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Pulmonary immune cells in health and disease: lymphocytes

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Pulmonary immune cells in health and disease: lymphocytes. C. Agostini, M. Chilosi, R. Zambello, L. Trentin, G. Semenzato. ©ERS Journals Ltd 1993.

ABSTRACT: Immunological defence mechanisms of the lung are provided by several different types of immunocompetent cell. Among these, the most important are lymphocytes, alveolar macrophages and neutrophils.

This review focuses mainly on the pattern of physiological activities of pulmonary lymphocytes in the local host defences. The article provides an overview of the events leading to the recruitment, homing and activity of lymphocytes in the lower respiratory tract. The pathways through which the pulmonary lymphoid system recognizes, destroys and contributes to removing potentially harmful inhaled antigenic materials are also briefly discussed.

The final section of the article highlights the pathogenetic role envisaged for lymphoid cells in disease states, in the context of the known biology of these cells. *Eur Respir J., 1993, 6, 1378–1401.*

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Much progress has been made in the last decade toward phenotypic, functional and molecular characterization of human lymphocytes. The identification of leucocyte surface molecules that divide lymphocytes into functionally distinct subsets, and the definition and cloning of several lymphokines regulating inflammatory and immune responses are among the remarkable advances that have had a relevant impact on the understanding of the functional role of lymphocytes in different physiological and pathological situations. The definition of the molecular basis of antigen recognition and the characterization of factors regulating lymphocyte homing have also helped in the understanding of the processes by which lymphocytes seek out and localize inflamed tissues.

We will, herein, mainly summarize the current concepts of the recruitment, homing and activity of lymphocytes in the lower respiratory tract. In particular, we shall describe the pattern of physiological activities shown by the lung lymphocyte subsets in response to immunological challenge. Furthermore, we will emphasize the mechanisms by which the lymphoid system recognizes, destroys, and contributes to removing, potentially harmful inhaled antigenic materials. The pathogenetic role envisaged for lymphoid cells in disease states will also be reviewed, in the context of the known biology of these cells.

It is important to state that full credit for the new advances in the comprehension of the role of the different lymphocyte subsets in pulmonary host defence mechanisms must be given to the technique of bronchoalveolar lavage (BAL). Providing access to cell populations within the lower respiratory tract, this technique has allowed researchers to shed some light on the complex network of interactions between cellular components of the pulmonary immune system and their by-products, in particular the cytokines.

Origin, maturation and differentiation of pulmonary lymphocytes

According to their specific properties and discrete functions, human lymphocytes are functionally compartmentalized into primary, secondary and tertiary lymphoid organs. Primary lymphoid tissues (*i.e.* bone marrow and thymus) represent the organs accounting for the production of mature "virgin" or "naive" B- and T-lymphocytes. Secondary lymphoid tissues (*i.e.* lymph nodes, spleen, Peyer's patches of the intestinal mucosa, and organized lymphoid tissue in the respiratory tract) are sites in which homing and secondary differentiation of virgin to memory T- and B-cells takes place. Antigen accumulation and processing occur mainly in secondary lymphoid tissues, and these processes are modulated by several accessory cells, specialized for presentation of antigens to both virgin and memory cells. Tertiary lymphoid tissues are considered to be all other body tissues that normally have few or absent lymphoid elements, but that, upon local antigenic stimulation, represent potential sites of accumulation of memory lymphocytes, that have previously been generated in secondary lymphoid organs.

The great majority of organized lymphoid tissue in the lung is represented by follicles that are located throughout the bronchial tree, as far down as the small bronchioles (the so called bronchus-associated lymphoid tissues (BALT)) [1, 2]. These structures can be considered specialized sites for secondary lymphoid differentiation, and are constituted by B-cell germinal centres, surrounded by T-cells, macrophages and dendritic cells. They share morphological characteristics with Peyer's patches of the intestine, and form strict associations with mucosal epithelium. In pulmonary follicles, naive B- and T-cells localize, differentiate into memory and effector lymphocytes, and continuously traffic until they respond to their cognate antigen. In this respect, contiguity is crucial between the respiratory epithelium and lymphoid follicles, which allows antigens to pass across the epithelial barrier and to contact cells with antigen presenting capacity (*i.e.* B-cells, macrophages, dendritic cells).

The molecular events controlling the traffic of lymphocytes from the primary lymphoid tissues to the intrafollicular lymphocyte pool are poorly characterized. As mentioned below, it is believed that expression of molecules involved in lymphocyte-endothelial cell recognition (leucocyte function associated antigen (LFA)-1, intercellular adhesion molecule (ICAM)-1, (α4-integrins, LFA-3, cluster designation (CD)44, leucocyte adhesion molecule (LAM)-1, CD45RO) and the consequent binding of naive lymphocytes to tissue-specific vascular adhesion molecules might play a role in determining the tendency of a given lymphocyte subset to localize into the lung [3, 4]. Furthermore, local epithelial regulatory factors, with the potential to regulate lymphocyte homing and production of chemoattractant cytokines (including interleukin (IL)-8 and platelet derived growth factor-4) are likely to co-operate in the compartmentalization of B- and T-cell subsets into pulmonary follicles.

The lung parenchyma represents a paradigmatic tertiary lymphoid tissue, that normally contains only a few lymphoid elements. In the normal nonsmoking adult, more than 90% of the cells within the alveolar spaces and interstitium are macrophages, whilst lymphocytes vary from 5–10% of the entire BAL cell population. As in other tertiary lymphoid organs, pulmonary lymphocytes are memory cells, sparsely distributed along the submucosa and lamina propria. Lymphoid effector cells are also present above the epithelial membrane, between the epithelial cells and within the interstitium. Finally, some lymphocytes are intimately associated with the alveolar lumen, in a fashion analogous to intraepithelial leucocytes of the gastrointestinal tract. These "intraepithelial" pulmonary lymphocytes are considered to be preactivated cells, which are in a sort of alert state that precedes their further functional activation whenever foreign antigenic material enters the respiratory tract.

As extensively specified below, unwanted antigens induce the clonal expansion of intra-alveolar precursor effector cells and the migration of memory lymphocytes, leading to a local accumulation and differentiation of antigen-specific T- and B-lymphocytes with effector specializations (immunoglobulin secretion, cytotoxic activity, delayed type hypersensitivity response, immunoregulatory activity, *etc.*). Another short-term consequence of the antigen recognition by pulmonary memory B- and T-cells is their functional activation and upregulation of the surface expression of several accessory molecules, including activation-dependent integrins of the α4 (CD49d) and β2 classes (CD18/LFA-1) and the member of the hyaluronate-binding receptor family, CD44 [5]. Keeping in mind that these molecules help the interaction of lymphocytes with the cellular and extracellular matrix components, it is likely that the heightened expression of adhesion molecules allows effector cells to extravasate efficiently from secondary lymphoid tissues to the inflamed pulmonary parenchyma.

In synthesis, we can assume that pulmonary lymphocytes continuously traffic throughout two functionally distinct lymphoid compartments: BALT tissue, *i.e.* the afferent lymphoid area, where antigens first enter the system and initiate an immune response; and the remainder of the lung parenchyma, where differentiated memory T- and B-cells that have developed in the secondary follicles travel for new interaction with inciting antigens. The increase of pulmonary memory T-cells after antigen challenging can be the consequence either of local proliferation or of migration from secondary lymphoid tissues (BALT and lymph nodes draining the pulmonary parenchyma). Both these functions are probably mediated by the interaction of lymphocytes with accessory cells. In fact, pulmonary intraepithelial and interstitial T-cells can recognize antigens with high efficiency, when presented by "professional" major histocompatibility complex (MHC) Class II dendritic cells [6]. In turn, as observed in other epithelial systems [7, 8], pulmonary dendritic cells can capture and transfer antigens from the airway epithelium to BALT and draining lymph nodes. Here, after antigen presentation and activation, resting T-cells can proliferate, and the repertoire of adhesive and homing receptors necessary for their migration to lung parenchyma is upmodulated (see below).

Membrane receptors and surface markers

In the last few years, it has been possible to characterize lymphocytes recovered from the BAL of healthy subjects and patients with lung disorders, by using a variety of monoclonal antibodies (MoAbs). Phenotypic data have provided a great body of information directly related to the differentiation and functional activities of the pulmonary lymphoid system. In this section, we present an overview of surface molecules that are expressed by pulmonary lymphocytes. MoAbs reacting with cell surface antigen of human leucocytes were recently listed, at the IVth International Human Leukocyte Differentiation Antigen Workshop in 1989, in which 78 human leucocyte differentiation antigens were officially recognized and clustered under the designation numbers from CD1a to CDw78 [9]. For the reader's convenience, in table 1, we have listed MoAbs (both clustered and nonclustered) which will be discussed in this chapter, with related reactivities and specificities.

Table 1. – Clustered and nonclustered monoclonal antibodies which have been discussed in this paper, with related reactivity and antigen specificity

Monoclonal antibody	Reactivity	Comments
CD2	T-cells, some NK cells	Receptor molecule for sheep red cells, involved in cell adherence after binding to LFA-3
CD3[A,F]	T-cells	Associated with the TCR
CD4[A,F]	T-helper/inducer cells, some macrophages	Class II MHC receptor, receptor for HIV-1
CD8[A,F]	T suppressor/cytotoxic cells	Class I MHC receptor
CD10	Lymphoid progenitors	Neutral endopeptidase
CD11a	Leucocytes	α-chain of the LFA-1 antigen binds ICAM-1 (CD54) and ICAM-2
CD11b	T-cell subsets, some NK cells, granulocytes, macrophages	α-chain of Mac-1 (complement receptor; C3bi)
CD11c	Weakly expressed on T-cells and B-cells, present on granulocytes and macrophages	α-chain of LFA-1 complex
CD16	NK cells, granulocytes, macrophages	Low affinity Fc receptor for IgG (FcRIII)
CD18	Leucocytes	β-chain of LFA-1 complex
CD19[A,F]	All B-cells	Transmembrane polypeptide
CD20[A,F,P]	B-cells after pro-B-cell stage	Putative involvement in ion transport
CD23	Strongly expressed on IL-4 activated B-cells, weakly expressed on mature B-cells	Low affinity receptor for IgE
CD25[A,F]	Activated T-cells, NK cells, some macrophages	p55 of IL-2R
CD29	T-cell subsets	Integrin β-1-chain, involved in cell adhesion, CD29+ T-cells are memory cells, CD4+/CD29+ cells provide help for antibody production
CD34[A,F,P]	Capillary endothelial cells, myeloid precursors	Single-chain transmembrane glycoprotein
CD38	Thymocytes, activated T-cells, plasma cells	Type II integral membrane glycoprotein
CD44	T-cells, neutrophils	Homing receptor belonging to the adhesion receptor family
CD45RA (220 kD isoform)	T-cell subset, B-cells, macrophages	T-cells expressing this antigen are naive or virgin T-cells
CD45RO[A,F,P] (180 kD isoform)	T-cell subset, B-cells, macrophages	T-cells expressing this antigen are memory or primed T-cells
CD49d	Activated T-cells and B-cells, monocytes, platelets	Integrins of the α4 class, associated with CD29 in VLA-2 complex
CD54	T-cells, macrophages endothelium cells, dendritic cells	ICAM-1, is the ligand for LFA-1, receptor for rhinovirus
CD56	NK cells	Isoform of neural adhesion molecule
CD57	Granular lymphocytes, some T-cells and B-cells	Unknown function

Table 1 cont....

Monoclonal antibody	Reactivity	Comments
CD58	Leucocytes	LFA-3, ligand for CD2 on T-cells
CD68[A,F,P]	Monocytes/macrophages	Excellent marker for tissue macrophages
CD69	Activated B-cells and T-cells, activated macrophages, NK cells	Involved in early events of lymphocyte activation
TCRδ1, TCR-γ/δ-1	All γ/δ cells	Associated with the TCR
BB3	γ/δ cell subsets	Identifies $V\delta2^+$, γ/δ cells
HLA-DR	T-cells, B-cells, macrophages and other APC	MHC Class II antigen
LAM-1, ELAM-1	Leucocytes	Selectins belonging to the adhesion receptor family
B-ly-7	Activated T-cells	Analogous to the integrin family
VLA-1	Activated T-cells	VLA antigen, integrin belonging to the adhesion receptor family
S6F1	Cytotoxic T-cells	Epitope of LFA-1 antigen
Ki67, PCNA[A,F,P]	Proliferating cells	Antigens expressed during G1, S, G2 and M cell cycle phases
Tu27	NK cells, activated T-cell and B-cells	p75 chain of IL-2 receptor
VCAM	Activated endothelium	Mediates leucocyte adhesion to endothelium in inflammation
Cytokeratin[A,F,P]	Epithelial cells	Immunohistochemical marker

[A]: immunohistochemical marker useful in the characterization of cell components in lymphoid lung lesions; [F]: frozen material; [P]: paraffin material. CD: cluster designation; NK: natural killer; LFA: leucocyte function associated antigen; TCR: T-cell receptor; MHC: major histocompatibility complex; HIV-1 human immunodeficiency virus-1; ICAM: intercellular adhesion molecule; Fc: complement fragment; Ig: immunoglobulin; IL: interleukin; IL-2R: interleukin receptor; VLA: very late activation antigen; HLA-DR: human leucocyte antigen-DR; APC: antigen presenting cell; LAM: leucocyte adhesion molecule; ELAM-1: endothelial leucocyte adhesion molecule; VCAM: vascular cell adhesion molecule. PCNA: proliferating cell nuclear antigen.

Expression of B-related markers

As in other secondary lymphoid tissues, highly differentiated B-cells largely predominate in the germinal centres of BALT follicular aggregates. In contrast, within alveolar spaces and in the diffuse pulmonary parenchyma, less than 5% of the lymphocytes are B-cells bearing surface immunoglobulins (IgM and, rarely, IgD). In normal pulmonary tissue, there are also scattered cells showing intracytoplasmic immunoglobulins, and secreting immunoglobulins into the surrounding microenvironment. In particular, IgG and IgA secreting cells can be detected in the lavage fluid of healthy subjects, whilst plasma cells with intracytoplasmic IgM are usually very rare [10, 11]. IgE secreting plasma cells are present only in the pulmonary tract of atopic individuals [12].

Recent studies on the expression of cell surface differentiation antigens by pulmonary B-cells, have shown that they are CD19+/CD20+/CD10-, showing the phenotype of mature circulating B-cells [13]. Whether pulmonary CD19+/CD20+ cells carry activation markers related to the B-cell compartment, such as CD25 (*i.e.* the p55 kD glycoprotein of the receptor for IL-2) or CD23 (the IgE complement fragment (Fc) receptor II) remains to be determined.

Expression of T- and natural killer (NK)-related markers

Under normal conditions, the T-cell population resident in the lung parenchyma is smaller than the macrophage population. Up to 30×10^3 lymphocytes·ml^{-1} can be recovered from the BAL fluid of normal individuals, and T-cells account for the majority of these lymphocytes. In fact, 75–90% of BAL lymphocytes express the sheep blood red cell-related CD2 determinant and bear CD3/T-cell receptor (TCR) antigen. This latter antigen represents the structure of the T-cell that is involved in the recognition of inhaled antigens in a MHC-restricted manner.

Two types of TCR independently expressed on different

human T-cell subsets are known. In most T-cells, TCR is constituted by two distinct transmembrane heterodimers formed by α and β binding chains [14]; more recently, it has been demonstrated that a small subset of T-cells expresses γ/δ heterodimers [15]. Using WT31 MoAb that recognizes the α/β TCR, we recently demonstrated that the majority of BAL T-cells from healthy individuals co-express the TCR α/β [13]. By contrast, only a few normal lung cells (about 5%) stain with the MoAb TCRδ1, that recognizes a common epitope of the δ chain apparently expressed by all TCR γ/δ cells [16, 17]. TCR γ/δ cells can be further subdivided in two non-overlapping populations, termed *Vδ1* and *Vδ2*, based on the absence or presence, respectively, of an interchain disulphide bond [18]. Under physiological conditions BB3+ *Vδ2* related T-cells account for about 70% of pulmonary γ/δ cells (our unpublished data).

When evaluated on cell suspensions recovered from the BAL of healthy nonsmoking individuals, both CD4 helper-related and CD8 cytotoxic/suppressor-related cells are present in approximately the same proportions as in peripheral blood (pulmonary ratio CD4/CD8≈2). Interestingly, a decreased CD4/CD8 ratio may be observed in the BAL of asymptomatic cigarette smokers [19]. CD4 and CD8 molecules are functionally associated with the TCR, and represent two structures that are involved in cell-cell adhesion and in the signal transduction during T-cell activation. In particular, CD4 is a receptor for a monomeric part of MHC Class II products (human leucocyte antigen (HLA)-DR, DQ and DP) expressed on pulmonary antigen presenting cells (such as lung B-cells, alveolar and interstitial monocyte-macrophages, dendritic cells, activated T-cells, endothelial cells, and some epithelial cells), whilst CD8 binds Class I MHC molecules (HLA-A, B and C) on the surface membrane of the target cells. Notably, the CD4 molecule is also the receptor for human immunodeficiency virus-1 (HIV-1), and it is believed that this structure might contribute to the spread of HIV-1 infection into the pulmonary microenvironment [20–22]. In fact, besides being expressed by pulmonary T-helper cells, CD4 molecules are also coexpressed on the surface membrane of alveolar macrophages and lung fibroblasts, *i.e.* two of the predominant cell populations harbouring HIV-1 in the lower respiratory tract [23].

Recent studies on the expression of cytotoxic markers by pulmonary cells led to the realization that the respiratory mucosa is equipped with cytotoxic cells that can undergo activation whenever challenged by foreign antigens. As better specified in the section devoted to the biological function of lung lymphocytes, several different lymphocyte-mediated cytotoxic mechanisms might potentially be active in the lung, including NK cell cytotoxicity, antigen-specific cytotoxic T-lymphocyte (CTL) activity, and lymphokine activated killer (LAK) activity. In healthy individuals, approximately 10% of BAL lymphocytes express the CD57 NK-related markers, and only a small proportion of cells (5%) express the CD56 NK-associated markers [24]. Interestingly, while the majority of peripheral blood cytotoxic cells with NK activity are large granular lymphocytes that bear CD16 and lack expression of the CD3/TCR, few CD3-/CD16+ cells are present in the pulmonary microenvironment [24] (fig. 1).

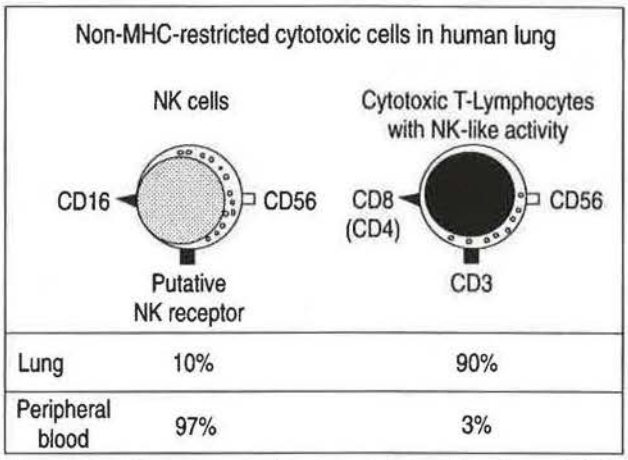

Fig. 1. – Comparison of the phenotypic profile of non-MHC-restricted cytotoxic cells isolated from the peripheral blood and in the lower respiratory tract of healthy individuals. Whilst the great majority of circulating non-MHC-restricted cytotoxic cells is represented by CD3-/CD16+/CD56+ large granular lymphocytes, pulmonary CD56+ granular lymphocytes coexpress the CD3 molecule and the α/β TCR in high proportion (≈90%), showing the phenotype that characterizes T-cells with NK-like activity. MHC: major histocompatibility complex; CD: cluster designation; TCR: T-cell receptor; NK: natural killer.

Constitutive expression of accessory molecules and activation markers by pulmonary T-cells

Several accessory molecules are constitutionally expressed on the surface of pulmonary T-lymphocytes. These structures have been involved in non-antigen specific homing of T-cells to the secondary lymphoid tissues, in the process of antigen specific activation and in the localization of T-cells to the sites of inflammation.

As discussed previously in the section on the origin of pulmonary lymphocytes, the process of non-antigen specific compartmentalization of T-cells involves the interaction of homing receptors on the surface of "virgin" lymphocytes (CD44, LFA-1 and very late activation (VLA) antigens) to organ-specific endothelial molecules (known as the vascular adressin) of the secondary lung lymphoid tissues [3–5, 25]. The conversion of naive CD45RA T-cells to memory pulmonary CD45RO T-cells coincides with the increase in the surface expression of adhesion structures (two to fivefold) [26]. In turn, CD45RO T-cells that express high levels of CD2, LFA-1, LFA-3, VLA and CD44 antigens acquire the ability to selectively localize within the pulmonary parenchyma [27].

Another important characteristic of the pulmonary T-cell population concerns its state of activation. Several T-cells sparsely distributed throughout the pulmonary parenchyma have undergone prior activation. This is supported by the findings that lymphocytes recirculating within the lung tissue are CD45RO memory T-cells, expressing high levels of adhesion receptors, including selectins and leucocyte integrins (figs 2 and 3) [27, 28]. However, in nonsmoking normal subjects also a subset of pulmonary lymphocytes (5%) stains with HLA-DR MoAb. Moreover, a similar proportion of lung T-cells

bears activation antigens, such as VLA-1 [27–29], the above reported memory phenotype (CD45RO/CD29) [27, 30], and molecules analogous to the integrins such as B-ly-7 and CD69 [31]. Finally, discrete amounts of normal T-cells express the p55 chain of the IL-2 receptor (CD25). Coupled with recent findings about the secretory capabilities of BAL T-cells, these data confirm the concept that pulmonary lymphocytes *per se* represent a class of preactivated memory cells.

Recruitment and activation

An optimal host response against pathogenic stimuli depends on the rapid homing, to sites of inflammation, by memory lymphocytes that have been previously primed in secondary lymphoid tissues. The first step in this process is the binding of memory cells to endothelium. In fact, the increased adhesiveness shown by activated T-cells to pulmonary endothelium probably represents the critical factor in recruiting antigen-specific T-cells to sites of inflammation (fig. 4a). More particularly, it is believed that T-cells with effector functions, after interaction with their inciting antigen, extravasate to the pulmonary inflamed areas. In this context, there is general agreement that the recruitment is the result of the binding of LFA-1 integrin, expressed on the cell surface of memory lymphocytes, to ligands expressed by the inflamed endothelium (including ICAM-1 and ICAM-2) [32, 33]. The system VLA-4/vascular cell adhesion molecule (VCAM)-1 represents another complex of molecules that is involved in the lymphocyte-endothelium adhesion during inflammation [34].

The expression both of ICAMs and VCAM-1 on pulmonary endothelium is induced by inflammatory mediators, that are locally released by lung immunocompetent cells following antigenic challenge. Under certain conditions, cytokines (such as IL-1, interferon-γ (IFN-γ) and tumour necrosis factor-α (TNF-α)), and bacterial products (including lipopolysaccharide) can upregulate the expression of messenger ribonucleic acid (mRNA) for adhesion molecules on endothelial cells (fig. 4a) [35]. Through the release of these molecules, antigen-activated T-cells, alveolar and interstitial macrophages, and other mucosal and intra-alveolar accessory cells (dendritic and endothelial cells) expand the pool of memory cells within the inflamed area. These newly recruited effector cells can, thus, more efficiently respond to a secondary antigenic challenge to the previously recognized antigen.

The expression of accessory molecules also influences several functional properties of pulmonary T-cells [36]. T-cell-mediated cytotoxicity, T-cell proliferation and T-dependent B-cell proliferation are dependent on the ability of T-lymphocytes to bind target material. In particular, LFA-1/ICAM and CD2/LFA-3 systems have the physiological role of enhancing the weak interactions between TCR and its potential targets. Other molecules

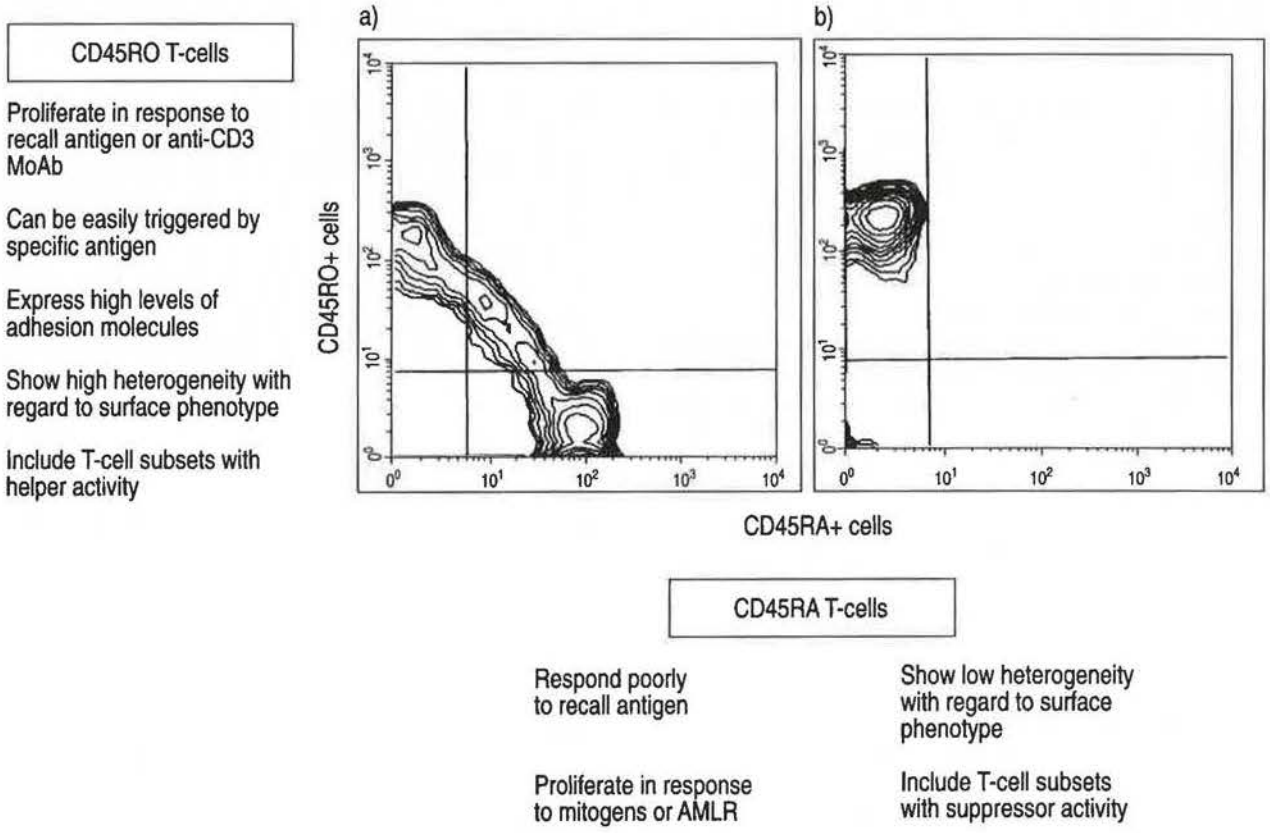

Fig. 2. – Phenotypic profile of CD45RO+ and CD45RA+ T-cell subpopulations: a) in peripheral blood; and b) in the lower respiratory tract. All lymphocytes retrieved from the BAL of this representative healthy subject are represented by CD45RO+ T-cells that express high levels of adhesion receptors and show functional capabilities of memory cells. MoAb: monoclonal antibody; AMLR: autologous mixed lymphocyte reaction.

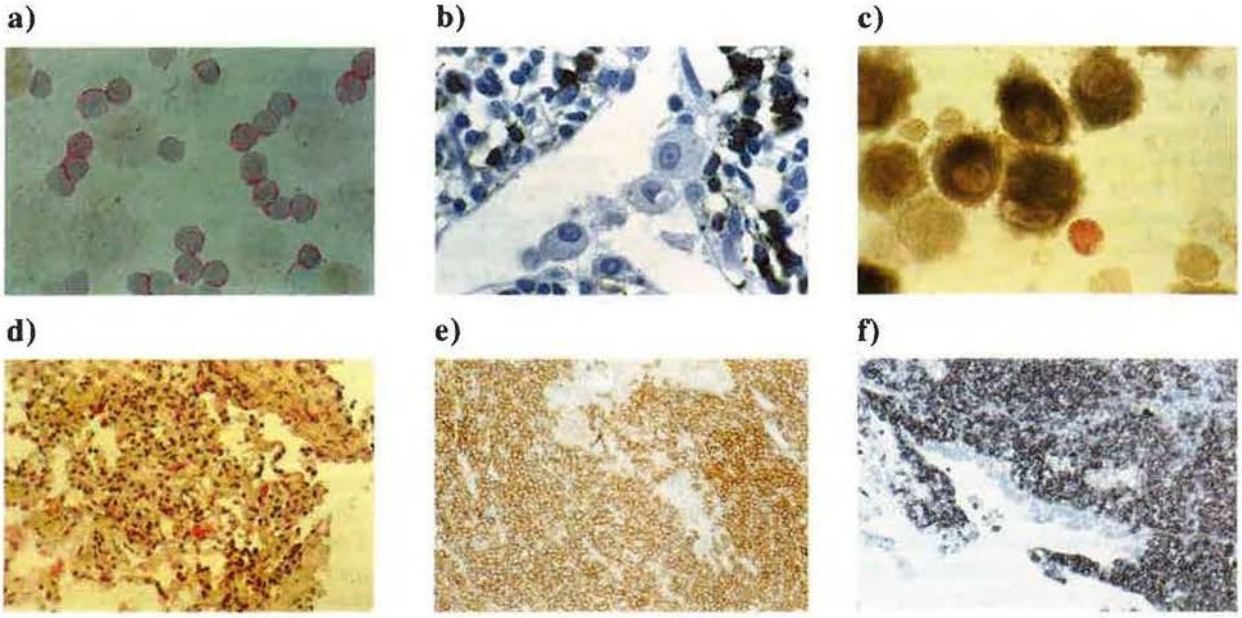

Fig. 3. – a) Cytospin of bronchoalveolar lavage (BAL) cells recovered from a sarcoid patient with high intensity alveolitis, immunostained with the alkaline phosphatase/anti-alkaline phosphatase (APAAP) technique (1,000×). b) Paraffin section from the transbronchial biopsy of a patient with active sarcoidosis, immunostained using the immunoperoxidase technique with anti-CD45RO monoclonal antibody (400×). Both BAL lymphocytes (red) and lymphocytes within the lung parenchyman (brown) are CD45RO positive "memory" cells. c) Cytospin of BAL cells isolated from a patient with active sarcoidosis, immunostained using APAAP technique with a monoclonal antibody recognizing the cell proliferation related marker Ki67 (1,000×). Note that the positivity for Ki67 is restricted to lymphocytes (red). Alveolar macrophages are recognized by morphology and by their strong nonspecific esterase (alpha-naphthyl-acetate-esterase, ANAE, brown/black staining). d) Cryostat section of the transbronchial biopsy from a patient with hypersensitivity pneumonitis, immunostained using the APAAP technique with anti-CD8 monoclonal antibody (100×). Most lymphocytes infiltrating the lung are positive (red). e and f) Cryostat section (e, 250×) and paraffin section (f, 250×) from the open lung biopsy of a patient with primary B-cell pulmonary low grade non-Hodgkin's lymphoma, using the immunoperoxidase technique with anti-kappa (e) and anti-CD20 (f) antibodies. This sample is characterized by the presence of a diffuse infiltration of CD20+/kappa+ neoplastic lymphocytes.

which are involved in T-cell recognition and proliferation include VLA-4 and CD44 antigens. Thus, the function of adhesion structures constitutionally expressed on the surface of pulmonary T-lymphocytes is not restricted to the process of tissue localization but even operates in concert with the TCR in the processing of potentially harmful antigens that have broken through the mucosal barrier of the respiratory tract.

In synthesis, a complex series of changes in phenotype and function accompanies the antigenic activation of T-cells at sites of inflammation. Antigen-dependent activation increases the expression of CD2, CD18, CD29, CD38, CD44, CD45RO, CD54 and CD58, class II MHC antigens, and VLA determinants on the cell surface of pulmonary T-cells. Activated pulmonary T-cells mediate antigen-specific effector functions and, with other pulmonary immunocompetent cells, secrete increased amounts of biological response modifiers that favour the tissue localization of newly recruited antigen-specific memory cells.

Biological cell functions and interactions with other cells

The pulmonary microenvironment is continuously exposed to a chaotic mix of antigens, but an efficient immune response mounts only to neutralize and destroy those few antigens that are potentially damaging for the lung. When an unwanted antigen enters the pulmonary tract, specialized antigen presenting cells (APC) are engaged, and a complex cascade of intercellular communications occurs between APC (alveolar and interstitial macrophages and, to a lesser extent, dendritic and endothelial cells) and effector T-lymphocytes. We now know, that antigen recognition represents the first step in the complex sequence of events that lead to cell migration and activation at sites of inflammation. Following attachment of antigen to the APC, the molecule is ingested by endocytosis, degraded, and part of its molecule is transported to the cell surface membrane. The antigen-derived molecule binds to the MHC molecule, *i.e.* the structure expressed on the surface membrane of APC that physically presents the foreign peptide to T-cells. As mentioned above, antigens presented in association with class I MHC determinants (HLA-A, B, and C) are recognized by CD8 cells, whilst CD4 cells recognize the foreign peptide only when it is presented in the context of a class II MHC (HLA-DR, DP, DQ) [37].

Following antigen recognition, discrete B- and T-lymphocyte subpopulations develop functional effector capabilities (immunoglobulin synthesis, cytotoxic activity, *etc.*), whilst other T-cell subsets acquire immunoregulatory functions that govern the magnitude and/or the duration of the effector cell responses. In addition, the interaction between pulmonary lymphocytes and alveolar macrophages results in the generation of a number of biological response modifiers, that are responsible for the recruitment of peripheral blood lymphocytes, monocytes

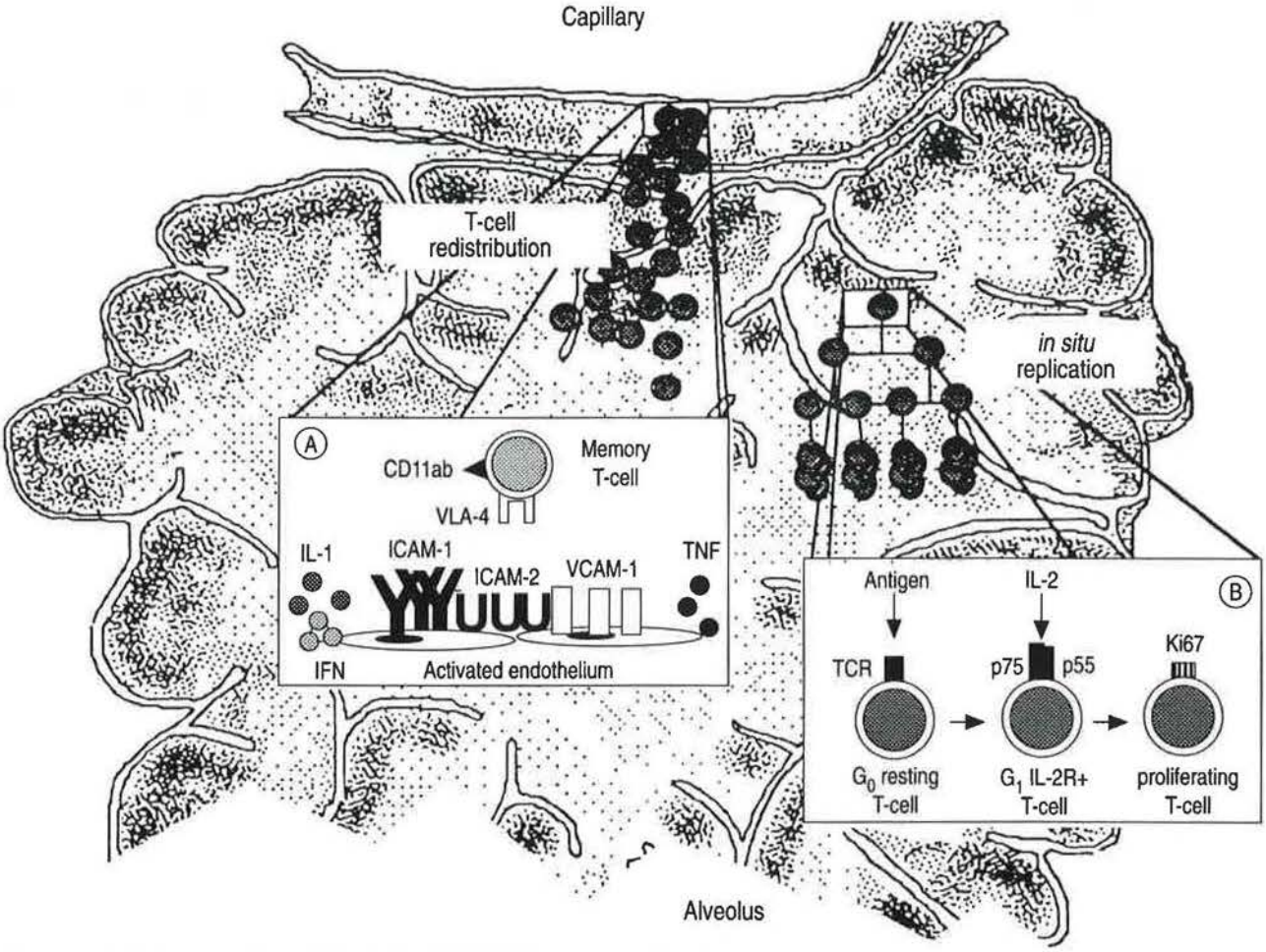

Fig. 4. – Mechanisms possibly involved in the development of T-cell alveolitis. Panel A: T-cell redistribution; and Panel B *in situ* IL-2 dependent proliferation of pulmonary T-cells. Panel A: T-cell recruitment is the result of the binding of LFA-1 (CD11a) and VLA-4 expressed on the cell surface of primed lymphocytes to ligands expressed by the inflamed endothelium (including ICAM-1 and 2, VCAM-1). The consequent binding of memory T-cells to cytokine-activated endothelium determines the tendency of a given lymphocyte subset to localize at sites of inflammation. Panel B: The induction of the IL-2 synthesis involves an early competence step that is triggered by foreign antigens. After activation of a quiescent lung T-cell, IL-2 mRNA accumulates and the lymphokine is rapidly secreted into the pulmonary microenvironment. Because of the repeated exposure of the T-cell to specific antigens, T-lymphocyte express maximal levels of the high affinity membrane receptor for IL-2 (formed by two distinct subunits, *i.e.* the p55 and p75 chains). The binding of IL-2 to its receptors initiates the progression of the cell cycle leading the the replication of the T-cell. VLA-1: very late activation antigen-1; IL-1: interleukin-1; ICAM: intercellular adhesion molecule; VCAM-1: vascular cell adhesion molecule; TNF: tumour necrosis factor; IFN: interferon; LFA-1: leucocyte function associated antigen-1; IL-2: interleukin-2; TCR: T-cell receptor; IL-2R: interleukin-2 receptor; mRNA: messenger ribonucleic acid.

and polymorphonuclears and for the further activation of surrounding immunocompetent cells.

Thus, the effectiveness of the lung immune system is dependent on the ordered differentiation of the lymphocyte subpopulations, which acquire different functional capabilities under antigenic pressure. Furthermore, networks of interacting cytokines are responsible for controlling the state of activation of all local immunocompetent cells. This section will focus on the highly complex heterogeneity of the lymphocyte subsets, and on the pattern of lymphokine production within the lower respiratory tract.

B-cell immune response

Whenever an inhaled antigen escapes the defence barriers of the upper respiratory tract, antigen-specific B-cells initiate the synthesis of immunoglobulins throughout the tracheobronchial tree and on the alveolar surface. IgA and IgG represent the two predominant immunoglobulins that can be found in the BAL. IgA molecules are present mainly in their secretory form, and are involved in the clearance of microorganisms from the respiratory tract, whilst IgG opsonins, specific for bacteria, facilitate the phagocytosis by alveolar macrophages, helping in the elimination of most pathogens [38, 39]. However, specific IgG is mandatory for the killing of Gram-negative microorganisms, for instance *P. aeruginosa* or *E. coli*. It is also possible that IgG may induce antibody dependent cellular cytotoxicity (ADCC) against various microorganisms and tumour cells [40]. This activity is mediated by multiple cell types, having on their surface membrane the Fc receptors for IgG as a common denominator. They include granular lymphocytes, T-cell subsets, activated macrophages and neutrophils.

Functional assessments of BAL B-lymphocytes have demonstrated that they are responsive to T-dependent B-mitogens, such as pokeweed mitogens (PWM), and can be elicited to synthesize immunoglobulins *in vitro* (see below). Taking advantage of this technique, it has been possible to study the ability of pulmonary B-cells to produce antibodies. Despite the high amounts of immunoglobulins which are present in BAL, it has been shown that immunoglobulin secreting cells are rare in the alveolar space [41]. Mucosal plasma cells secreting IgA predominate in the upper airway. By contrast, IgG is produced actively only by B-cells of lung parenchyma, whilst intraepithelial cells do not synthesize IgG *de novo*. It is generally assumed that, following antigenic challenge, immunoglobulins are produced in the upper airway (IgA) or pulmonary interstitium (IgG) and then released into the alveolar space.

We shall see in the following paragraphs, that an efficient release of soluble mediators (IL-2, IL-4, IL-6) by T-cells with helper activity is required for the activation and expansion of B-cells, whilst antigen-specific and antigen-nonspecific suppressor CD8 cells counteract the effect of helper T-cells on B response.

Immunoregulatory properties of pulmonary T and NK cells

The ultimate goal of the T-cell activation is to induce the generation of different sets of T-cell subpopulations with antigen specificity (helper, suppressor-inducer, suppressor, contrasuppressor cell). Detailed analysis of the T-effector subsets in the lung have demonstrated that the great majority of CD4 lymphocytes retrieved from healthy subjects express the phenotype that is characteristic of memory cells with helper activity (CD4+/CD29+/CD45RO) [29]. These cells are capable of providing help for immunoglobulin synthesis *in vitro*, but cannot mediate suppression, delayed-type hypersensitivity, or cytolysis (fig. 2). By contrast, pulmonary CD4+/CD45RA+ can activate CD8 suppressor cells, thus slowing down specific antibody responses driven by helper CD4+ T-cells. They also mediate delayed-type hypersensitivity mechanisms and cell-mediated cytotoxic activity. Data obtained in patients with interstitial lung diseases (ILD) and pulmonary viral infections, suggest that both these helper and suppressor-inducer CD4+ cell subsets are involved in the induction of responsiveness and tolerance to micro-organisms and antigens. This is particularly evident in patients with sarcoidosis, whose BAL lymphocytes express the CD4+/CD29+/CD45RA- associated phenotype, show the ability to provide immunoglobulin synthesis in a PWM-driven B-cell differentiation assay and synthesize several biological response modifiers [42, 43].

The role of suppressor cells in the modulation of pulmonary immune responses is less clear. Whilst there is no doubt that the phenomenon of the T-mediated suppression also exists in the lung, the means by which suppressor CD8 cells control the size and the duration of the pulmonary immune activation is not fully characterized. For instance, some lung CD8 cell populations show an *in vitro* suppressor activity on B-cells, but in other experimental systems the same CD8 cells generate cytotoxic effector cells. It is also difficult to differentiate CD8 suppressor cells from CD8 cytotoxic cells, in terms of phenotype, since both cells may carry similar surface markers. The scenario is further complicated by the fact that it is still unknown whether complementary circuits of contrasuppressor CD8 cells (*i.e.* the cell subset that protects T-helper cells from the suppressor cells) take place in the lung. Clarification of the immunoregulatory networks occurring in the pulmonary tract of healthy subjects and patients with pulmonary disorders should be possible within the next few years.

As previously mentioned, the pulmonary immune system comprises two major classes of lymphoid cytotoxic effectors: 1) antigen-specific CTL that recognize target cells *via* the CD3-Ti complex and require the expression of MHC-gene products on targets; and 2) non-MHC restricted cytotoxic cells that may lyse certain tumour and viral infected targets without prior sensitization [13, 24]. In resting conditions, only a minority of BAL cells are MHC-restricted CTL [24]. Although pulmonary CTL are very few in the lung of normal individuals, they do not behave as bystander cells. These cells play a central role in anti-viral immunity, and are directly involved in the protection against many bacterial and protozoan infections. CTL are also essential for protection against tumours, and can play a part in the pathogenesis of some pulmonary diseases, because under certain circumstances they can mediate the lysis of normal constituents of the lung parenchyma [44]. Furthermore, CTL have been involved in mechanisms leading to host graft rejection following allogeneic lung transplantation (see below).

Unlike specific CTL that increase only during conditions resembling host invasion, non-MHC-restricted cells are present in discrete numbers under normal conditions also. As discussed above (fig. 1), whilst the major part of circulating non-MHC-restricted cells are CD3 negative, lung CD56 and CD57 cells coexpress the CD3 molecule and the α/β TCR in high proportion, showing the phenotype that characterize T-cells with NK-like-activity. From a functional point of view, these cells show a low but detectable cytolytic activity against the NK-sensitive K-562 targets [45, 46]. Notably, the non-MHC-restricted cytotoxic activity can be demonstrated only following removal of alveolar macrophages from the cell population being studied, since pulmonary macrophages exert a strong suppressive activity on the *in vitro* NK activity [46]. Progenitors of lymphokine-activated killer cells (LAK) cells can also be easily demonstrated among lymphocytes that home on the lower respiratory tract. In fact, IL-2 treatment of BAL cells generates a spontaneous killing of NK-resistant targets, and we have recently demonstrated that the effector cell population contains both T-cells with NK-like activity and CD8 T-cells [47].

With all these data taken together, we can postulate a model for the cytotoxic mechanisms that take place in the lung following antigenic challenge. Non-MHC-restricted cells represent the first line of the pulmonary host defence against tumour and microbial infections. These cells are appointed to destroy foreign material in

a nonspecific manner. If this first line of defence is overwhelmed by the non-self molecules, alveolar macrophages phagocytize the non-self molecules, and initiate the process of antigen presentation to T-cells. This leads to the production of a large repertoire of immunomodulatory molecules, including IL-1, IL-2, IFN-γ and TNF-α, that potentiate the non-MHC-restricted lysis, induce LAK activity, and favour the expansion and diversification of specific CTL, able to provide a specific cytotoxic response against foreign antigens. The release of cytokines is also accompanied by changes in the expression of adhesion molecules on effector cells, and this contributes to the magnitude of the cytotoxic response.

Lymphokine pattern in the lung

Thus, most of the functions shown by T-cells are mediated by the *in situ* release of a series of biological response modifiers. In fact, after antigenic activation, pulmonary T-lymphocytes acquire the capacity to produce a broad repertoire of lymphokines. These molecules act as critical mediators of cell functions and cell-cell communications, by influencing many physiological cell properties, including proliferation, differentiation and activation of other immunocompetent cells, chemotaxis, connective tissue metabolism, *etc.* [48]. Only the lymphokines that might be relevant to the function of the pulmonary immune system are mentioned here (table 2).

Interleukin 2. There is general agreement that IL-2 can be considered the pivotal molecule among the several lymphokines that are actively released by pulmonary T-cells. The role of IL-2 in the pulmonary immune system, as in other organs, is to expand activated T-cell populations through a series of well-established events (fig. 4b) [49]. Besides providing the relevant proliferative stimulus for activated T-cells, IL-2 is also involved in the regulation of immunoglobulin production and in the enhancement of the potential capabilities of pulmonary cytotoxic cells. Furthermore, inasmuch as some alveolar macrophages normally express a low density p55 interleukin receptor (IL-2R) and the addition of IL-2 to activated macrophages increases expression of granulocyte and granulocyte macrophage colony stimulating factor (G-CSF and GM-CSF) gene, it is thought that IL-2 might be involved in the activation of some functional capabilities of alveolar macrophages [50–52].

Interleukin 4. This lymphokine is a cofactor for cell proliferation of multiple cell lineages, and enhances the production of IgG1 and IgE by B-cells. It also induces the expression of class II MHC antigens on surface membrane of accessory cells, and acts in synergism with IL-2 in stimulating the growth of T-cells [53]. The extent to which T-cells produce IL-4 in the human lung has not been completely defined.

Interleukin 5. By producing IL-5, pulmonary T-cells promote IgE and IgA production [54]. This lymphokine also has activity on the differentiation and activation of eosinophils. At the present time, it is believed that IL-5 might represent a crucial factor in the development of asthma in atopic individuals [55].

Interleukin 6. IL-6 represents a stimulator for B-cell growth and for T-cell proliferation [56]. IL-6 is produced by different cell populations, including activated T-lymphocytes and macrophages, endothelial cells, and fibroblasts. It may cause fever and the active synthesis of acute-phase proteins. The cytokine also has weak antiviral interferon-like effects. An intriguing possibility is that IL-6 may be involved in lung diseases characterized by *in situ* proliferation of fibroblasts.

Interleukin 9. IL-9 is a T-cell derived lymphokine that maintains T-cell proliferation independently from other growth factors, such as IL-2 and IL-4 [57]. Because this lymphokine enhances the IL-3 mediated growth of mast cell precursors, and promotes IL-6 production by mast cells, it has been speculated that IL-9 may function *in vivo* as an enhancing factor for the local mast cell activity.

Interleukin 10. This molecule is released by activated T-helper cells and, as recently demonstrated, by some B-cell subsets [58]. It shows inhibitory activity on the release of IFN-γ and IL-2 by TH1 cells (see below). Furthermore, IL-10 stimulates mast cell growth and regulates the accessory function of antigen presenting cells. Its role in pulmonary pathobiology is still unknown.

Interleukin 12. This lymphokine has been shown to stimulate the proliferation and the lytic activity of activated T-cells and NK cells [59]. Furthermore, it synergizes with IL-2 in the generation of LAK cells. Activation of CD3+ T and CD56+ NK cells results in an upregulation of IL-12 receptor expression. In turn, the IL-12/IL-12 receptor binding provides a co-stimulatory signal for enhancing the cytotoxic activity of killer cells. The role of IL-12 in pulmonary immune responses is presently under investigation.

Interleukin 13. This recently discovered lymphokine strongly inhibits IL-6 secretion induced by lipopolysaccharide (LPS) in peripheral blood monocytes [60]. A marked inhibition is also seen for the mRNA expression of several other cytokines in LPS-treated monocytes, including IL-1, TNF-α, and IL-8. IL-13 also increases the proliferative activity and the CD23 expression on B-cells [60]. Whether the anti-inflammatory function of IL-13 may be crucial in limiting inflammatory responses in the lower respiratory tract deserves further investigation.

Interferon-γ. Besides its well-established antiviral activities, IFN-γ has multiple effects on the pulmonary immune system [61]. It enhances the accessory function of antigen presenting cells, increases the cytotoxic function, both in macrophages and lymphocytes, and regulates the secretion of other lymphokines. Because of its multiple effects, this molecule represents a key factor in the regulation of various mucosal immune responses in the lung.

Table 2. – List of lymphokines that can be released by pulmonary lymphocytes under physiological conditions and/or during inflammation

Lymphokine	Main cell source	Main biological activity
IL-2	Helper T-cells (TH1), some NK cells	Induces cell proliferation, promotes cytokine production, activates macrophages, stimulates immunoglobulin synthesis in B-cells, evokes cytotoxic activity
IL-3	T-cells (TH1 and TH2)	Multilineage colony stimulating factor
IL-4	T-cells (TH2)	Involved in IgE synthesis, involved in growth of T, B and myeloid cells
IL-5	T-cell subsets (TH2)	Involved in growth and activation of eosinophils
IL-6	Macrophages, fibroblasts, T-cell subsets (TH2)	Stimulates immunoglobulin synthesis, proinflammatory factor
IL-9	T-cell subsets (TH1)	Costimulator for T-cell proliferation, regulates mast cell growth
IL-10	T-cell subsets (TH2), B-cells,	Inhibits cytokine synthesis in TH1 cells, regulates mast cell growth and macrophage differentiation
IL-12	Accessory cell populations	Regulates NK activity
IL-13	Activated T-cell subsets	Inhibits cytokine synthesis in monocyte-macrophages, regulates B-cell proliferation
GM-CSF, G-CSF, M-CSF	Activated macrophages, T-cells (TH1 and TH2)	Multilineage colony stimulating factors
IFN-γ	Activated macrophages, T-cell subsets (TH1), NK cells	Regulator of B-cell function, activates cytotoxic cells, mesenchymal cell growth
TNF-β	Macrophages, activated T-cells	Anti-tumoral and viral activity, involved in cell recruitment, involved in cytotoxic mechanisms, proinflammatory factor
TGF-β	Macrophages, T-cell subsets, platelets	Growth factor for fibroblasts, induces collagen and fibronectin synthesis, immunesuppressive factor

IL: interleukin; GM-CSF: granulocyte macrophage colony stimulating factor; G-CSF: granulocyte colony stimulating factor; M-CSF: macrophage colony stimulating factor; IFN-γ: gamma-interferon; TNF-β: tumour necrosis factor-β; TGF-β: transforming growth factor-β.

Colony stimulating factors. GM-CSF, G-CSF, macrophage colony stimulating factor (M-CSF) and IL-3 are soluble mediators that are able to induce the growth and differentiation of progenitors of myeloid and macrophage precursors in the bone marrow, facilitating the accumulation and differentiation of cells belonging to phagocyte lineages in lung tissues [62]. Furthermore, GM-CSF modulates the cytokine production by human mononuclear cells, and enhances the antigen presenting capacity on pulmonary accessory cells [63].

Transforming growth factor-β. Transforming growth factor-β (TGF-β) is mainly secreted by monocyte-macrophages and activated lymphocytes, including pulmonary lymphocytes [64]. This potent immunosuppressive molecule exerts chemotactic activity for monocytes, and modulates the synthesis and the effect of several other molecules, such as IL-1, IL-2, IL-3, GM-CSF, IFN-γ and TNF-α. The few data available in humans suggest that this molecule is constitutively released in the respiratory tract.

Tumour necrosis factor-β. The wide secretory repertoire of pulmonary lymphocytes includes TNF-β. As with macrophage-derived TNF-α, this product of activated lymphocytes is involved in cytotoxicity mechanisms and plays a role in endotoxic shock [65]. Furthermore, it behaves as a stimulator and regulator of synthesis and release of other lymphokines. To date, little is known of the *in vivo* synthesis of TNF-β in the lung, but it is likely that it participates in the local cytotoxicity mechanisms.

Subdivision of pulmonary T-cell subpopulations based on lymphokine production. Studies in animal models have shown that activated CD4 T-cells can be subdivided into two broad types of helper cells, called TH1 and TH2, based on the pattern of lymphokine production [66, 67]. Murine TH1 cells secrete IL-2, IL-12, IFN-γ and TNF-β but not IL-4, IL-5 and IL-6, whilst TH2 lymphocytes produce IL-4, IL-5, IL-6, IL-9 and IL-10. Both cells produce GM-CSF and IL-3. The two subpopulations also differ from a functional point of view. In fact, TH1 lymphocytes elicit delayed type hypersensitivity reaction and help in IgG synthesis, but through the release of IFN-γ inhibit the cytokine release by TH2 lymphocytes and the IgE synthesis. In contrast, TH2 cells release IL-10, that inhibits the proliferative activity of TH1 lymphocytes.

The possibility that the pattern of lymphokine production may vary in the lung of healthy individuals and patients with different pulmonary diseases has been apparent since the initial description of the functional dicotomy of activated TH1 and TH2 CD4+ cells. As specified in the section devoted to the role of lymphocytes during pulmonary diseases, recent studies have raised the possibility that different types of pulmonary inflammation are characterized by an influx of cells that are committed to release specific cytokines (fig. 5). It is not clear whether under physiological conditions there are differences in the pattern of cytokine production between CD4 cells homing in the different compartments of the pulmonary microenvironment.

Macrophage-derived cytokines with influence on functional activity of pulmonary lymphocytes. Normal lung macrophages are capable of producing detectable amounts of IL-1α and IL-1β, even if in a lower quantity than that released by peripheral blood monocytes. Experimental data have suggested that an increased expression of IL-1 mRNA by alveolar macrophages may lead to the activation of pulmonary T-cells [68]. It is likely that the role of IL-1 in the pulmonary immune system is to provide accessory growth factor for CD4 memory T-cells.

It has recently been demonstrated that human alveolar macrophages may contribute to the host defence mechanisms, by the synthesis and release of a neutrophil chemotactic cytokine, called IL-8. When activated by LPS or other cytokines, including TNF-α, alveolar macrophages show an increased expression of IL-8 mRNA in a kinetic and dose-dependent manner [69]. Since this macrophage-derived cytokine can also favour the local accumulation of lymphocytes [70], an intriguing hypothesis, yet to be tested, is that the production of this factor might play a part in the recruitment of memory T-cells at sites of pulmonary inflammation.

A general consideration is needed to conclude the topic of the lymphokine pattern in the lung. It should be noted that most of the cytokines considered so far are not produced by pulmonary T-cells alone, but are potentially released by many other pulmonary cells. For instance, the cytokine IL-6 is produced not only by pulmonary T-cells but also by activated macrophages, endothelial cells and fibroblasts. Furthermore, increasing evidence suggests that several of the T-derived lymphokines are not cell lineage specific. For instance, the lymphokine IL-4 acts upon T- and B-cells, as well as on myeloid cells. Thus, the interrelationship between the various lymphokines results in a complex network of effects on the pulmonary microenvironment, that is further complicated by the fact that an individual induction signal results in the synthesis and release of lymphokines that can display overlapping and even contradictory cell regulatory actions. Therefore, any physiological or pathological alteration of the cytokine network leads to the release of a cascade of interacting extracellular signalling proteins, with complex and variable consequences on the surrounding pulmonary environment. Further understanding of the role of lymphokines in the lung under physiological conditions could form the basis for a better comprehension of the pathogenesis of several pulmonary disorders.

Laboratory techniques for the study of pulmonary lymphocytes

A wide array of laboratory assays are now available for accurately testing the phenotypic and functional profile of every cell component of the human immune system. This section will review laboratory tests that are currently applied in studying the immune competence of pulmonary lymphocytes.

Histological and immunohistological examination of lung biopsies

Histological and immunophenotypic characterization of lymphocytes in lung biopsies is considered of paramount importance for final diagnosis in different pathological conditions. Among these, are the differential diagnosis of primary lymphoid lung lesions (malignant lymphomas, pseudolymphomas and lymphoid interstitial pneumonia), and the search for granulomas in the differential diagnosis of sarcoidosis and other interstitial lung diseases. The most direct approach to the study of lung parenchyma is provided by open-lung biopsy [71], but the less invasive transbronchial biopsy is now preferred in most cases. In fact, the knowledge of physiopathological mechanisms involved in reactive and neoplastic lymphoid diseases has been significantly expanded due to the possibility of direct and detailed analysis of different cell components *in situ*.

Immunohistochemical methods that increase the discriminating power of morphological analysis have been successfully applied to cryostat and paraffin embedded specimens, providing important data on the changes in the pulmonary microenvironment. A large number of MoAbs are currently available, that allow the characterization of different lymphoid B and T subpopulations, macrophages, dendritic cells, as well as other components of the lung parenchyma and extracellular matrix (table 1 superscripts indicate MoAbs useful for characterizing different cell types in lung biopsies). Many immunophenotypic markers can be used only on frozen-tissue sections, since routine fixation and embodiment procedures destroy antigenic determinants. Nevertheless, a number of MoAbs recognizing

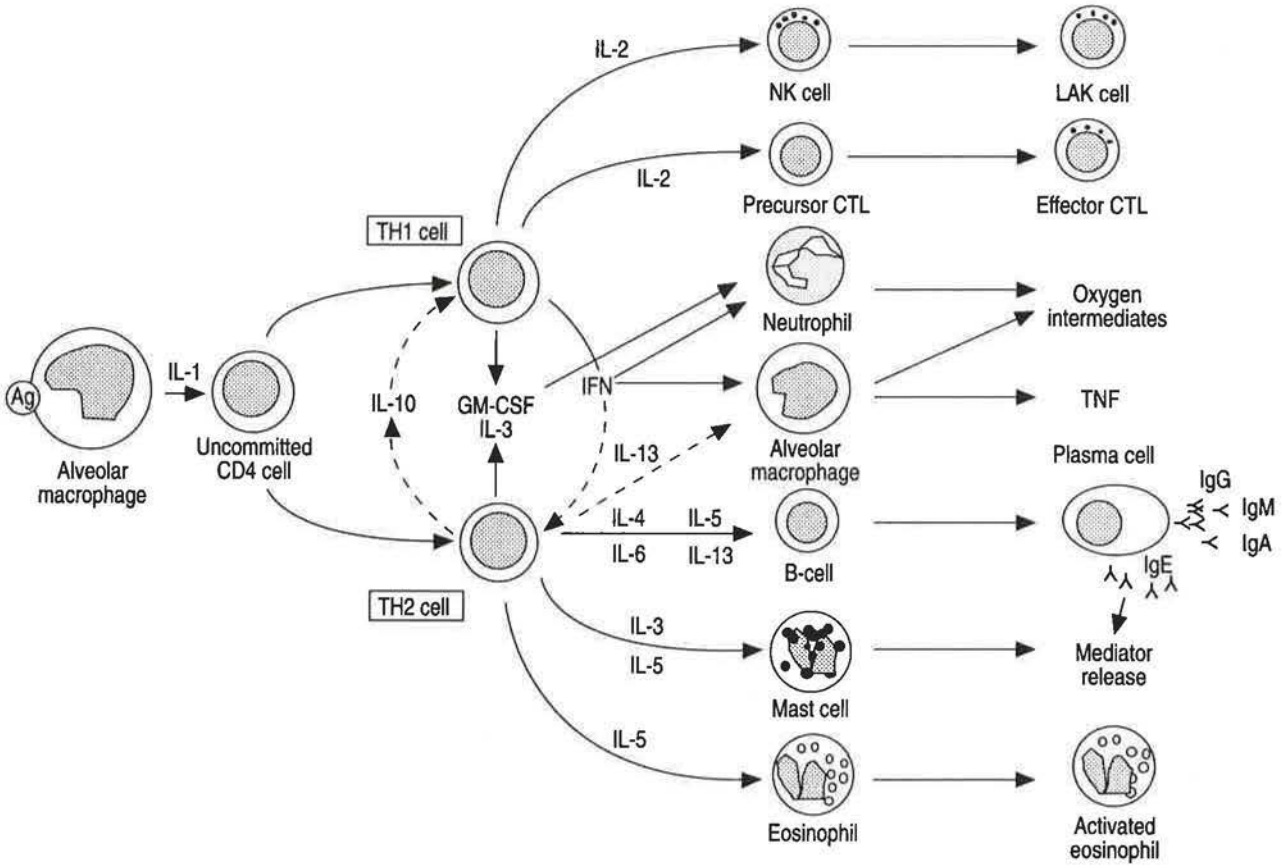

Fig. 5. – Schematic representation of lymphokines that are locally released by TH1 and TH2 pulmonary cells. Interaction between biological response modifiers and surrounding immunocompetent cells results in a complex network of effects on local immune system. The solid arrows indicate positive signals, whilst broken arrows indicate negative or inhibitory activities on target cells. For simplicity only the major activities are shown. The chart shows that some lymphokines display overlap of functions, whilst others show contradictory regulatory actions on target cells. IL: interleukin; IFN: interferon-γ; GM-CSF: granulocyte-macrophage colony stimulating factor; TNF: tumour necrosis factor-α; TH: T-helper; CTL: cytotoxic T-lymphocyte; NK: natural killer; LAK: lymphokine activated killer; Ag: antigen; Ig: immunoglobulin.

relevant fixation-resistant epitopes are available, which can be used for research and diagnostic purposes. In particular, double marker and triple marker analyses can be performed on the same section, thus providing additional information that can be helpful in resolving the micro-environment complexity of reactive lesions [72].

Immunohistochemical analysis has been widely used on lung biopsies to study the *in situ* distribution of T-cell subsets in sarcoidosis and hypersensitivity pneumonitis, confirming the data obtained by BAL analysis. The simultaneous detection of differentiation antigens, together with cell-cycle related proteins (Ki67, proliferating cell nuclear antigen, PCNA), allows for the precise analysis of proliferating cells within sarcoid lesions [73]. In addition, immunohistochemistry helps by providing information on the normal and pathological distribution of extracellular matrix components within the lung. This is a field of growing interest for understanding the complex mechanisms of lymphocyte recirculation between secondary lymphoid tissues and the lung [74]. A significant increase in sensitivity of granuloma detection can be obtained, even if a limited number of markers are routinely applied in the histological analysis of transbronchial biopsies from patients with clinical and or laboratory features suggestive of sarcoidosis [75].

Cellular analysis by flow cytometry

Flow cytometers are instruments used to analyse single samples of cell suspensions. The technique combines the light scatter and volume measurements of cell populations under study, with the use of fluorescence-labelled MoAbs. Mononuclear cells larger and/or smaller than lymphocytes and neutrophils can be identified, gated out electronically, and the cell analysis can be selectively concentrated on the lymphoid component of the BAL sample (fig. 6).

This approach has its clinical application in the study of lung diseases which are characterized by an alveolitis [76]. In particular, flow cytometers have been widely used for the phenotypic analysis of B, T and NK lymphocytes accumulating in the lower respiratory tract of patients with interstitial lung disease (ILD), HIV-1 infection, asthma, and lung transplant recipients (see below). Flow cytometry can also provide additional information about the activation state of pulmonary immunocompetent cells. By combining the use of two or three differently coloured fluorochromes (*i.e.* fluorescein, phycoerythrin, allophycocyanin, Texas red, peridinin chlorophyl protein) it is possible to simultaneously evaluate the presence of differentiation and activation markers on BAL cells. One such example is the use of two colour immunofluorescence

technique, with HLA-DR MoAb paired with anti CD4 MoAb, to assess disease activity in patients with sarcoidosis [77]. Other potential applications of the flow cytometry analysis in the study of BAL cell suspensions are listed in table 3, but the clinical value of these tests remains to be defined.

Tests used to assess functional activities of pulmonary T, B and NK lymphocytes

A variety of tests are now available for determining functional activities of pulmonary T, B and NK lymphocytes. The proliferative activity of T-cells can be measured following incubation of BAL lymphocytes with stimuli that provide a polyclonal proliferative signal (including phytomitogens such as phytohaemagglutinin, concanavalin A and anti-CD3 MoAb). In these proliferation assays, the cellular deoxyribonucleic acid (DNA) synthesis by T-cells is usually assessed by measuring radioactive ^{3}H-uptake. Another promising approach for studying the immunoregulatory role of T-cells in lung is the analysis of the functional properties of BAL-derived T-cell clones obtained by limiting dilution from patients with inflammatory lung diseases [78].

In vitro tests to evaluate the B-cell competence in the pulmonary compartment are also available. These tests used to determine immunoglobulin synthesis by B-cells following incubation of BAL cells with T-dependent B-mitogens, including pokeweed mitogen or staphylococcal protein A. Immunoglobulin levels are then measured on the conditioned media by immunoenzymatic or radio-immunological methods.

The determination of the lysis of NK-sensitive target cells (such as the widespread erythroleukaemia cell line K-562) is the method of choice to evaluate NK activity in the lung. Target cells labelled with ^{51}Cr are cocultured with effector BAL cells, and the radio-activity released by lysed targets is measured in supernatants with a gamma counter.

Although most of these functional tests are well standardized, only a few of them have established clinical reliability in monitoring the clinical course of immunologically-mediated lung diseases. One exception is the primed lymphocyte test (PLT), a mixed leucocyte culture (MLC) test that is currently employed in clinical monitoring of rejection following lung transplants (see below) [79].

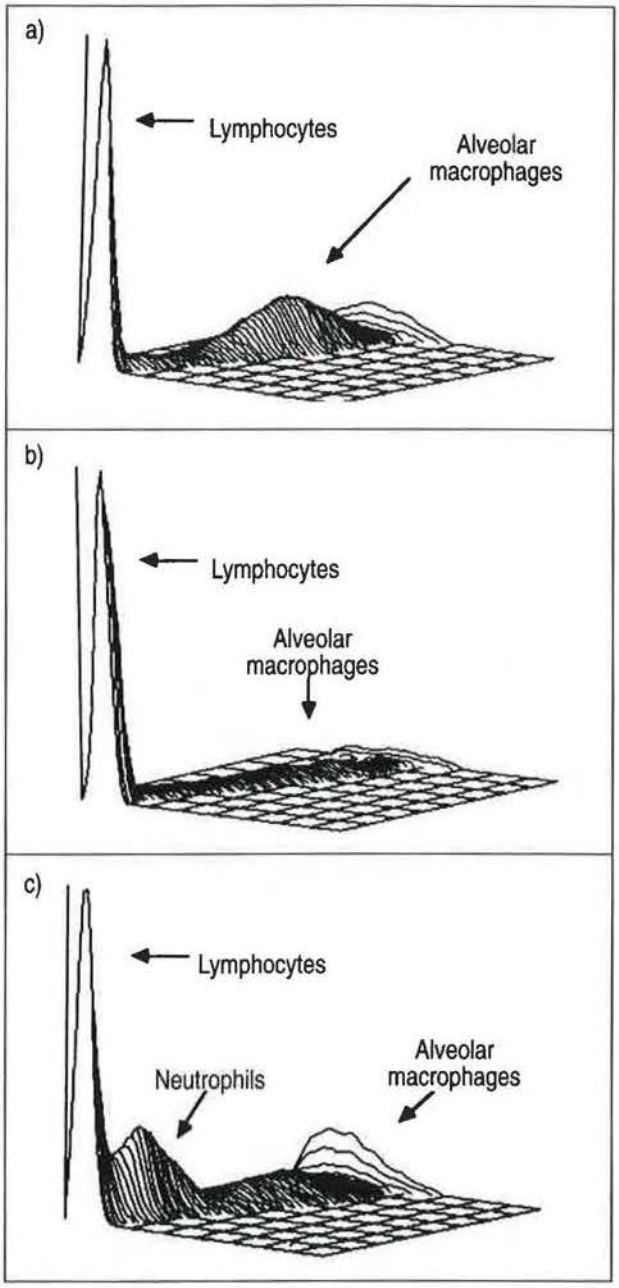

Fig. 6. – Side scatter and forward scatter cytogram for bronchoalveolar lavage (BAL) cells recovered from: a) a healthy subject; b) a patient with lymphocyte alveolitis; and c) a patient with neutrophil alveolitis. The arrows indicate the localization of lymphocytes, macrophages and neutrophils.

Genotypic analysis

As outlined in the preceding chapters, pulmonary immunology has greatly benefited from the advent of molecular biological techniques. In particular, taking advantage of the cloning of a myriad of immunologically relevant genes (including those encoding for leucocyte markers, T-cell receptors, and other accessory molecules, cytokines and cytokine receptors, MHC antigens, *etc.*) it has been possible to study the role of the distinct cell subsets in immune mechanisms that lead to inflammation of the lower respiratory tract. Despite this, molecular biological assays devoted to the study of the lung immune system have not yet achieved routine application in clinical pneumology, and are currently used only for basic immunological research. Figure 7 shows an example of the possible application of the molecular biological techniques in the studies of pulmonary immunocompetence. The Northern blot analysis and the following densitometry analysis are used to compare the amount of cytokine messages for a series of cytokines expressed by alveolar macrophages (AMs) recovered both from patients with interstitial lung disease and healthy subjects.

However, it should be noted that molecular biological tests can be used not solely for the study of the immune

Table 3. – Main applications of flow cytometry analysis of BAL cells

Analysis of the expression of differentiation and activation markers by T, B and NK lymphocyte subsets and alveolar macrophages
Monitoring clinical course of alveolitis in interstitial lung disease
Assessment of prognosis in HIV-1 seropositive patients with pulmonary involvement
Separation of individual cell subsets through sorting
Cell cycle analysis of proliferative activity of pulmonary cells
Determination of the expression of cytokine receptors by using fluorochrome-labelled cytokines

BAL: bronchoalveolar lavage; NK: natural killer; HIV-1: human immunodeficiency virus-1.

system. They are particularly useful when the demonstration of the presence of infectious microorganisms in the pulmonary microenvironment is mandatory. In fact, specific polymerase chain reaction technique and/ or *in situ* hybridization methods are currently available for the detection of known DNA or ribonucleic acid (RNA) sequences of several pathogens. For instance, these methods can demonstrate the presence of mycobacteria or *Pneumocystis carinii* sequences in BAL cells recovered from the lower respiratory tract of HIV-1 infected subjects with opportunistic infections [80, 81].

Role of pulmonary lymphocytes in human disease

It is now widely accepted that pulmonary T and B lymphocytes play a role in the pathogenesis of a number of pulmonary disorders. In this section, we will briefly describe the main results obtained in the last decade in terms of B, T and NK abnormalities in lung diseases.

Functional properties of pulmonary B-cells in some lung diseases

The impairment of the pulmonary B-cell system may lead to an increased susceptibility to certain kinds of respiratory infections [82]. One example is the association between the defect of IgG production and recurrent pulmonary infections, and another example is the high virulence of those pathogenic bacteria that can elaborate IgA protease, which destroys lung immunoglobulins. Another important cause of dysfunction of pulmonary B-cells is represented by HIV-1 infection. The levels of IgG and IgA are significantly increased in the BAL of patients with acquired immune deficiency syndrome (AIDS). Furthermore, using the reverse haemolytic plaque assay, YOUNG *et al.* [83] were able to demonstrate the presence of B-cells spontaneously secreting aberrant immunoglobulins in the lung of HIV-1 infected subjects.

B-lymphocytes producing high amounts of immunoglobulins can be isolated from the BAL of patients with some ILD. A polyclonal increase of BAL immunoglobulins is usually observed in patients with sarcoidosis; this finding is in keeping with the helper nature of lymphocyte infiltration, which characterizes this disease [84]. The BAL of patients with hypersensitivity pneumonitis (HP) contains large quantities of precipitating antibodies against the allergens causing the inflammatory process [85]. Goodpasture's syndrome and idiopathic pulmonary fibrosis represent another two examples of pulmonary disorders that are associated with a local release of immunoglobulins and deposition of immune complexes. Finally, the production within the respiratory tract of antineutrophil cytoplasmic autoantibodies (ANCA) has been claimed to be involved in the immunopathogenesis of pulmonary involvement during Churg-Strauss syndrome and Wegener's granulomatosis [86].

Pulmonary T-cells in patients with interstitial lung diseases

Changes in the frequency of T-cell subsets have been reported in many different ILD (table 4) [87–98]. Perhaps the most prominent are the immunological abnormalities that occur during sarcoidosis and HP. Both of these diseases are characterized by the accumulation of T-lymphocytes within the alveolar structures of the lung [82–98]. More specifically, lymphocytes usually belong to the helper-related CD4 T-cell subset in patients with sarcoidosis, whereas an intra-alveolar accumulation of CD8 cells with cytotoxic activity represents the hallmark of HP [82, 85].

In this respect, it must be stated that in the past 10 yrs there has been considerable enthusiasm in using the phenotypic profile of BAL T-cells for prognostic and diagnostic ends. According to this method, patients with ILD have been subdivided into patients with high-intensity CD4 or CD8 alveolitis. Recent data suggest that the diagnosis of sarcoidosis can be made by BAL in about 50–60% of patients. These are those cases with high CD4/CD8 ratios above 3.5 [96]. Nevertheless, it should be noted that in patients with CD4 alveolitis the phenotypic profile of the lymphoid constituents of alveolitis does not permit discrimination between patients with sarcoidosis, berylliosis or asbestosis. Furthermore, rare but well-documented cases of patients with sarcoidosis can be found in whom a CD8 alveolitis takes place [99]. Again, as a consequence of the therapy or of the different clinical

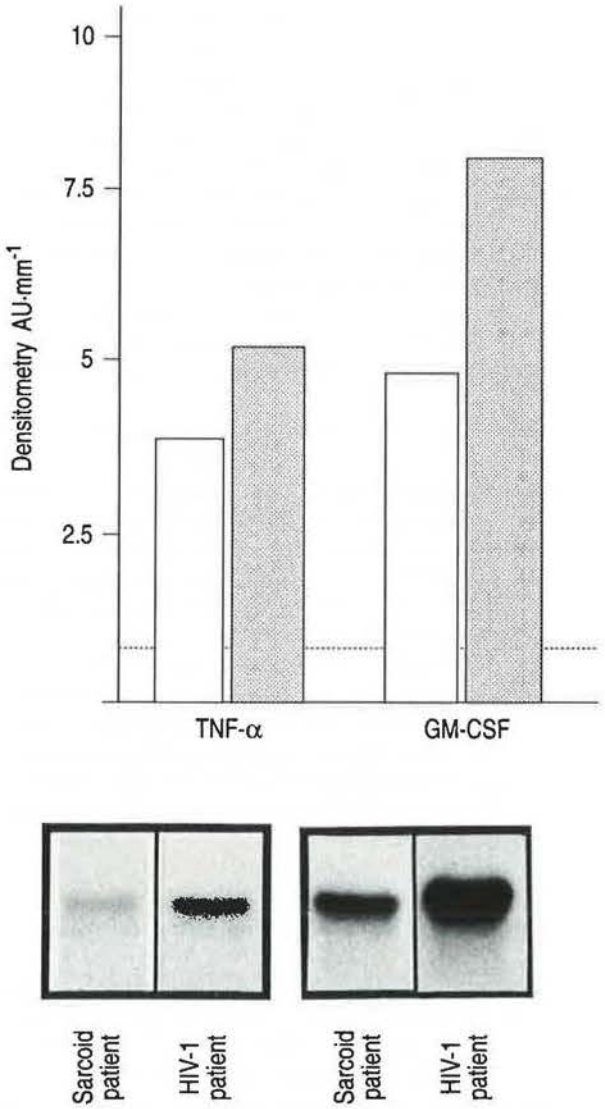

Fig. 7. – Northern blot analysis of TNF-α and GM-CSF mRNA expression by LPS-stimulated AMs from one patient with active sarcoidosis (lanes 1 and 3, respectively) and one patient with HIV-1 infection (lanes 2 and 4, respectively). Ten μg of total RNA were loaded in each line. The same amount was visualized by probing with actin probe. To compare the amount of cytokine messages induced by LPS on AMs, a densitometry analysis was performed. Dashed line indentifies one arbitrary unit (AU) corresponding to the densitometry value of cytokine mRNA expressed by AMs isolated from a healthy subject. As shown in the figure, the expression of TNF-α and GM-CSF messages in LPS-stimulated AMs isolated from patients with both HIV-1 infection and sarcoidosis is 3–8 fold higher than in LPS-stimulated AMs from the control subject. GM-CSF: granulocyte macrophage colony stimulating factor; LPS: lipopolysaccharide; AM: alveolar macrophage; HIV-1: human immunodeficiency virus-1. For further abbreviations see legend to figure 4.

phases of the ILD, the phenotypic profile of the alveolitis can change during the course of the disease [100]. In other words, immunological analysis of BAL cell constituents may contribute to a presumptive diagnosis by enhancing or decreasing the probability of a determined ILD, but definitive conclusions cannot be drawn.

From a pathogenetic point of view, two mechanisms account for the increased number of pulmonary T-cells in ILD (fig. 4a and b). The concept that a T-cell redistribution is involved in the development of sarcoid alveolitis is based on the observation that whilst circulating CD4 cells are diminished, pulmonary CD4+ lymphocytes dramatically increase in this disease, both in percentage and in absolute number. Concerning HP, recent experimental data in mice challenged with *Faeni rectivirgula* demonstrated that: 1) following antigenic instillation, the BAL cell number of Lyt2+ (CD8) cells gradually increased; 2) the depletion of circulating CD8 cells led to a decrease in pulmonary T-cells [101]. Although these findings were obtained in animal models, they suggest the hypothesis that a massive influx of CD8 cells specifically sensitized to the antigen also takes place in the lung of HP patients. The precise molecules and mechanisms that govern this migratory process of circulating T-cells to the lung are poorly understood. At present, the most reasonable candidates involved in the mechanisms leading to T-cell alveolitis during ILD are macrophage-derived factors displaying chemotactic activity for lymphocytes, including IL-1 and IL-8.

Regarding the concept of *in situ* replication, various findings are consistent with the hypothesis that IL-2 might act as a growth factor for T-lymphocytes infiltrating lung tissues of patients with HP and sarcoidosis. In both diseases, a heightened number of pulmonary lymphocytes express the IL-2 receptor. In particular, a large number of BAL lymphocytes from sarcoid patients are CD25+ (the p55 chain of the IL-2 receptor) [102, 103], whilst CD8 BAL cells from HP patients express the p75 subunit of the IL-2 receptor [104]. Both sarcoid and HP BAL lymphocytes are able to proliferate *in vitro* in response to IL-2 [105], and it has been demonstrated that sarcoid T-cells constitutionally synthesize and secrete IL-2 [106]. Furthermore, the demonstration that an increased number of BAL T-cells of sarcoid patients expresses the cell-cycle related Ki67 antigen supports the hypothesis that sarcoid T-cells are able to proliferate *in situ* [73] (fig.

Table 4. – Phenotype of lymphocytes recovered in BAL fluid of patients with different interstitial lung disorders (ILD)

Prevalence of CD4+ cells*	Prevalence of CD8+ cells*
Sarcoidosis	Hypersensitivity pneumonitis
Tuberculosis (infrequent)	AIDS
Berylliosis	Silicosis
Asbestosis	Histiocytosis X
Rheumatoid arthritis	ILD associated with collagen vascular diseases
Crohn's disease	Drug-induced pneumonitis
	Blastomycosis
	ILD associated with GvH disease
	ILD associated with HTLV-I related myelopathy
	Bronchiolitis obliterans orgainizing pneumonia (BOOP)

BAL: bronchoalveolar lavage; AIDS: acquired immune deficiency syndrome; GvH: graft *versus* host; HTLV-I: human T-cell leukaemia virus type-I. *: See [42, 87–98].

3c). It remains to be established whether other cofactors for the IL-2-mediated T-lymphocyte proliferation cooperate in the mechanisms accounting for the development of the lymphocyte alveolitis in these diseases, including IL-4, IL-6 and GM-CSF.

Interestingly, some investigators have focused their attention on the possibility of enumerating IL-2 producing BAL cells for prognostic ends in sarcoidosis [107]. More specifically, inasmuch as recent studies have demonstrated that the production of IL-2 is genetically determined in the lung of sarcoid patients (since the gene for IL-2 is turned on in the CD4+/HLA-DR+ subpopulation), it has been proposed that the count of BAL CD4+/HLA-DR+ cells may represent a useful method for defining different phases of the disease [108]. In fact, it has been demonstrated that the increase of the CD4+/HLA-DR+ cells, with respect to the control population, is statistically highly significant in the active phase of the disease, with the significance being less evident, or even lacking, in the split and inactive stages of the disease [108].

The expression of CD3/TCR complex by pulmonary T-cells from patients with ILD has recently been the subject of intense investigation. As in healthy subjects, T-cells isolated from BAL fluid of patients with active sarcoidosis express the α/β TCR-related determinant WT31. Conversely, an expansion of $V\delta1^+$ γ/δ cells can be demonstrated only in a small subset of patients with active sarcoidosis [109, 110], and analysis of junctional region sequences has revealed the clonal nature of this $V\delta1$ expansion [110]. Interestingly, the surface expression of CD3/TCR is downmodulated as compared to peripheral blood [111]. Furthermore, sarcoid BAL T-cells show increased levels of mRNA transcripts for the β-chain of TCR, that parallels the enhanced surface expression of a series of accessory molecules, including CD29, HLA-DR, VLA-1 and CD2. These findings collectively support the hypothesis that sarcoid cells behave as normal cells that are repeatedly stimulated by an unknown antigen through the α/β TCR.

We recently evaluated the expression of α/β and γ/δ TCR-related determinants in patients with HP [112]. Our study demonstrated an increase in the pulmonary γ/δ pool. In fact, the absolute number of these cells is markedly increased in HP lung, with respect to the values observed in controls. This finding suggests that γ/δ lymphocytes might have relevance in the local immune response against the offending agent causing HP. Since several recently published studies have reported that γ/δ cells are able to kill targets in a non-MHC-restricted manner, it is tempting to hypothesize that these cells participate in the cytotoxic response that takes place in the lung during hypersensitivity reaction.

Different groups have recently investigated the genomic configuration of the TCR in sarcoidosis and HP [112–116]. A marked quantitative bias in the usage of β-chain constant region segments (Cβ1 *versus* Cβ2) and variable elements ($V\beta8$, $V\beta14$, $V\alpha2,3$) by T-cells was demonstrated in sarcoid lung. Furthermore, a lung compartmentalization of $V\alpha2,3$ CD4 cells has recently been demonstrated, which was significantly linked to a discrete haplotype (HLA-DRw17 and HLA-DQw2) and to the course of the disease [115]. Collectively, these observations suggest that sarcoid CD4 cells are not randomly activated, but rather that they proliferate in response to a specific unknown antigenic pressure, that favours the cell proliferation of a limited number of clones. Because there are no indications of HLA sharing between sarcoid patients, these findings suggest the possibility that an entity behaving as a "superantigen" may be linked to the development of the sarcoid granulomatous damage [116]. Alternatively, it is possible that the preferential expression of particular *V* region segments may be due to the accumulation of T-effector cells that acquire a peculiar tropism for the pulmonary tract, in that they express certain *V* genes of the TCR.

By using a series of MoAbs recognizing different *V* regions of the TCR ($V\beta5$, $V\beta6$, $V\beta8$, $V\beta12$), we were unable to demonstrate any selective expansion of β-chain variable elements by BAL T-cells from HP patients [104]. Nevertheless, the Southern blot analysis of BAL T-cell DNA of HP patients revealed a polyclonal pattern that seems biased toward cells that have rearranged and possibly expressed particular $V\beta$ or $V\gamma$ genes. In fact, whilst in some patients an unbiased pattern of $V\gamma$ gene rearrangement was observed, others showed a preferential usage for $V\gamma8$ and/or $V\gamma2/V\gamma4$ rearrangement. Taken together, our data suggest that the relevant antigen(s) accounting for the hypersensitivity reaction elicits a response at lung level that induces an oligoclonal selection of T-cells.

Pulmonary T and NK cells during HIV-1 infection

Pulmonary involvement by HIV-1 infection is characterized by an adaptive increase in the number of antigen-specific and nonspecific CD8 cytotoxic cells [20–22, 117]. In fact, the phenotypic evaluation of BAL T-cell subpopulations has demonstrated that about 25% of subjects with early infection, and 50% of patients with advanced disease, show an increased number of pulmonary CD8+ cells [73]. In contrast, the pulmonary helper-related CD4+ lymphocytes are virtually absent in the lung of HIV-1 infected patients, resulting in a marked decrease in the pulmonary CD4/ CD8 ratio. There are some differences in the quantitative deficiency of pulmonary CD4 cells among patients with HIV-1 infection, since patients with earlier infection generally tend to have more CD4 cells, as compared to those who present with opportunistic infections.

CD8 cells accumulating in the lung of HIV-1 infected patients are represented by two populations of CTL: CD3+/CD8+/S6F1^{bright} MHC-restricted CTL expressing the D44 determinant and CD3+/CD8+/S6F1^{bright} T-cells bearing the NK-related CD56 and CD57 markers [97, 118]. From a functional point of view, it has been shown that CD3+/CD8+/D44+ CTL may lyse ^{51}Cr-labelled HIV-1 infected autologous AMs. The T-cell mediated lysis of AMs is MHC-restricted, because anti-HLA-A, B, C MoAbs are able to completely inhibit the cytolytic activity. Furthermore, the P815 target cell line experimentally transfected with HLA-A2 and the HIV-1-related gene

env, can be selectively lysed by BAL CD8 CTL isolated from HLA-A2/cross-matched HIV-1 seropositive patients [119]. Taken together, these findings lead to the assumption that CD3+/CD8+/D44+ cells represent a population of MHC-restricted CTL that recognize and attack HIV-1 antigens on the surface membrane of infected cells. In contrast to the HLA-restricted cytotoxic activity, mainly directed against HIV-1 proteins, the non-MHC-restricted machinery does not seem to take part in host defence mechanisms against HIV-1, but it rather contributes to the resistance against the development of AIDS-related pulmonary opportunistic infections.

The majority of pulmonary HIV-1 specific CTL coexpress the CD3 molecule and the α/β TCR, but we recently showed that in a subset of HIV-1 infected patients, a small but definite proportion of lung lymphocytes (ranging from 5 up to 40% of BAL T-cells) stained with the MoAb TCRδ1 [120]. TCR γ/δ cells homing in on the lung of these subjects show peculiar phenotypic characteristics. In fact, unlike peripheral blood TCR γ/δ lymphocytes, BAL TCR γ/δ cells coexpress the CD8 determinant and adhesion molecules in high proportion. Although the functional role of human TCR γ/δ cells still remains unknown, we believe that TCR γ/δ cells might play an important role in the pulmonary immune system of some AIDS patients, perhaps by the recognition of different sets of HIV-1 antigens than those recognized by TCR α/β cells.

The events leading to the activation and the expansion of CTL within the respiratory tract of HIV-1 infected patients are under investigation. BAL T-lymphocytes of HIV-1 seropositive patients with lymphocytic alveolitis bear the p75 chain of IL-2R, and proliferate in the presence of IL-2 [121]. This fact suggests that, as in other ILD, effector lymphocytes may accumulate within the lung through an IL-2 driven proliferation of pre-existing T-cells. Consistent with this interpretation, is the recent demonstration that an enhanced number of BAL T-cells of HIV-1 infected patients expresses the cell-cycle related Ki67 antigen [20]. Alternatively, the recruitment of specific CTL against virus-infected cells from the peripheral blood and BALT to the lung can be claimed as a second mechanism, that could lead to an adaptive increase of the lymphoid component of the alveolitis.

Pulmonary T-cells in patients with asthma

An area of current interest has been the role of pulmonary T-lymphocytes in the regulation of the inflammatory events associated with allergy and asthma. Activated CD4 T-helper cells, which show increased expression of the p55 chain of the IL-2R, have been found in bronchial biopsies obtained from mild atopic asthmatics [122–124]. Furthermore, a direct relationship has been demonstrated between the numbers of activated T-lymphocytes and eosinophils in BAL fluid [124]. Also, the degree of the disease seems to correlate with the activation state of the CD4 infiltrate [124]. In fact, the number of CD25+/CD4+ cells is higher in the BAL fluid of asthmatic patients with bronchial hyperresponsiveness than in those without [124]. Keeping in mind that following allergen inhalation the peripheral count of circulating CD4 lymphocytes usually decreases in asthmatic subjects [125], it has been proposed that a selective recruitment of activated CD4 T-lymphocytes to the lung takes place during the late phase reaction to allergen bronchial challenge.

As reported earlier, activated CD4 T-cells can be subdivided into two general classes of lymphokine producing cells, TH1 and TH2. The possibility that human TH2 cells promoting IgE synthesis may mediate asthmatic reaction has been apparent since the initial description of the functional dichotomy of activated CD4 cells. Nonetheless, a convincing demonstration that CD4 cells, which infiltrate the bronchial mucosa during asthma show a TH2 pattern of cytokine release was provided only recently [123, 126]. As compared to control subjects, patients with asthma have more BAL lymphocytes positive for mRNA for IL-2, IL-3, IL-4, IL-5 and GM-CSF. In contrast, activated CD4 lymphocytes isolated from asthmatic subjects do not synthesize IFN-γ. Taken together, these data indicate that atopic asthma is associated with activation in the lung of a peculiar CD4 cell subset that shows a TH2-like pattern of cytokine-gene expression.

Pulmonary T-cells in lung transplant recipients

Fibreoptic bronchoscopy, associated with BAL analysis and transbronchial lung biopsy is considered the first invasive procedure in establishing a rapid diagnosis of opportunistic and non-opportunistic infections, during the early postoperative period following lung transplantation. Apart from this, the analysis of cells recovered from BAL has contributed to understanding the role of T-lymphocytes in the pathogenesis of lung allograft rejection. An increased number of T-cells can be recovered from the lower respiratory tract of patients with acute rejection and/or viral infections [97, 127]. BAL T-lymphocytes obtained from these patients are predominantly CD4+/CD29+/CD45RA- cells, whilst a predominant CD8 phenotype can be observed in the cell population obtained from the BAL of patients with obliterative bronchiolitis (*i.e.* the obstructive airway disease which complicates the later phases of the posttransplant period) [128].

From a functional point of view, T-cells retrieved from the BAL or lymphocytes grown from transbronchial biopsies (TBB) of patients with an oncoming rejection episode exhibit donor-specific alloreactivity in primed lymphocyte test (PLT), and increased mitogen response [127, 129]. More specifically, it has been demonstrated that donor-specific reactivity is directed toward donor class II MHC antigens during periods of acute rejection, whilst the reactivity of BAL CD8 cells recovered from patients with obliterative bronchiolitis correlated specifically with donor class I MHC antigens [128]. Taken together, these data suggest that different T-cell subsets are involved in cellular mechanisms mediating acute and chronic rejection of lung allograft.

Immunological studies of the lymphocyte component of alveolitis have recently been used in clinical monitoring of recipients. In fact, recent reports indicate that

a high percentage of recipients, who during clinically quiescent periods exhibit a positive PLT and negative TBB histology, subsequently develop rejection episodes. This finding suggests that the study of cells derived from BAL could be of importance, not only in the investigation of the pathogenesis of the rejection mechanisms, but also to predict putative rejection episodes and monitor the efficacy of immunosuppressive treatment [130].

Primary malignant lymphomas of the lung

The complex nosography of primary lymphoid lung lesions has been a matter of debate for many years [131, 132], but it is currently on the way to better definition. On the basis of recent immunohistochemical and gene rearrangement studies, which define the clonality of B-cell infiltrations, it is now becoming clear that a large proportion of the localized lesions previously defined as "pseudolymphoma" and some of the diffuse infiltrations of lung (lymphoid interstitial pneumonia (LIP)) are true lymphomas [133–136]. Cytogenetic studies further support this view [137]. In the above lesions, the lung parenchyma is variably infiltrated by heterogeneous lymphocyte populations, with a relative predominance of small or medium-sized B-cells (fig. 3e and 3f), often characterized by clefts (centrocyte-like), or plasmocytoid features. Several lines of evidence suggest that primary malignant lymphomas of the lung arise from BALT, and can be included in the wider group of mucosa-associated lymphomas, together with gastric, thyroid and salivary-gland lymphomas [134]. This category of B-cell lymphomas is characterized by an indolent clinical course, and distinct pathological and phenotypic features, including a fairly broad cytological spectrum (defined as "intermediate", "monocytoid" or "centrocyte-like"), the presence of lymphoepithelial lesions, and frequent admixture of neoplastic B-lymphocytes with reactive germinal centres and polytypic plasma cells. This pattern suggests that these lymphomas can develop as complications of pre-existing reactive lymphoid infiltrates caused by infections, some cases of immunodeficiency or autoimmune diseases. The morphology and phenotype of centrocyte-like cells of low-grade BALT-lymphomas are similar to parafollicular or marginal zone B-cells with either "memory" or preplasma cell differentiation.

Recently, molecular analysis has been used to demonstrate that most low grade mucosa-associated lymphoid tissue lymphomas lack the bcl-2 gene rearrangement, typical of follicular cell lymphomas [138, 139]. On the other hand, rearrangement of the chromosome 11 bcl-1 locus is frequent in centrocytic lymphoma [140], but significant data are not available on bcl-1 involvement in primary low-grade lymphomas.

Primary high grade lymphomas in the lung are rare [135], often arising as transformation of low-grade B-cell lymphomas. These lymphomas are extremely aggressive, infiltrate the pulmonary parenchyma, and destroy airways and vascular components of the respiratory tract. Large areas of necrosis are common, and the prognosis is poor.

T-cell lymphomas are rarely found as primary lymphomas in the lung, and have been mainly described as angiocentric and angiodestructive processes, under the name of lymphomatoid granulomatosis [141]. These lesions, which are characterized by varying degrees of clinical aggressiveness, have recently been recognized as T-cell malignant lymphoma on the basis of phenotypic and rearrangement studies [142, 143].

Therapeutic perspective

Drugs able to suppress a specific immune response have been widely and effectively used for the treatment of several pulmonary diseases. At present, steroids still represent the therapy of choice in several ILD. They downmodulate the expression of IL-2R on target cells and production of IL-2 by relevant cells, mainly CD4 lymphocytes, which are equipped with the high affinity receptors for glucocorticoids. Furthermore, they inhibit most macrophage activities, including TNF-α and IL-1 release, accessory functions and tumoricidal activity. Cyclosporin A, a fungal-derived agent, which inhibits the production of interleukin-2 on CD4 cells and, thereby, impairs T-lymphocyte activation, and cytotoxic drugs (including azathioprine, methotrexate and cyclophosphamide) are commonly used for suppressive therapy.

It should be noted that these immunosuppressive drugs induce a nonspecific, generalized inhibition of host immune response. Recent advances in hybridoma and recombinant DNA technology can identify a number of new compounds showing the capability of modifying a "specific" immune function with net positive or negative effects on the activity of the immune system. This group of immunomodulatory drugs includes MoAbs reacting with determinants closely linked to the functional activity of T-cells. Monoclonal antibodies specific to pan-T structures (CD3, CD2), adhesion molecules (LFA-1) and cytokine receptors (anti IL-2R, CD25) are able to induce an effective and selective immunosuppression, and have been used in clinical trials for the treatment of allogeneic transplant rejection. Nevertheless, these MoAbs, which are produced from mice by hybridoma technology, cause significant side-effects, and this precludes their repeated use in widespread clinical trials. It is likely that the development of human MoAbs may produce large amounts of reagents with the specificity for a single epitope, but without the problems related to the nonhuman origin of the currently used antibodies. In this context, it is believed that human MoAbs could represent an ideal approach in the control of hypersensitivity mechanisms, which take place in the pulmonary microenvironment of patients with ILD and following lung transplantation.

Cytokines represent another group of immunomodulators which are expected to control the immune functions of pulmonary lymphocytes *in vivo*. Recombinant IL-2, GM-CSF, TNF-α, IFN-α and γ have already been utilized in different clinical trials for treatment of tumours, viral infections and immune deficiencies, and there has been clear evidence that these drugs show an *in vivo* immunomodulatory activity on circulating

mononuclear cells. However, studies extending these observations to the pulmonary compartment have yet to be reported. A preliminary study has demonstrated that a local activation of alveolar macrophages can be achieved following inhalation of aerosolized IFN-γ [144]. This study gives hope for the development of an organ-specific therapy for lung, using cytokines. Further advances in the understanding of the role of cytokines in lung immunity could provide the basis for developing rational therapies, involving the local use of a number of biological response modifiers.

Concluding remarks

Several pieces of evidence are emerging concerning the intriguing network of the pulmonary host defence mechanisms. It is now known that the pulmonary immune system functions in a complex manner, characterized by the constant activity of different cell populations, with effector and immunoregulatory functions. Furthermore, it has become clear that activation of intraepithelial and alveolar T-cells induces the *in situ* production of several interacting cytokines, that regulate a wide spectrum of biological events relevant to inflammation and cell growth. Furthermore, lymphocyte activation is intertwined with the transient increase in adhesiveness and migration of primed T-lymphocytes to inflamed areas. Dysregulation at any level of these networks of cell interactions, or alterations of the cascade of cytokines that are locally produced, can contribute to disease pathogenesis.

As more knowledge becomes available on the complex relationship between local immunity and some pulmonary diseases, it is hoped that we will gain novel models of treatment for the situations in which the functional impairment of the lung host defences contributes to disease pathogenesis. The recent availability of immunosuppressive drugs that alter the local production of biological response modifiers and/or effector functions of pulmonary lymphocyte subsets might help in attaining this goal in the diseases that result from local hypersensitivity reactions. A more complete understanding of the synergies between cytokines locally produced in health and disease will also help to plan therapeutic interventions with combinations of cytokines. Hopes of obtaining these goals are associated with the current revolution in the area of molecular biology, and the development of new immune techniques, which could improve our knowledge of the mechanisms that regulate immunological events in the lung micro-environment.

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