

**SERIES 'PULMONARY IMMUNE CELLS'**  
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## Pulmonary macrophages

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*Pulmonary macrophages. M-L. Lohmann-Matthes, C. Steinmüller, G. Franke-Ullmann. ©ERS Journals Ltd 1994.*

**ABSTRACT:** Interest in pulmonary macrophage research has greatly increased as is now possible not only to work with the easily accessible alveolar macrophages but also with macrophages prepared from lung tissue, such as the interstitial macrophages, dendritic cells and intravascular macrophages. A fascinating aspect is that, in one organ, the modulation of macrophage functions according to their anatomical localization can be studied. This article tries to review some of the modern aspects of research on pulmonary macrophages. These include localization and origin of the various subpopulations, membrane receptors and surface markers, arachidonic acid metabolism, antimicrobial activity, cytokine production and some aspects of macrophage involvement in sarcoidosis and idiopathic lung fibrosis.

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The lung provides a fitting environment for research on macrophages, for four reasons. Firstly, the lung has direct contact with the environment with respect to both injury and treatment. The alveoli are in a unique position in the body, where exogenous air encounters a thin cellular layer consisting of only about two cells beyond which immediate contact occurs with a refined organ with particular tasks, definitely requiring the structural integrity of the organ.

Secondly, the alveoli are the only part of the body where the phylogenetically older nonspecific defence system, represented by the alveolar macrophages, have the tasks of keeping the surfaces of the alveoli sterile and providing defence against any invasive agents. Furthermore, these alveolar macrophages can attract other cells of the nonspecific defence system when they are unable, successfully, to complete their task alone.

Thirdly, nowhere else in the organism do we meet four subpopulations of macrophages with different tasks and functions: the alveolar, the interstitial, and the intravascular macrophages, and the various accessory cells, such as dendritic cells or Langerhans' cells.

Fourthly, the lung is the only organ to which curative or modulating substances can be applied directly, so that they can exert their influence only in the target organ without affecting the whole organism.

### Localization and origin of lung macrophages

Based on their localization, the macrophages in the lung include four different types: 1) the alveolar macrophage; 2) the interstitial macrophage; 3) the intravascular macrophage; and 4) the dendritic cell. The alveolar macrophages have a unique localization in the body, since

they are placed within the alveolar surfactant film, which is produced by type II alveolar lining cells and is composed of phospholipids and proteins [1]. In addition, they are the only macrophages in the body which are exposed to air. They are located at the interphase between air and lung tissue, and represent the first line of defence against inhaled constituents of the air. They possess a high phagocytic and microbicidal potential. In the normal resting animal, they represent more than 90% of the cells of bronchoalveolar lavage (BAL) [2, 3]. Morphologically, most of them look like mature tissue macrophages [4]. These mature alveolar macrophages are the first to appear in the course of several washing procedures. Therefore, they may be less adherent than smaller cells, which appear at later stages of the washing procedure. The smaller cells look more like interstitial macrophages and may have only recently arrived in the alveoli [5].

Several methods have been described to separate alveolar macrophages from interstitial macrophages. LAVNIKOVA *et al.* [6] performed extensive washings, followed by cutting and desaggregation of the lung tissue and collagenase digestion, which resulted in a contamination of the interstitial population with only 0.3% of alveolar macrophages. LEHNERT *et al.* [7] and CROWELL *et al.* [8] first perfused the lungs of mice through the pulmonary artery, in order to remove blood monocytes. This procedure was followed by 12 BAL washing cycles, a mechanical disruption and digestion with collagenase. The contamination with alveolar macrophages was quantitated using an *in situ* phagocytosis assay with opsonized sheep erythrocytes, resulting in 4% contamination of interstitial cells with alveolar macrophages. In our laboratory, interstitial lung macrophages were isolated by 10 BAL washings, followed by perfusion through

the pulmonary artery in order to remove peripheral blood monocytes and instillation of collagenase into the pulmonary artery and the trachea. Finally the lung was cut and further digested in collagenase. Alveolar macrophages had been labelled previously *in vivo* using the dye PKH 26, which labelled 100% of the alveolar macrophages. The percentage of alveolar macrophages contaminating the interstitial macrophages could be defined by fluorescence-activated cell sorter (FACS), being about 3%.

The interstitial macrophage population is described by LAVNIKOVA *et al.* [6] as negative, or only weakly positive, with respect to esterase staining, as compared to alveolar macrophages which stain brightly positive for nonspecific esterase. In contrast, LEHNERT and co-workers [7, 8] described the interstitial cell as positive for nonspecific esterase. The interstitial macrophages are located in the lung connective tissue, and seem to have intermediate functional properties, both as cells of second line defence and antigen-presenting cells. With respect to Fc-receptor-dependent phagocytosis, interstitial and alveolar macrophages have been shown by LAVNIKOVA *et al.* [6] and by CROWELL *et al.* [8] to be equally effective. With respect to other functions, alveolar and interstitial macrophages are clearly different from each other. Interstitial cell functions, such as Fc-receptor-independent phagocytosis, production of cytokines, *e.g.* tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ) or interferon- $\alpha/\beta$  (IFN- $\alpha/\beta$ ), and production of oxygen radicals are reduced whereas accessory function and Class II expression are increased, compared to alveolar macrophages (Franke-Ullmann and Lohmann-Matthes, unpublished observations).

The dendritic cell, which may or may not belong to the macrophage lineage, is located in small numbers in the lung interstitial tissue [9, 10], and is similar to the dendritic cell which has been described in the spleen [11]. It is characterized by little, if any, phagocytic function, by high amounts of Class II antigen expression and by the absence of Fc- $\gamma$  receptors on the surface, and is specialized for antigen presentation and accessory function. In addition to their presence in the interstitial part of the lung, dendritic cells have been reported to form a network between the epithelial alveolar lining cells [12, 13]. Recently, Langerhans' cells have also been reported to be present in the alveoli of smokers [14, 15]. Dendritic cells are present from the 20th day of gestation, and are fully active as accessory cells at birth [16].

The fourth type of macrophage in the lung, intravascular macrophages, are located on the endothelial cells of the capillaries facing the bloodstream [17, 18]. In contrast to monocytes, they are highly phagocytic and are believed to remove foreign and damaging material which enters the lung *via* the bloodstream [19]. This cell type has been described in humans, cats, dogs and sheep but not in rodents.

### Origin of lung macrophages

The origin of lung macrophages has been discussed extensively. Two mechanisms apparently contribute to

the recruitment both of alveolar and interstitial macrophages, *i.e.* chemotactic attraction of monocytes from the lung blood pool and local replication in the lung. There is convincing evidence that bone marrow derived blood monocytes migrate into the alveoli both under steady-state conditions and during acute inflammation [20–23]. In the mouse and in humans it has been shown that, following bone marrow transplantation, donor macrophages repopulate the alveoli of the host within three months. These findings do not, however, rule out local replication of macrophages in the lung, since whole body irradiation, which precedes transplantation, inactivates all host cells in the lung which could have replicated. Therefore, donor monocytes are the only cells available to migrate to the lung interstitium and alveoli. Intratracheal instillation of bacille Calmetie-Guérin (BCG) and carbon as inflammatory stimuli were reported to induce a strong influx of monocyte-derived cells into the alveoli, one day later. Additional evidence for the origin of alveolar macrophages from monocytes has come from the observations of UNGAR and WILSON [24], and VAN FURTH and SLUITER [25], that monocytes indeed pass from the interstitium to the alveoli.

In addition to the recruitment of monocytes to the alveoli, several groups have reported local proliferation of cells of the macrophage lineage in the lung. *In vitro* data demonstrated that alveolar macrophages readily responded to colony-stimulating factors (CSFs) with proliferation [26, 27]. The same is likely to be the case with interstitial macrophages; however, this is more difficult to demonstrate clearly because interstitial macrophages already proliferate spontaneously *in vitro*. This spontaneous proliferation may be due to other contaminating cell types which produce CSF *in vitro*. Lung fibroblasts and endothelial cells are known to produce CSF, and lung-conditioned media have been used as a source for CSF. When granulocyte-macrophage colony-stimulating factor (GM-CSF) is applied intranasally, a fourfold increase in proliferating alveolar macrophages and a tenfold increase in proliferating interstitial macrophages can be observed using bromodeoxyuridine-labelling. (Franke-Ullmann and Lohmann-Matthes, unpublished observations).

Under normal *in vivo* conditions, BITTERMAN *et al.* [28] have shown that 0.5% of the alveolar macrophages incorporate  $^3\text{H}$ -thymidine. Under subacute or chronic inflammatory conditions, a 10–15 fold increase of  $^3\text{H}$ -thymidine-incorporating macrophages occurs. Using the carbon inflammatory model, mentioned above, ADAMSON and BOWDEN [29] have shown that there is a biphasic response, with monocyte influx into the alveoli on day 1, and proliferation of macrophages at later times in the interstitium. Additional evidence for *in situ* replication of lung macrophages comes from experiments using the carbon inflammatory model in leucopenic mice. After instillation of carbon, these mice did not respond with an early influx of monocytes, but the number of alveolar macrophages increased due to local proliferation [30]. These data were confirmed by SHAMI *et al.* [31]. Recently, PFORTE *et al.* [32] have reported proliferation of alveolar macrophages in patients suffering from sarcoidosis or

interstitial lung fibrosis. In summary, local proliferation of cells of the macrophage system can occur both in the alveoli and in the lung interstitium. Under normal conditions, the contribution of these cells to the inflammatory reaction may be moderate. However, under conditions of bone marrow impairment, local proliferation may be intensified replacing systemic reactions to a certain degree, a mechanism which has been shown to occur similarly in the liver of adult mice [33].

Alveolar macrophages are easily accessible by BAL, whereas preparation of other lung macrophage populations is difficult and time-consuming. For that reason, most functional data so far published have been performed with alveolar macrophages. Therefore, in this review, most functional aspects of lung macrophages will concern the alveolar macrophage.

### Membrane receptors and surface markers

Macrophages possess a broad variety of membrane receptors, through which they interact with a large number of different molecules [34–36]. Opsonization is an essential mechanism in enhancing phagocytosis of micro-organisms. Three groups of receptors play an important role in opsonization: the Fc receptors, complement receptors and lectin receptors [37–40].

Three receptors for the Fc portion of immunoglobulin G (IgG) molecules have been described on alveolar macrophages [41]: Fc $\gamma$ RI a high affinity receptor for monomeric IgG; Fc $\gamma$ RII, a low affinity receptor [42] for IgG and aggregated IgG; and Fc $\gamma$ RIII, a low affinity receptor for aggregated IgG. On human alveolar macrophages, binding sites for the Fc portion of IgG1 and IgG3 are more frequent than for IgG2 and IgG4 [42]. In addition to Fc- $\gamma$  receptors for IgG, Fc receptors for immunoglobulin E (IgE) have been shown to be present on rat and human alveolar macrophages [43], and Fc receptors for immunoglobulin A (IgA) [44] have recently been described on mouse, rat and human alveolar macrophages [44]. *Via* these Fc receptors, macrophages have access to all antigens which are recognized by antibodies. In addition to the presence of Fc receptors on alveolar macrophages, they also bear membrane-bound, cytophilic IgG [45] and IgA [46], which play a role in nonopsonized phagocytosis.

The second group of receptors involved in opsonization are the complement receptors. Three complement receptors have been described on alveolar macrophages. The most important is the CR1 (CD35) receptor, which binds C3b with high affinity, and iC3b and C4b with low affinity [37, 47]. CR3 (MAC-1,  $\alpha$ -chain CD11b,  $\beta$ -chain CD18) binds iC3b with high affinity, and C3dg and C3d with low affinity. In addition, it binds  $\beta$ -glucan in the cell wall of fungi and bacteria. CR4 ( $\alpha$ -chain CD11c,  $\beta$ -chain CD18) binds iC3b but not  $\beta$ -glucan. For opsonized phagocytosis to occur *via* the CR4, additional structures must be recognized on micro-organisms [48]. Also, the alveolar lining surfactant, a product of alveolar type II cells, has opsonizing activity [49, 50].

The third group of phagocytosis-associated receptors, the lectin-binding receptors, are numerous on alveolar

macrophages. They react with lectins on micro-organisms and plant cells. Resting alveolar macrophages have a characteristic binding pattern for lectins, which is different from peripheral monocytes. Four lectin-receptors are expressed significantly less on alveolar macrophages than on monocytes (PNA, UEA-1, BSL 1, SIA) [51].

Many other receptors are also present on alveolar macrophages (table 1). There are receptors for macrophage-activating cytokines, such as interleukin-1 (IL-1), and TNF, which in addition to being secreted by macrophages (see below) also bind to the receptors of the same cell in an autocrine fashion, resulting in activation. The most prominent macrophage-activating cytokine is interferon- $\gamma$  (IFN- $\gamma$ ), which also binds to macrophages *via* a receptor. Interleukin-2 (IL-2) has also been shown to bind to receptors on alveolar macrophages and on peripheral blood monocytes [52]. Many other cytokine-receptors have been described on alveolar macrophages, including receptors for macrophage-deactivating cytokines such as interleukin-4 (IL-4) and interleukin-10 (IL-10) [53]. Furthermore, the growth factors CSF-1 and GM-CSF interact with macrophages through specific receptors [54]. Apart from inducing proliferation, both growth factors cause limited activation [55]. Much information has accumulated concerning signal transduction as a result of receptor/ligand interaction using growth factors [56] and macrophage-activating factors, such as IFN- $\gamma$  [57], as ligands.

Compared to activating cytokines, deactivating cytokines are less well-characterized with respect to receptor/ligand interactions and the events following thereafter [58]. At present, the most important deactivating cytokines are IL-10 and transforming growth factor- $\beta$  (TGF- $\beta$ ). IL-4 has an intermediate position, since some macrophage functions are induced by IL-4 (major histocompatibility complex (MHC) class II expression) and others are inhibited, *e.g.* production of TNF. In addition to other producer cell types, the deactivating molecules IL-10 and TGF- $\beta$  are also produced by the macrophages themselves, under appropriate stimulation. It is, as yet, unclear whether and to what degree macrophages may induce their own deactivation *via* an autocrine feedback mechanism. Data on macrophage deactivation have so far only been obtained with peritoneal macrophages and cell lines, but not with lung macrophages. Future research on mechanisms of deactivation of lung macrophages may open additional possibilities of local lung therapy.

In addition to receptors, there are surface markers on the outer membrane of pulmonary macrophages. These markers are, in part, associated with a certain function or differentiation status, such as the transferrin receptor (CD71); others label particular cell types, such as the markers for lung interstitial dendritic cells or suppressor macrophages [59, 60]. Membrane glycoproteins of the integrin- $\beta$  family are critical for the ability of macrophages to migrate and to establish cell/cell contact [61–64]. The adhesion associated molecules CD11a, CD11b and CD11c have been demonstrated to be present on alveolar macrophages to varying degrees. CD11a was found on about 92% of alveolar macrophages, CD11b was only present on 52% under resting conditions, but was raised

Table 1. – Ligands recognized by alveolar macrophages *via* receptors

<b>Immunoglobulins</b> IGg1, IgG2a (murine) IgG2b, IgG3 (murine) IgG1, IgG3 monomers (Human) IgG complexes (human) IgE, IgA (murine and human)	<b>Complement</b> C3b, iC3b, C4b, C3d, C5a
<b>Proteins</b> Fibronectin, fibrin Lactoferrin, transferrin GM-CSF, CSF-1 Interferon- $\gamma$ , IL-4, IL-1Ra IL-2, insulin	<b>Lipoproteins</b> Low density lipoprotein $\beta$ -very low lipoprotein
<b>Surface markers</b> Class II molecules, CD11a, CD11b, CD11c, CD14, CD18, CD54 Molecules recognized by monoclonal antibodies: 25F9 (mature macrophages), 27E 10 (inflammatory macrophages), Ki M2, Ki M8 (mature macrophages), RM31 (inflammatory macrophages), RFD1, RFD7, RFD9	<b>Lectins with specificity for</b> $\alpha$ -linked galactose residues N-acetylgalactosamine residues N-acetylglucosamine residues $\alpha$ -linked fucose residues Mannose residues N-acetylneuramine residues

Ig: immunoglobulin; GM-CSF: granulocyte-macrophage colony-stimulating factor; IL: interleukin.

to 83% in patients with active sarcoidosis, and correlated with the numbers of lymphocytes present in the BAL of sarcoidosis patients [65]. CD11c was present on about 90% of alveolar macrophages. The CD18 molecule representing the  $\beta$ -chain of the CD11 group was found on 88% of alveolar macrophages. Intercellular adhesion molecule-1 (ICAM-1) (CD54) was upregulated to 84% in the presence of BAL lymphocytosis associated with sarcoidosis, compared to an expression on 39% of resting BAL alveolar macrophages [65].

From the markers supposed to characterize mature macrophages, only the marker recognized by the 25F9 monoclonal antibody [66] had a stable expression on alveolar macrophages of over 90%, whereas the transferrin receptor, the markers recognized by Ki M8, RFD7, RFD9, 27E 10 and RM 3/1 were upmodulated in the presence of lymphocytes in the BAL, probably indicating an activated status [65]. The same was reported for CD14, the receptor for the lipopolysaccharide (LPS) binding protein [67], and Ki M2. Also the MHC class II expression is under the influence of BAL lymphocytes or cytokines [65]. Thus, the most stable surface markers of alveolar macrophages are CD11a, CD11c, CD18 and 25F9; whereas many, if not most, of the other surface markers of alveolar macrophages are upregulated in the presence of BAL lymphocytes, either in terms of numbers of positive alveolar macrophages or in terms of intensity of membrane expression [65].

#### *Arachidonic acid metabolism in alveolar macrophages*

Human as well as animal alveolar macrophages have been shown to produce metabolites of arachidonic acid, both along the cyclo-oxygenase pathway (the thromboxanes and prostaglandins) and the lipoxygenase pathway (the leukotrienes and hydroxyecosatetraenoic acids HETEs) [68, 69]. Both kinds of metabolites play a role in the modulation of inflammatory reactions. Cyclo-oxygenase products secreted by alveolar macrophages

include thromboxane  $A_2$ , measured as its stable metabolites thromboxane  $B_2$ ,  $PGE_2$ ,  $PGD_2$  and  $PGF_{2\alpha}$ , lipoxygenase products include leukotriene  $B_4$  and 5-HETE. The largest amount of such released metabolites comprises thromboxane  $A_2$  ( $TxA_2$ ), which is released in  $ng \cdot mg^{-1}$  cell protein, five times more than leukotriene  $B_4$  ( $LTB_4$ ), which has the second largest concentration [68].

The precise role of macrophage activation with respect to the production of these metabolites is not yet fully clear. Alveolar macrophages, collected from rats following one day of *in vivo* treatment with LPS, produced significantly more  $LTB_4$  and 5-HETE than alveolar macrophages from control animals [70]. Similarly, human alveolar macrophages primed *in vitro* with LPS showed an enhanced production of  $LTB_4$  following stimulation with calcium-ionophore A23187 [71]. The macrophage activator,  $IFN-\gamma$ , was also shown to prime rat alveolar macrophages for enhanced release of lipoxygenase pathway products, without causing a direct secretion of leukotrienes [72]. The pattern of activation with respect to arachidonic acid metabolism appears to depend on the kind of stimulus used. Whereas  $IFN-\gamma$  has only preactivating properties (at least at the low dosage tested) LPS has been shown to either preactivate macrophages to enhance release of the lipoxygenase product  $LTB_4$ , or, at higher dosages, to directly activate alveolar macrophages to enhance release mainly of cyclo-oxygenase pathway products, such as thromboxane  $B_2$  ( $TxB_2$ ) and prostaglandin  $E_2$  ( $PGE_2$ ) [73].

It was demonstrated that alveolar macrophages from rats which had been exposed to silica inhalation *in vivo* spontaneously produced  $TxB_2$  and  $PGE_2$  *in vitro*. Upon *in vitro* stimulation with LPS, the secretion of these cyclo-oxygenase products was strongly enhanced [74]. In contrast, *in vitro* stimulation with silica enhanced production of metabolites of the lipoxygenase pathway and decreased the production of the products of the cyclo-oxygenase pathway [73]. From these *in vitro* data, one may assume that silica can increase arachidonic acid

metabolites like  $\text{LTB}_4$  which promote fibroblast proliferation *in vitro* [75], either directly or by stimulating the production of IL-1 [76] and TNF [77]. These have been shown to promote fibroblast proliferation, and suppress arachidonic acid metabolites which decrease or inhibit fibroblast proliferation *in vitro*, like  $\text{PGE}_2$  [77, 78]. Thus, substances which modulate the arachidonic acid metabolism, such as silica, may play a direct role in the promotion of lung fibrosis [79].

#### *Production of reactive oxygen intermediates (ROIs) by alveolar macrophages*

Alveolar macrophages produce  $\text{O}_2$  metabolites, such as superoxide anion ( $\text{O}_2^-$ ), hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) and hydroxyl radical ( $\text{OH}^\cdot$ ), in association with phagocytosis [80]. Alveolar macrophages can be activated to produce reactive oxygen intermediates (ROIs) by many substances, including phorbol myristate acetate (PMA), zymosan and immunoglobulin G (IgG) immune complexes. In addition, they can be activated or primed by cytokines (IFN- $\gamma$ , platelet-derived growth factor (PDGF), GM-CSF, TNF,  $\text{LTB}_4$ ) [80–83] to enhance secretion of ROIs. ROIs play an important role in both the intracellular and extracellular defence mechanisms directed against micro-organisms. This capacity to produce ROIs is a prerequisite of the nonspecific anti-infectious defence system, as can be deduced from the high susceptibility for infectious diseases in individuals suffering from defective ROI producing enzyme systems, *e.g.* chronic granulomatous disease (CGD) [84]. In addition to their role in antimicrobial defence, ROIs may play a role in lung injury, since they have been shown to be cytotoxic for fibroblasts [85].

In this context, however, not only macrophage- or polymorphonuclear neutrophil (PMN)-derived ROIs must be taken into account, but also ROIs which are secreted *via* the action of related enzyme systems by nearly all cell types, *e.g.* endothelial cells, fibroblasts and mesangial cells, upon stimulation by macrophage products such as TNF and IL-1 [86, 87]. ROI production using enzyme systems other than the classical nicotinamide adenine dinucleotide phosphate reduced (NADPH) oxidase system is quantitatively less than the production of ROIs by the typical producer macrophages and PMNs. However, although producing a lower level of ROI, the production is continuous over a long time period and may, thus, contribute to chronic lung injury. In this situation, macrophages would only be responsible in an indirect way, by supplying stimulating substances.

#### *Antimicrobial activity of lung macrophages*

With respect to the killing of micro-organisms, alveolar macrophages have the most important role of all pulmonary macrophages. They are ideally located in a strategically important area, where exogenous air with all its contaminants comes into contact with a surface of only one or two cells, the alveolar lining cells, which

form an extremely thin barrier between the air and lung interstitial tissue. Therefore, a high degree of protecting potential is a prerequisite for the functional integrity of this organ. The fact that under normal conditions almost no infections of the lung occur, demonstrates the high efficiency of the system. The efficiency of microbial killing by alveolar macrophages depends on the species of the micro-organisms [88], and on the size of the inoculum [89]. Indeed, this cell with strong microbicidal and phagocytic potential, can clear certain bacteria, viruses and fungi more efficiently than others.

The microbicidal efficiency of alveolar macrophages also depends on the quantity of the inoculum. A small number of micro-organisms ( $10^5$  organisms) can be eliminated by the alveolar macrophages alone, whereas an inoculum of  $10^6$  induces a modest influx of PMNs to the alveoli, attracted by chemotactic substances produced by macrophages. PMNs and alveolar macrophages together have a greater capacity to clear micro-organisms ( $10^7$  organisms) than macrophages alone [89]. However, this barrier can also be overcome by an exceedingly large inoculum of  $10^8$  micro-organisms. In this situation, successful clearance can be achieved following activation of T- and B-lymphocytes.

However, not only the quantity but also the species of micro-organism seems to play a role, *e.g.* *Staphylococcus aureus* is cleared in the lung predominantly by alveolar macrophages, since the clearance of this bacterium occurs equally well in neutropenic mice [88]. In contrast, the killing of *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* was suppressed in neutropenic mice [88]. Certain bacteria, such as *S. aureus*, are phagocytosed and subsequently killed intracellularly by the alveolar macrophages without prior activation, while others, such as *Mycobacteria* spp., *Listeria* spp., and *Legionella pneumophila*, are readily phagocytosed by the alveolar macrophages but no intracellular killing occurs [90]. These phagocytosed bacteria then continue to multiply in the nonactivated macrophage, and will only be cleared when the macrophages are activated by cytokines such as interferon [90].

The immunocompromised host is particularly susceptible to such infections, since they lack T-lymphocyte-derived macrophage-activating cytokines. Thus, an intratracheal inoculum, of *e.g.* *Listeria monocytogenes*, will readily multiply inside the alveolar macrophages unless alveolar macrophages are activated locally by intratracheal application of interferon (unpublished observation). The situation is similar in the *Legionella* system [90]. Recently, it has been reported that, following an intratracheal infection with *Legionella pneumophila*, local activation of the alveolar macrophages successfully reduces the bacterial burden in the lung [91].

Aerogenic fungal infections of the lung can also be cleared by resting, or more efficiently by activated, alveolar macrophages in conjunction with PMNs.

Fungi, such as *Cryptococcus neoformans*, can be cleared by alveolar macrophages in small numbers. For a larger size of inoculum PMNs are recruited in addition [92]. Killing of fungi is apparently not predominantly performed by ROIs, since it has been shown that alveolar

macrophages producing much less ROI than peritoneal macrophages are more efficient in killing *Candida albicans* [93]. It has also been shown with Kupffer cells that the use of ROI scavengers hardly affected the killing of *Candida* spp. [93].

Viral respiratory infections are a frequent event in humans of all age groups. Whereas, in the nonimmuno-compromised host, influenza virus is the most prominent causative agent for viral lung disease, in the immuno-compromised host, adenoviruses, respiratory syncytial viruses (RSV) and Herpes viruses, such as cytomegal viruses (CMV) and Herpes simplex virus (HSV), play an additional important role, since the control of viral infections by specific T-lymphocytes cannot take place in the immunosuppressed organism.

Alveolar macrophages also represent the first line barrier against infection with respiratory viruses. The interaction of alveolar macrophages with respiratory viruses has been looked at extensively *in vitro* [94–97]. It has been shown that macrophages are infected to a limited degree by respiratory viruses, such as CMV, HSV and RSV [96]. The infection induces a quick cytokine response, the magnitude of which depends, as with bacteria and fungi, on the type of virus and the size of the inoculum [98]. Usually, a high amount of IFN- $\alpha/\beta$  is produced, which may protect neighbouring cells from being infected. Also, interleukin-6 (IL-6) and TNF are produced in large amounts by alveolar macrophages infected with RSV [95]. When Balb/c mice are infected intratracheally with  $10^4$  plaque-forming units (PFU) of RSV, alveolar macrophages produce chemotactic substances to attract granulocytes, which arrive at the alveoli about 6 h after experimental infection [97, 98]. PMNs can kill phagocytosed viruses, *e.g.* by ROIs which are produced as a response to viral infection. However, the overall respiratory burst in response to other stimuli is reduced in virally-infected alveolar macrophages and granulocytes, where their antibacterial and antifungal potential is reduced [97, 99]. This effect may explain the observation of an increased susceptibility of hosts suffering from respiratory viral infection to a subsequent bacterial infection [100, 101]. Innate defence mechanisms by alveolar macrophages and PMNs have been shown to play an essential role in the primary defence against viral infection, and activation [99] of these two cell types enhances innate resistance. Mechanisms, by which alveolar macrophages may interfere with early stages of virus/target cell interaction, viral replication and spread, may include phagocytosis and inactivation of the virus in the lysosomal compartment, defective virus replication in the alveolar macrophages, and cytotoxicity against a virus-infected cell, mediated by alveolar macrophages and PMNs either directly or with the aid of antibody.

When alveolar macrophages were compared with interstitial macrophages with respect to interaction with RSV, it was found that interstitial macrophages were more susceptible to viral infection [97]. In contrast, the cytokine response measured as production of interferon and TNF was reduced compared with that of alveolar macrophages after RSV infection [97] (see below).

This observation fits well with the idea of a different

involvement of pulmonary subpopulations in first line defence against micro-organisms. Alveolar macrophages are optimally equipped for the first line of nonspecific defence, whereas interstitial macrophages with their higher Class II expression and better accessory function (Franke-Ullmann and Lohmann-Matthes, unpublished data) are more adapted for the initiation of specific immune reactions. Finally, dendritic cells are exclusively restricted to that task.

### Cytokines produced by pulmonary macrophages

Most of the data accumulated come from studies with alveolar macrophages because of their easy accessibility [102]. Alveolar macrophages have been reported to produce IL-1 [103], IL-6 [104], TNF [105], TGF- $\beta$  [106], fibroblast growth factors (FGF) [107], chemotactic factors [108, 109], PDGF [110], and CSFs [111]. The production of the individual cytokines is influenced to a great extent by the stimulus used. The most widely used stimuli are bacterial lipopolysaccharides and viruses.

#### *Alveolar macrophages*

The most prominent cytokines produced by alveolar macrophages are those which are involved in antimicrobial and antiviral activity, such as TNF- $\alpha$ , IFN- $\alpha$  and IFN- $\beta$ , oxygen radicals [112] and nitric oxides [113]. In addition to this antimicrobial potential, stimulated alveolar macrophages secrete chemotactic factors to attract granulocytes, such as interleukin-8 (IL-8) [109], macrophage inflammatory proteins 1 and 2 (MIP-1 and MIP-2) [108], LTB<sub>4</sub> and PDGF [110]. As discussed before, small inocula of micro-organisms may be cleared by alveolar macrophages alone. For larger inocula to be cleared, granulocytes freshly attracted by the above-mentioned chemotactic factors are needed. In addition to these cytokines, which are important for nonspecific resistance against infective agents, stimulated alveolar macrophages produce IL-1 $\alpha$  and IL-1 $\beta$  [103], IL-6 [104] and TGF- $\beta$  [106]. Several cytokines secreted by alveolar macrophages have fibrogenic activity. PDGF, TGF- $\beta$ , FGF [114], TNF and also fibronectin [115] stimulate fibroblast proliferation. Also, IL-1 has been reported to be involved in fibroblast growth, although it may act through the induction of other factors [116]. These same molecules also play a role in the synthesis of collagen and the production of connective tissue [116]. For a detailed discussion of the functional properties of these mediators and cytokines, specialized reviews on cytokines should be consulted [102].

Besides bacterial lipopolysaccharides, respiratory viruses are very efficient in stimulating cytokine production by alveolar macrophages. It has been reported that respiratory syncytial virus stimulates human and mouse alveolar macrophages production of TNF, IL-1, IL-6, IL-8 and IFN- $\alpha/\beta$  [97, 100, 117–121]. Viral infection of macrophages not only induces antiviral activity *via* production of IFN- $\alpha/\beta$  but also mounts an inflammatory

reaction, which may activate and protect other target cells against the virus. This has been shown, not only for RSV, but also for many other viruses, such as influenza A [100], Sendai virus [118], Newcastle disease virus [119] and cytomegalovirus [120]. This nonspecific inflammatory reaction may represent the first line barrier against viral infections [121]. In the case of influenza virus, it has been shown that the virus-induced TNF production is strongly enhanced when LPS is given simultaneously [100]. This may suggest that the severe manifestations of a combined viral/bacterial infection are partially due to the adverse effects of high TNF release [100, 101].

#### Effect of smoking on alveolar macrophages

When functions of alveolar macrophages are evaluated, smoking has to be considered as a major confounding factor [122]. Number of macrophages, morphology, surface markers, motility, content and releasability of lysosomal enzymes may be influenced by smoking. Smokers macrophages have been reported to spontaneously release a chemotactic molecule for neutrophils [123]. Accessory function and IL-1 secretion have been reported to be diminished in smokers macrophages [122].

#### Functional comparison between alveolar and interstitial macrophages

Little data is available on functional parameters of interstitial lung macrophages. Morphologically, alveolar macrophages are described as mature large cells which resemble tissue macrophages [4, 124]. The interstitial macrophages, in contrast, are described as smaller and to resemble more closely peripheral blood monocytes [6, 125]. Both macrophage populations have been reported to produce fibrogenic molecules [106, 107, 110, 126]. With respect to phagocytic capacity, both populations are equally active, using the Fc-receptor-dependent EA system [6, 8].

We compared some functional properties of alveolar and interstitial mouse macrophages in our laboratory. In the Fc receptor independent phagocytosis of *Saccharomyces cerevisiae*, alveolar macrophages were clearly more effective than interstitial macrophages (unpublished observations). Regarding cytokine production, alveolar macrophages were more effective in producing cytokines which are involved in antimicrobial defence, such as TNF- $\alpha$ , interferon, oxygen radicals and nitric oxides (fig. 1). Interstitial macrophages on the other hand, were more active in secreting IL-6 and IL-1, and showed a higher Class II expression along with a stronger accessory function (fig. 2) (Franke-Ullmann and Lohmann-Matthes, unpublished data).

In summary, alveolar macrophages appear to be better equipped for their antimicrobial task, whereas interstitial macrophages, although also having an antimicrobial potential, show a more pronounced capacity with respect

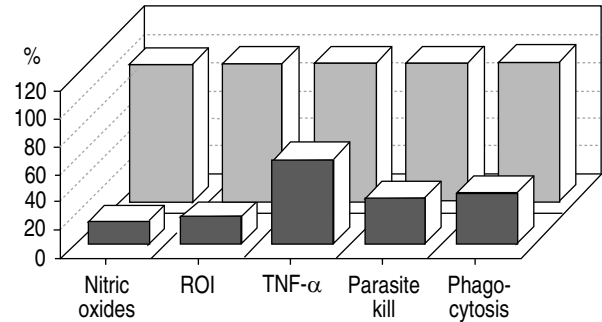


Fig. 1. – Comparison of functions of alveolar and interstitial macrophages. Cells had been treated the same way, *i.e.* alveolar macrophages were subjected to the same enzymatic treatments necessary to prepare the interstitial macrophages. All functions were tested in relevant bioassays. ■: interstitial macrophages; □: alveolar macrophages. Columns are referred to from left to right. Columns 1–3 after 5  $\mu$ g LPS, Columns 4 and 5 without stimulation. ROI: reactive oxygen intermediate; TNF- $\alpha$ : tumour necrosis factor- $\alpha$ ; LPS: lipopolysaccharide.

to immunoregulatory functions. The third population, the dendritic cells, have exclusive immunoregulatory and stimulating properties.

#### Lung macrophages in sarcoidosis and idiopathic lung fibrosis

Many reports exist demonstrating the presence of activated macrophages in sarcoidosis and pulmonary fibrosis. In sarcoidosis, alveolar macrophages seem to be activated *in situ*, since they have been demonstrated to produce *in vitro* high amounts of TNF- $\alpha$  and IL-1 [127, 128]. These cytokines may upregulate the production of GM-CSF and CSF-1, which are elevated in the BAL of sarcoidosis patients [32, 54]. In sarcoidosis, alveolar macrophages have an increased proliferative activity as measured by Feulgen stain and Ki 67 positivity, an antibody which recognizes a nuclear proliferation antigen [32]. The production of ROIs was found to be increased by alveolar macrophages of patients with sarcoidosis [129], and several surface markers to be upregulated, such as CD54, KiM2, CD71, CD11b, and RFD9. In the granuloma of sarcoidosis patients IL-1Ra is produced spontaneously, which may modulate the activity of the spontaneously produced IL-1 [131]. The

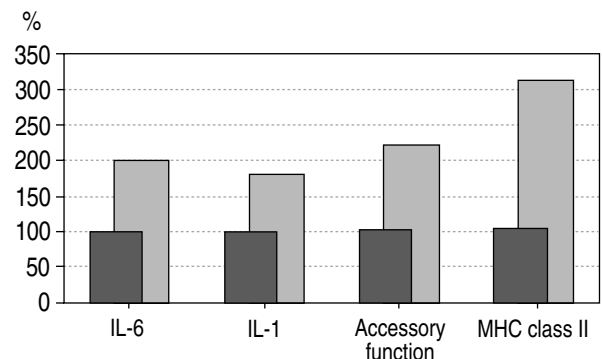


Fig. 2. – Comparison of functions of alveolar and interstitial macrophages. ■: alveolar macrophages; □: interstitial macrophages. Columns are referred to from left to right. Columns 1 and 2 after 5  $\mu$ g LPS, Columns 3 and 4 without stimulation. IL: interleukin; MHC: major histocompatibility complex; LPS: lipopolysaccharide.

co-operation between IL-1 and IL-1Ra is, in addition, modulated by IL-4, which reduces IL-1 production and increases production of IL-1Ra [132]. In contrast to the activated alveolar macrophages, the peripheral blood monocytes of the same patients were quiescent [130].

Also, in idiopathic lung fibrosis, several functions of lung macrophages are upregulated, and *in situ* activation of macrophages is discussed. The production of ROIs by alveolar macrophages is increased [133]. Enhanced proliferation of alveolar macrophages is observed, as in sarcoidosis patients [32]. In addition, a strong spontaneous production of the fibrogenic molecule PGDF by alveolar macrophages has been described [110]. In idiopathic lung fibrosis, increased numbers of neutrophils are present in the lung parenchyma. Alveolar macrophages have been demonstrated to release *in vitro* spontaneously chemotactic activity for neutrophils [134]. Recently, elevated levels of messenger ribonucleic acid (mRNA) for IL-8 have been reported to be present in such alveolar macrophages. This mechanism may be responsible for the recruitment and activation of neutrophils in idiopathic lung fibrosis [135]. Lectin-binding is also different from that of normal alveolar macrophages. Four lectins which have low binding in the normal alveolar macrophage show elevated binding in macrophages from patients with idiopathic lung fibrosis [51]. This type of binding has been reported to resemble the binding characteristics of monocytes [51]. Since idiopathic lung fibrosis is characterized by inflammatory conditions of alveoli and lung parenchyma, a higher percentage of freshly migrated monocytes from peripheral blood may be present in the lung.

All these data suggest that *in situ* activated pulmonary macrophages may be involved in sarcoidosis and idiopathic lung disease.

### Therapeutic aspects

The following remarks refer exclusively to animal models, since that is what the authors of this article are experienced in.

Macrophages in the alveolar space and in lung can have low activation, which results in an enhanced susceptibility to infections. This occurs under T-cell immunosuppression, when the macrophage activating T-cell cytokines are absent. Such a lack of activation may be treated by intratracheal or inhaled application of macrophage-activating substances, such as interferon- $\gamma$ . However, the success of the treatment depends on the quantity of the macrophage activators applied, which regulates whether or not only the alveolar or both alveolar and interstitial macrophages are activated. Since the alveolar macrophages carry out the first line antimicrobial defence, it would usually be desirable to activate only these cells. Figure 3 gives an example from our laboratory for such a local activation of lung macrophages *in situ*. Macrophages were activated by IFN- $\gamma$ , in a dose-dependent way, to secrete IL-6. We have successfully treated immunosuppressed rats suffering from an intra-tracheally applied *Listeria monocytogenes* infection by the intratracheal application of interferon (unpublished observation).

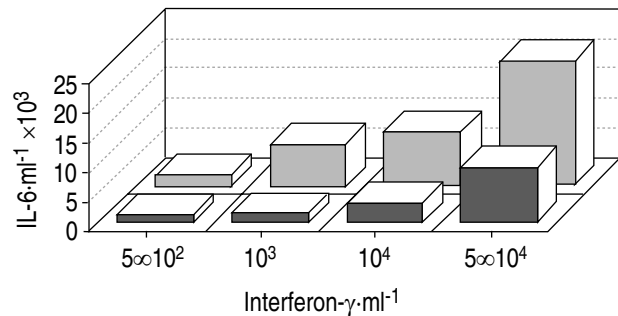


Fig. 3. – Dose-dependency of macrophage activation on the amount of activating substance. Rats were treated intra-tracheally with varying amounts of rat Interferon gamma. 18 h later alveolar and interstitial macrophages were prepared and tested using a bioassay for their interferon gamma-induced production of Interleukin. ■ : interstitial macrophages; □ : alveolar macrophages. IL-6: interleukin 6.

Similar data have been obtained with *Legionella pneumophila* in the immunosuppressed host [91]. Preliminary data with RSV infection in the mouse indicate that activation of alveolar macrophages may reduce the viral load in the lung, (unpublished observation).

In contrast, when lung macrophages are activated *in vivo*, as in sarcoidosis or interstitial lung fibrosis, the inhalative application of deactivating substances, such as IL-10 or TFG- $\beta$ , may be useful in the future. The established therapy with cortisone suppresses several macrophage functions. With the macrophage-deactivating substances one must take into account that they are often selective in their functions. For example, IL-10 mainly deactivates the IL-6 and TNF- $\alpha$  gene,

Regarding more generalized immunosuppression, which affects not only T-lymphocytes but also haematopoietic cells of the myelomonocytic lineage, it may be of benefit to apply GM-CSF or CSF-1 locally. However, the precise conditions required to recruit only the cell type needed, and to achieve proliferation only in the desired compartment, *e.g.* in the alveoli, has not yet been worked out in the animal model.

The lung is an organ bearing the danger of direct contact with the environment with all its possible damaging influences. On the other hand, this exposure allows optimal direct "targeting" of the lung by inhalation of therapeutic agent(s), without a "co-treatment" of the whole body.

### References

1. Johnsson S, Musher DM, Goree A, Lawrence EC. Human alveolar lining material and antibacterial defense. *Am Rev Respir Dis* 1986; 133: 136–142.
2. Reynolds HY. Bronchoalveolar lavage (state of art). *Am Rev Respir Dis* 1987; 135: 250–263.
3. Daniele RP, Dauber JH, Altose ND, Rowlands DT, Gorenberg DJ. Lymphocyte studies in asymptomatic cigarette smokers: a comparison between lung and peripheral blood. *Am Rev Respir Dis* 1977; 116: 997–1005.
4. Zwilling BS, Campolito LB, Reiches NA. Alveolar subpopulations identified by differential centrifugation on a discontinuous albumin density gradient. *Am Rev Respir Dis* 1982; 125: 448–452.



5. Hohan A, Dauber JH, Diamond MS, Daniele RP. Separation of bronchoalveolar cells from the guinea-pig on continuous gradients of Percoll: functional properties of lung macrophages. *J Reticuloendoth Soc* 1983; 33: 157-164.
6. Lavnikova N, Prokhorova S, Helyar L, Laskin DL. Isolation and partial characterization of subpopulations of alveolar macrophages, granulocytes, and highly enriched interstitial macrophages from rat lung. *Am J Respir Cell Mol Biol* 1993; 8: 384-392.
7. Lehnert BE, Valdez Y, Holland L. Pulmonary macrophages: alveolar and interstitial populations. *Exp Lung Res* 1985; 9: 177-185.
8. Crowell RE, Heaphy E, Valdez Y, Mold C, Lehnert BE. Alveolar and interstitial macrophages in the murine lung. *Exp Lung Res* 1992; 18: 435-446.
9. Holt PG, Schon-Hegrad MA, Oliver J. MHC class II-antigen-bearing dendritic cells in pulmonary tissues of the rat. *J Exp Med* 1988; 167: 262-274.
10. Hance AJ. Pulmonary immune cells in health and disease: dendritic cells and Langerhans' cells. *Eur Respir J* 1993; 6: 1213-1220.
11. Steinman RM, Cohn ZA. Identification of a novel cell type in peripheral lymphoid organs of mice. *J Exp Med* 1974; 139: 380-397.
12. Nicod LP, Lipscomb MF, Weissler JC, Lyons CR, Albertson J, Toews GB. Mononuclear cells in human lung tissue. Characterization of a potent accessory cell not obtained by bronchoalveolar lavage. *Am Rev Respir Dis* 1987; 136: 818-823.
13. Holt PG, Schon-Hegrad MA, Oliver J, Holt BJ, McMenamin PG. A contiguous network of dendritic antigen-presenting cells within the respiratory epithelium. *Int Arch Allergy Appl Immunol* 1990; 91: 155-159.
14. Soler PA, Basset F, Hance AJ. Cigarette smoking-induced changes in the number and differentiated state of pulmonary dendritic/Langerhans' cells. *Am Rev Respir Dis* 1989; 139: 1112-1119.
15. Casolaro MA, Bernaudin JF, Saltini C, Ferrans VJ, Crystal RG. Accumulation of Langerhans' cells on the epithelial surface of the lower respiratory tract in normal subjects in association with cigarette smoking. *Am Rev Respir Dis* 1988; 137: 406-411.
16. McCarthy KM, Gong JL, Telford JR, Schneeberger EE. Ontogeny of Ia+ accessory cells in fetal and newborn rat lung. *Am J Respir Cell Mol Biol* 1992; 6: 349-356.
17. Warner AE, Barry BE, Brain JD. Pulmonary intravascular macrophages in sheep. Morphology and function of a novel constituent of the mononuclear phagocyte system. *Lab Invest* 1986; 55: 276-288.
18. Dehring DJ, Wismar BL. Intravascular macrophages in pulmonary capillaries of humans. *Am Rev Respir Dis* 1989; 139: 1027-1029.
19. Warner AE, Molina RM, Brain JD. Uptake of blood-borne bacteria by pulmonary intravascular macrophages and consequent inflammatory responses in sheep. *Am Rev Respir Dis* 1987; 136: 683-690.
20. Godleski J, Brain JD. The origin of alveolar macrophages in mouse radiation chimaeras. *J Exp Med* 1972; 136: 630-643.
21. Blusse van Oud Albas A, van Furth R. Origin, kinetics and characteristics of pulmonary macrophages in the normal steady-state. *J Exp Med* 1979; 149: 1504-1518.
22. Thomas ED, Ramberg RE, Sale GE, Sparkes RS, Golde DW. Direct evidence for bone marrow origin of the alveolar macrophage in man. *Science* 1976; 192: 1016-1018.
23. Blusse van Oud Albas A, van der Linden-Schrever B, van Furth R. Origin and kinetics of pulmonary macrophages during an inflammatory reaction induced by intra-alveolar administration of aerosolized heat-killed BCG. *Am Rev Respir Dis* 1983; 128: 276-281.
24. Ungar J, Wilson GR. Monocytes as a source of alveolar macrophages. *Am J Pathol* 1985; 11: 681-691.
25. van Furth R, Sluiter W. Distribution of blood monocytes between a marginating and a circulating pool. *J Exp Med* 1986; 163: 474-479.
26. Chen BDM, Mueller M, Chou TH. Role of GM-CSF in the regulation of murine alveolar macrophage proliferation and differentiation. *J Immunol* 1988; 141: 139-144.
27. Golde DW, Byers LA, Finley TN. Proliferative capacity of human alveolar macrophages. *Nature* 1974; 247: 373-375.
28. Bittermann P, Adelberg S, Goodmann S, Saltzman L, Chrystal RG. Role of macrophage replication in modulating the increased number of alveolar macrophages in chronic inflammatory lung disorders. *Am Rev Respir Dis* 1983; 127(Suppl.): A60.
29. Adamson IYR, Bowden DH. Role of monocytes and interstitial cells in the generation of alveolar macrophages. II. Kinetic studies after carbon loading. *Lab Invest* 1980; 42: 518-524.
30. Evans M, Shami SG, Martinet M. Enhanced proliferation of pulmonary alveolar macrophages after carbon instillation in mice depleted of blood monocytes by strontium. *Lab Invest* 1986; 54: 154-159.
31. Shami SG, Martinez LA, Evans MJ. The role of migrating inflammatory cells in proliferation of lung interstitium and epithelium. *Chest* 1986; 89(Suppl.): 170-173.
32. Pforte A, Gerth C, Voss A, et al. Proliferating alveolar macrophages in BAL and lung function changes in interstitial lung disease. *Eur Respir J* 1993; 6: 951-955.
33. Decker T, Baccarini M, Lohmann-Matthes ML. Liver associated precursor cells proliferative under impairment of regular hematopoiesis. *Eur J Immunol* 1988; 18: 697-703.
34. Sibille Y, Reynolds HY. Macrophages and polymorphonuclear neutrophils in lung defense and injury. *Am Rev Respir Dis* 1990; 141: 471-501.
35. Fels AOS, Cohn ZA. The alveolar macrophage. *J Appl Physiol* 1986; 60: 353-369.
36. Koren HS, Becker S. Antimicrobial defense mechanisms. In: Parent RA, ed. *Comprehensive Treatise on Pulmonary Toxicology*. Boca Raton, FL, CRC Press, 1992; pp. 747-769.
37. Reynolds HY, Atkinson JP, Newball HH, Frank MM. Receptors for immunoglobulin and complement on human alveolar macrophages. *J Immunol* 1975; 114: 1813-1819.
38. Davis-Scibienski C, Beaman BL. Interaction of alveolar macrophages with *Nocardia asteroides*: immunological enhancement of phagocytosis, phagosome-lysosome fusion and microbicidal activity. *Infect Immun* 1980; 30: 578-587.
39. Goldstein EW, Lippert W, Warshauer D. Pulmonary alveolar macrophage, defender against bacterial infection of the lung. *J Clin Invest* 1974; 54: 519-528.
40. Hoidal JR, Schmeling D, Peterson PK. Phagocytosis, bacterial killing and metabolism by purified human lung phagocytes. *J Infect Dis* 1981; 144: 61-71.
41. Anderson CL, Looney RL. Human leucocyte IgE Fc receptors. *Immunol Today* 1986; 7: 264-271.

42. Naegel GP, Young RK, Reynolds HY. Receptors of human IgG subclasses on human alveolar macrophages. *Am Rev Respir Dis* 1984; 129: 413–418.
43. Melewicz FM, Kline LE, Cohen AB, Spiegelberg HE. Characterization of the IgG Fc receptors for IgE on human alveolar macrophage. *Clin Exp Immunol* 1982; 49: 364–370.
44. Gaudie J, Richards C, Lamontagne L. Fc receptors for IgA and other immunoglobulins on resident and activated alveolar macrophages. *Mol Immunol* 1983; 20: 1029–1037.
45. Verbourgh HA, Hoidal JR, Nguyen BYT, Verhoef J, Quie PG, Peterson PK. Human alveolar macrophage cytophilic immunoglobulin G-mediated phagocytosis of protein A-positive staphylococci. *J Clin Invest* 1982; 69: 63–69.
46. Sibille Y, Chatelain B, Staquet P, Merrill WW, Delacroix DL, Vaerman JP. Surface IgA and Fc-alpha receptors on human alveolar macrophages from normals and patients with sarcoidosis. *Am Rev Respir Dis* 1989; 139: 740–747.
47. Myones BL, Dalzell JG, Hogg N, Ross GD. Neutrophil and monocyte surface p150.95 has iC3b receptor activity resembling CR3. *J Clin Invest* 1988; 82: 640–649.
48. Wright SD, Craigmyle L, Silverstein SC. Fibronectin and serum amyloid P stimulate C3b and C3b-mediated phagocytosis in cultured monocytes. *J Exp Med* 1983; 158: 1338–1346.
49. Robertson B. Interaction of pulmonary surfactant and alveolar macrophages in the nonspecific defence system of the lung. *Eur J Respir Dis* 1980; 61(Suppl. 108): 16–18.
50. O'Neill S, Lesperance E, Klas DJ. Rat lung lavage surfactant enhances bacterial phagocytosis and intracellular killing by alveolar macrophages. *Am Rev Respir Dis* 1984; 130: 225–230.
51. Meyer KC, Powers C, Rosenthal N, Auerbach R. Alveolar macrophage surface carbohydrate expression in interstitial lung disease as determined by lectin binding profiles. *Am Rev Respir Dis* 1993; 148: 1325–1334.
52. Hancock WW, Muller WA, Cotran RS. Interleukin-2 receptors are expressed by alveolar macrophages during pulmonary sarcoidosis and are inducible by lymphokine treatment of normal human lung macrophages, blood monocytes and monocyte cell lines. *J Immunol* 1987; 138: 185–191.
53. Sone S, Yanagawa H, Nishioka Y. Interleukin-4 as a potent down-regulator for human alveolar macrophages capable of producing tumour necrosis factor and interleukin-1. *Eur Respir J* 1992; 5: 67–72.
54. Kreipe H, Radzun J, Heidorn K, et al. Proliferation, M-CSF and M-CSF receptor expression of alveolar macrophages in active sarcoidosis. *Lab Invest* 1990; 62: 697–703.
55. Ralph P, Ladner MB, Wang AM, Kawasaki ES, Stanley ER, Broxmeyer HE. The molecular and biological properties of the human and murine members of the CSF-1 family. In: Webb DR, Pierce CW, Cohen S, eds. *Molecular Basis of Lymphokine Action*. Clifton, New Jersey, Humana, 1987; pp. 299–311.
56. Baccarini M, Sabatini DM, App H, Rapp UR, Stanley ER. CSF-1 stimulates temperature-dependent phosphorylation and activation of the RAF-1 proto-oncogene product. *Embo J* 1990; 9: 3649–3657.
57. Decker T, Lew DJ, Mirkovitch J, Darnell JE. Cytoplasmic activation of GAF, an IFN- $\gamma$  regulated DNA-binding factor. *Embo J* 1991; 10: 927–932.
58. Lehmann J, Seeger D, Strehlow I, Schindler C, Lohmann-Matthes ML, Decker T. Interleukin-10-induced factors belonging to the p91 family of proteins bind to interferon-gamma responsive promoter elements. *J Immunol* 1994; (in press).
59. Poulter LW, Campbell DA, Munro C, Janossy G. Discrimination of human macrophages and dendritic cells using monoclonal antibodies. *Scand J Immunol* 1986; 24: 351–357.
60. Spiteri MA, Poulter LW. Characterization of immune inducer and suppressor macrophages from the normal lung. *Clin Exp Immunol* 1991; 83: 157–162.
61. Hoogsteden HC, van Hal PThW, Wijkhuijs JM, Hop W, Hilvering C. Expression of the CD11/CD18 cell surface adhesion glycoprotein family and MHC class II antigen on blood monocytes and alveolar macrophages in interstitial lung disease. *Lung* 1992; 170: 221–233.
62. Albert RK, Embree LJ, McFeely JE, Hickstein DD. Expression and function of  $\beta_2$ -integrins on alveolar macrophages from human and nonhuman primates. *Am Rev Respir Dis* 1992; 7: 182–189.
63. Montefort S, Holgate ST. Adhesion molecules and their role in inflammation. *Respir Med* 1991; 85: 91–99.
64. Striz I, Wang YM, Kalaycioglu O, Costabel U. Expression of alveolar macrophage adhesion molecules in pulmonary sarcoidosis. *Chest* 1992; 102: 882–886.
65. Striz I, Wang YM, Svarkova I, Trnka L, Sorg C, Costabel U. The phenotype of alveolar macrophages and its correlation with immune cells in bronchoalveolar lavage. *Eur Respir J* 1993; 6: 1287–1294.
66. Zwadlo G, Brocker EB, von Bassewitz DB, Feige U, Sorg C. A monoclonal antibody to a differentiation antigen present on mature human macrophages and absent from monocytes. *J Immunol* 1985; 134: 1487–1491.
67. Wright SD, Ramos RA, Tobias PS, Ulevitch RJ, Mathison JC. CD14, a receptor for complexes of LPS and LPS-binding protein. *Science* 1990; 249: 1431–1433.
68. McDermott J, Kelsey CR, Wadell KA, et al. Synthesis of leukotriene B<sub>4</sub> and prostanoids by human alveolar macrophages: analysis by gas chromatography/mass spectrometry. *Prostaglandins* 1984; 27: 163–177.
69. Fels AOS, Pawlowski NA, Cramer EB, King TKC, Cohn ZA, Scott WA. Human alveolar macrophages produce leukotriene B<sub>4</sub>. *Proc Natl Acad Sci USA* 1982; 79: 7866–7870.
70. Takahashi H, Abe M, Hashimoto S, Takayama K. *In vitro* effects of lipopolysaccharides on alveolar and peritoneal macrophages of rat: superoxide anion generation and 5-lipoxygenase metabolism of arachidonic acid. *Am J Respir Cell Mol Biol* 1993; 8: 291–298.
71. Suzuki K, Yamamoto T, Sato A, et al. Lipopolysaccharide primes human alveolar macrophages for enhanced release of superoxide anion and leukotriene B<sub>4</sub>: self-limitations of the priming response with protein synthesis. *Am J Respir Cell Mol Biol* 1993; 8: 500–508.
72. Meslier L, Aldrich Aj, Bigby TD. Effect of interferon- $\gamma$  on the 5-lipoxygenase pathway of rat lung macrophages. *Am Rev Respir Dis* 1992; 6: 93–99.
73. Koren HS, Joyce M, Devlin RB, Becker S, Driskoll K, Madden MC. Modulation of eicosanoid production by human alveolar macrophages exposed to silica *in vitro*. *Environ Health Persp* 1992; 97: 77–83.
74. Phan SH, McGarry BM, Loeffler KM, Kunkel SL. Regulation of macrophage-derived fibroblast growth factor release by arachidonite metabolites. *J Leukocyte Biol* 1987; 42: 106–113.

75. Mohr C, Davis GS, Graebner Hemenway DR, Gemsa D. Enhanced release of prostaglandin E<sub>2</sub> from macrophages of rats with silicosis. *Am J Respir Cell Mol Biol* 1992; 6: 390–396.
76. Rola-Pleszczynski M, Lemaire I. Leukotrienes augment interleukin-1 production by human monocytes. *J Immunol* 1985; 135: 3958–3961.
77. Driskoll KE, Higgins JM, Laytart MJ, Crosby LL. Differential effects of mineral dusts on the *in vitro* activation of alveolar macrophage eicosanoids and cytokine release. *Toxicol In Vitro* 1990; 4: 284–288.
78. Elias JA, Rossmann MD, Zurier RB, Daniele RP. Human alveolar macrophage inhibition of lung fibroblast growth: a prostaglandin-dependent process. *Am Rev Respir Dis* 1985; 131: 94–99.
79. Clark JG, Kostal KM, Marino BA. Bleomycin-induced pulmonary fibrosis in hamsters: an alveolar macrophage product increases fibroblast prostaglandin E<sub>2</sub> and cyclic adenosine monophosphate and suppresses fibroblast proliferation and collagen production. *J Clin Invest* 1985; 72: 2082–2091.
80. Fantone JC, Ward PA. Role of oxygen-derived free radicals and metabolites in leucocyte-dependent inflammatory reactions. *Am J Pathol* 1982; 107: 397–418.
81. Kemmerich B, Rossing TH, Pennington JE. Comparative oxidative microbicidal activity of human blood monocytes and alveolar macrophages and activation by recombinant interferon-gamma. *Am Rev Respir Dis* 1987; 136: 266–270.
82. Goldstein IM, Roos DD, Kaplan HB, Weissman G. Complement and immunoglobulins stimulate superoxide production by human leucocytes independently of phagocytosis. *J Clin Invest* 1975; 57: 836–841.
83. Sumimoto H, Takeshige K, Minakami S. Superoxide production of human polymorphonuclear leucocytes stimulated by leukotriene B<sub>4</sub>. *Biochem Biophys Acta* 1984; 803: 271–277.
84. Cohen MS, Isturitz RE, Malech HL, *et al.* Fungal infection in chronic granulomatous disease: the importance of the phagocyte in defense against fungi. *Am J Med* 1981; 71: 59–67.
85. Hoidal JR, Fox RB, LeMarbe PA, Perri R, Repine JE. Altered oxidative metabolic responses from asymptomatic cigarette smokers. *Am Rev Respir Dis* 1981; 123: 85–89.
86. Meier B, Radeke HH, Selle S, *et al.* Human fibroblasts release reactive oxygen species in response to IL-1 or TNF. *Biochem J* 1989; 263: 539–545.
87. Meier B, Cross AR, Hanock JT, Kaup FJ, Jones OTG. Identification of a superoxide generating NADPH-oxidase system in human fibroblasts. *Biochem J* 1991; 275: 241–245.
88. Rehm SR, Gross GN, Pierce AK. Early bacterial clearance from murine lungs: species-dependent phagocyte response. *J Clin Invest* 1980; 66: 194–203.
89. Onofrii HNM, Toews GB, Lipscomb MF, Pierce AK. Granulocyte/alveolar macrophage interaction in the pulmonary clearance of *Staphylococcus aureus*. *Am Rev Respir Dis* 1983; 127: 335–342.
90. Horwitz MA, Siverstein SC. Activated human monocytes inhibit the intracellular multiplication of Legionnaires' disease bacteria. *J Exp Med* 1981; 154: 1618–1627.
91. Skerrett SJ, Martin TR. Intratracheal IFN- $\gamma$  augments pulmonary defenses against experimental legionellosis. *Am Rev Respir Dis* 1994; 149: 50–58.
92. Gadebush HH, Gikas POW. The effect of cortisone on experimental pulmonary cryptococcosis. *Am Rev Respir Dis* 1965; 92: 64–72.
93. Decker T, Lohmann-Matthes ML, Baccarini M. Heterogenous activity of immature and mature cells of the murine monocyte/macrophage lineage derived from different anatomical districts against yeast-phase *Candida albicans*. *Infect Immun* 1986; 54: 477–486.
94. Panuska JR, Midulla F, Cirino NM, *et al.* Virus-induced alteration in macrophage production of tumor necrosis factor and prostaglandin E<sub>2</sub>. *Am Phys Soc* 1990; 259: 396–402.
95. Becker S, Soukup J, Quay J. Cytokine (TNF, IL-6, IL-8) production by RSV-infected human alveolar macrophages. *J Immunol* 1991; 147: 4307–4312.
96. Becker S, Soukup J, Yankaskas JR. RSV infection of human primary nasal and bronchial epithelial cell cultures and bronchioalveolar macrophages. *Am J Respir Cell Mol Biol* 1992; 6: 369–374.
97. Franke-Ullmann G, Pfortner C, Walter P, *et al.* Alteration of pulmonary macrophage function by RSV infection *in vitro*. *J Immunol* 1994; (in press).
98. Fujisawa H, Tsuru S, Taniguchi M, Zinnaka Y, Nomoto K. Protective mechanisms against pulmonary infection with influenza virus. I. Relative contribution of PMN and alveolar macrophages to protection during the early phase of intranasal infection. *J Gen Virol* 1987; 68: 425–431.
99. Rouse BT. Role of adaptive immune defense mechanisms in Herpes simplex resistance. In: Rouse BT, Lopez C, eds. Immunobiology of HSV Infection. Boca Ration, FL, CRC Press, 1983.
100. Nain M, Hinder F, Gong JH, Bender A, Sprenger H, Gemsa D. TNF production of influenza A virus infected macrophages and potentiating effect of LPS. *J Immunol* 1990; 145: 1921–1927.
101. Babiuk LA, Lawman MJP, Bielefeldt Ohmann H. Viral-bacterial synergistic interaction in respiratory disease. *Adv Vir Res* 1988; 35: 219–226.
102. Kelley J. Cytokines of the lung (state of the art). *Am Rev Respir Dis* 1990; 141: 765–788.
103. Kern JA, Lamb RJ, Reed JC. IL-1 $\beta$  gene expression in human monocytes and alveolar macrophages from normal subjects and patients with sarcoidosis. *Am Rev Respir Dis* 1988; 137: 1180–1184.
104. Jordana M, Richards C, Irving LB, Gauldie J. Spontaneous *in vitro* release of alveolar macrophage cytokines after the intratracheal instillation of bleomycin in rats. *Am Rev Respir Dis* 1988; 137: 1135–1140.
105. Panuska JR, Hertz MI, Taraf H, Villani A, Cirino NM. RSV infection of alveolar macrophages in adult transplant patients. *Am Rev Respir Dis* 1992; 145: 934–939.
106. Assoian RK, Fleurdelys BE, Stevenson HC. Expression and secretion of TGF- $\beta$  by activated human macrophages. *Proc Natl Acad Sci USA* 1987; 84: 6020–6024.
107. Gospodarowicz D, Ferrara N, Schweigerer L, Neufeld G. Structural characterization and biological functions of fibroblast growth factor. *Endocrin Rev* 1987; 8: 95–114.
108. Driskoll K, Hassenbein DG, Carter J, *et al.* Macrophage inflammatory proteins 1 and 2: expression by rat alveolar macrophages, fibroblasts and epithelial cells and after mineral dust exposure. *Am J Respir Cell Mol Biol* 1993; 8: 311–318.
109. Standiford TJ, Kunkel SL, Kasahara K, Milia MJ, Rolfe MW, Strieter RM. IL-8 gene expression from human

- alveolar macrophages: the role of adherence. *Am J Respir Cell Mol Biol* 1991; 5: 579–585.
110. Martinet Y, Rom WN, Grotendorst GR. Exaggerated spontaneous release of PDGF by alveolar macrophages from patients with idiopathic pulmonary fibrosis. *N Engl J Med* 1987; 317: 202–209.
  111. Becker S, Devlin RB, Haskill S. Differential production of TNF, M-CSF and IL-1 by human alveolar macrophages. *J Leukocyte Biol* 1989; 45: 353–361.
  112. Aerts C, Wallaert B, Grosbois JM, Voisin C. Release of superoxide anion by alveolar macrophages in pulmonary sarcoidosis. *Ann NY Acad Sci* 1986; 465: 193–200.
  113. Jorens PG, van Overveld FJ, Bult H, Vermeire PA, Herman AG. L-arginine-dependent production of nitrogen oxides by rat pulmonary macrophages. *Eur J Pharmacol* 1991; 200: 205–209.
  114. Bittermann PB, Rennard SJ, Hunninghake GW, Crystal RG. Human alveolar macrophage growth factor for fibroblasts: regulation and partial characterization. *J Clin Invest* 1982; 70: 806–822.
  115. Bittermann PB, Rennard SJ, Adelberg S, Crystal RG. Role of fibronectin as a growth factor for fibroblasts. *J Cell Biol* 1983; 97: 1925–1932.
  116. Kovacs E. Fibrogenic cytokines: the role immune mediators in the development of scar tissue. *Rev Immunol Today* 1991; 12: 17–23.
  117. Roberts Jr LJ, Prill AH, Mann TN. IL-1 and IL-1 inhibitor production by human macrophages exposed to influenza virus and respiratory syncytial virus. *J Exp Med* 1986; 163: 511–519.
  118. Aderka D, Holtman H, Tokar L, Hahn T, Wallach D. TNF induction by Sendai virus. *J Immunol* 1986; 136: 2938–2945.
  119. Lorence RM, Rood PA, Kelley KW. Newcastle disease virus as an antineoplastic agent: induction of TNF and augmentation of its cytotoxicity. *J Natl Cancer Inst* 1988; 80: 1305–1310.
  120. Dudding L, Haskill S, Clark BD, Auron PE, Sporn S, Huang SE. Cytomegalovirus infection stimulates expression of monocyte-associated mediator genes. *J Immunol* 1989; 143: 3343–3351.
  121. Morahan PS, Morse S. Macrophage-virus interaction. In: Proffitt MR, ed. *Virus Lymphocyte Interactions: implications for Disease*. Amsterdam, Elsevier, 1979; pp. 17–35.
  122. Costabel U, Guzman J. Effect of smoking on bronchoalveolar lavage constituents. *Eur Respir J* 1992; 5: 776–779.
  123. Hunninghake GW, Crystal RG. Cigarette smoking and lung destruction. *Am Rev Respir Dis* 1983; 128: 833–838.
  124. Bowden DH. Macrophages, dust and pulmonary disease. *Exp Lung Res* 1987; 12: 89–107.
  125. Kobzik L, Godleski J, Barry BE, Brain JD. Isolation and antigenic identification of hamster lung interstitial macrophages. *Am Rev Respir Dis* 1988; 138: 908–914.
  126. Brody AR, Bonner JC, Overby LH, et al. Interstitial pulmonary macrophages produce PDGF that stimulates rat lung fibroblast proliferation *in vitro*. *J Leukocyte Biol* 1992; 51: 640–648.
  127. Hunninghake GW. Release of IL-1 by alveolar macrophages of patients with active pulmonary sarcoidosis. *Am Rev Respir Dis* 1984; 129: 569–576.
  128. Mornex JF, Leroux C, Greenland T, Ecochard D. From granuloma to fibrosis in interstitial lung diseases: molecular and cellular interactions. *Eur Respir J* 1994; 7: 779–785.
  129. Schaberg T, Rau M, Stephan H, Lode H. Increased number of alveolar macrophages expressing surface molecules of the CD11/CD18 family in sarcoidosis and idiopathic pulmonary fibrosis is related to the production of superoxide anions by these cells. *Am Rev Respir Dis* 1993; 147: 1507–1513.
  130. Müller Quernheim J, Pfeifer S, Männel D, Strausz J, Ferlinz R. Lung restricted activation of the alveolar macrophage/monocyte system in pulmonary sarcoidosis. *Am Rev Respir Dis* 1992; 145: 187–192.
  131. Rolfe MW, Standiford TJ, Kunkel SL, et al. IL-1Ra expression in sarcoidosis. *Am Rev Respir Dis* 1993; 148: 1378–1384.
  132. Galve-de Rochemonteix B, Nicod LP, Chicheportiche R, Lacraz S, Baumberger C, Dayer JM. Regulation of IL-1Ra, IL-1 $\alpha$  and IL-1 $\beta$  production by human alveolar macrophages with PMA, LPS, and IL-4. *Am J Respir Cell Mol Biol* 1993; 8: 160–168.
  133. Strausz J, Müller-Quernheim J, Stepling H, Ferlinz R. Oxygen radical production by alveolar inflammatory cells in idiopathic pulmonary fibrosis. *Am Rev Respir Dis* 1990; 141: 124–128.
  134. Hunninghake GW, Gadek JE, Lawley TJ, Crystal RG. Mechanisms of neutrophil accumulation in the lungs of patients with idiopathic pulmonary fibrosis. *J Clin Invest* 1981; 68: 259–269.
  135. Carre PC, Mortensen RL, King TE, Noble PW, Sable CL, Riches DWH. Increased expression of the interleukin-8 gene by alveolar macrophages in idiopathic pulmonary fibrosis. A potential mechanism for the recruitment and activation and neutrophils in lung fibrosis. *J Clin Invest* 1991; 88: 1802–1810.