binding analysis. We demonstrated a defective production of NKCF and we showed that cytotoxic effector cells bind the targets, but do not kill them. Interestingly, and these data recently produced, the cytotoxic function improves following in vitro treatment of effector cells with recombinant IL-2 [36]. In conclusion, pulmonary NK cells from patients with HIV infection are quantitatively increased, maintain the capacity to bind sensitive targets but, perhaps in a progressive way, they lose the property to release the cytotoxic factors involved in the NK lytic machinery. These findings suggest that the defective spontaneous cytotoxicity observed in the lung cells from AIDS patients was not due to their inability to bind the targets but was consequent to their failure to release the soluble molecule (NKCF) which is mandatory for the efficiency of the cytotoxic machinery. Triggering the lung effectors with recombinant IL-2 enhances the NK function and elicits LAK activity. This might suggest the design of new strategies for the treatment of AIDS-associated pulmonary complications.

As far as the macrophage component of alveolitis in HIV patients is concerned, we provided several pieces of evidence in support of the concept that resting alveolar macrophages from HIV infected patients constitutively
release tumour necrosis factor (TNF) [37]. In fact, highly purified alveolar macrophages recovered from the BAL of these patients exhibited high levels of cell-mediated cytotoxicity against U937 targets (TNA-alpha sensitive), and the addition of a polyclonal anti-TNF-alpha antibody resulted in a significant inhibition of the target lysis. Furthermore, we demonstrated that alveolar macrophages from HIV patients generate supernatants containing TNA-alpha, and express detectable levels of messenger ribonucleic acid (mRNA) transcripts for this lymphokine. Since normal alveolar macrophages do not express mRNA for TNA-alpha or produce this molecule unless they are triggered with LPS or gammaLFN, our observations are consistent with the hypothesis that alveolar macrophages from HIV-1 infected patients are primed to synthesize this molecule in vivo. Considering the role of TNF in the human lung [38] our data suggest that the in situ hyperproduction might play a role in the pathogenesis of AIDS-related pulmonary complications [37].

Other conditions

All of these studies are of course also aimed at further elucidating the comprehension of cell populations normally resident in the lung. For instance, a variety of observations indicate that lung lymphocytes are somewhat different from circulating peripheral blood lymphocytes in that they are activated, perhaps being in a sort of alert state that introduces further functional activation whenever a foreign antigen, or a cell not recognized as self, enters the respiratory tract [39]. With regard to the issue of abnormal, neoplastic cells present in the pulmonary parenchyma, another area of our investigation deals with the pulmonary involvement in other conditions in which cytotoxic cells might be of relevance, including lung cancer [40]. In this area, studies recently performed in our laboratory addressed the question of whether LAK cells, in addition to killing neoplastic cells, might to some extent provide a lysis of normal cells. Although LAK cells have been supposed to spare the normal counterpart, the issue is still controversial since some data do not support this concept [41].

To verify the hypothesis that LAK cells contribute to some of the side-effects related to IL-2/LAK immunotherapy, we took the toxicity of LAK cells against pulmonary alveolar macrophages into consideration. We demonstrated that human LAK cells generated in vitro following incubation of peripheral blood mononuclear cells with recombinant IL-2 were able to lyse normal autologous or allogeneic alveolar macrophages [42]. The finding that LAK cells are cytotoxic to normal, non-transformed alveolar macrophages indicates that the pathogenetic mechanisms involving this self-addressed lysis activity could account for some adverse reactions at lung level related to LAK/IL-2 immunotherapy [42].

Further areas of investigation include the evaluation of models of lymphocytic growth and cellular recruitment at lung level and this can be investigated both by taking advantage of molecular biological technology and by evaluating recently discovered new lymphokines (including IL-4, IL-6, and IL-8) and/or adhesion molecules (such as those related to the superfamily of adhesive receptors called integrins). The study of the specificity of local immune responses and the possibility of modulating them are also goals of our research in future years.

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*Immunologie des lamaladies interstitielles pulmonaires: réactions cellulaires dans le poumon des patients atteints de sarcoidose, de pneumopathie d'hypersensibilité, et d'infection par V.I.H.* G. Semenzato.

**RÉSUMÉ:** Ce travail résume les recherches entreprises dans notre laboratoire et les résultats obtenus au sujet de l'immunologie des maladies pulmonaires interstitielles. Les secteurs d'investigation ont compris principalement la sarcoidose, la pneumopathie d'hypersensibilité et, plus récemment, les atteintes pulmonaires dans le SIDA. Chez les patients atteints de sarcoidose, deux mécanismes principaux rendent compte de l'alvéolite, en l'occurrence une prolifération cellulaire in situ et une redistribution cellulaire provenant du sang périphérique vers les sites d'activité de la maladie, y compris le poumon. Ces observations concernent à la fois les lymphocytes (cellules en relation avec les "CD4 assistants") et les macrophages, et conduisent à la formation et assurent le maintien de granulomes sarcoïdiques. Chez les patients atteints de pneumopathie d'hypersensibilité, les infiltrats pulmonaires sont caractérisés par des cellules portant le phénotype suppresseur/cytotoxique. L'augmentation de cellules douées de ces caractéristiques dans le poumon de ces patients est susceptible d'être en rapport avec une réponse immune locale à un stimulus antigénique. Dans le poumon de patients atteints de SIDA, nous avons trouvé également une alvéolite lymphocytaire discrète, caractérisée par un phénotype en relation avec les CD8 cytotoxiques. Le rôle des actions cytotoxiques en relation avec les lymphocytes et les macrophages, et qui agissent au niveau pulmonaire dans le SIDA, est en cours d'évaluation. L'analyse des cellules obtenues par lavage, principalement les lymphocytes et les macrophages, à la fois en ce qui concerne le phénotype de surface, les évaluations fonctionnelles *in vitro* et l'analyse moléculaire, a fourni de nouvelles vues sur la pathogénie des maladies interstitielles pulmonaires citées plus haut. *Eur Respir J.,* 1991, 4, 94–102.