Pulmonary macrophages

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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.

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. 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-α (TNF-α) or interferon-α/β (IFN-α/β), 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-γ 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 Calmette-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 H-thymidine. Under subacute or chronic inflammatory conditions, a 10–15 fold increase of 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 microorganisms. 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]: FcγRI a high affinity receptor for monomeric IgG; FcγRII, a low affinity receptor [42] for IgG and aggregated IgG; and FcγRIII, 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-γ 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, cytotrophic 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, α-chain CD11b, β-chain CD18) binds iC3b with high affinity, and C3dg and C3d with low affinity. In addition, it binds β-glucan in the cell wall of fungi and bacteria. CR4 (α-chain CD11c, β-chain CD18) binds iC3b but not β-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-γ (IFN-γ), 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-γ [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-β (TGF-β). 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-β 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-β 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

<table>
<thead>
<tr>
<th>Immunoglobulins</th>
<th>Complement</th>
<th>Lipoproteins</th>
<th>Lectins with specificity for</th>
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<tr>
<td>IgG1, IgG2a (murine)</td>
<td>C3b, C3c, C4b, C4d, C5a</td>
<td>Low density lipoprotein</td>
<td>α-linked galactose residues</td>
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<tr>
<td>IgG2b, IgG3 (murine)</td>
<td></td>
<td>β-very low lipoprotein</td>
<td>N-acetylgalactosamine residues</td>
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<td>IgG1, IgG3 monomers (Human)</td>
<td></td>
<td></td>
<td>N-acetylglucosamine residues</td>
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<tr>
<td>IgG complexes (human)</td>
<td></td>
<td></td>
<td>α-linked fucose residues</td>
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<td>IgE, IgA (murine and human)</td>
<td></td>
<td></td>
<td>Mannose residues</td>
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<td>N-acetylturanine residues</td>
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<td>Proteins</td>
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<td>Fibronectin, fibrin</td>
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<td>Lactotransferrin</td>
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<td>GM-CSF, CSF-1</td>
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<td>Interferon-γ, IL-4, IL-1Ra</td>
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<td>IL-2, insulin</td>
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<td>Surface markers</td>
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<td>Class II molecules, CD11a, CD11b, CD11c, CD14, CD18, CD54</td>
<td>Molecules recognized by monoclonal antibodies:</td>
<td>25F9 (mature macrophages), 27E 10 (inflammatory macrophages), Ki M2, Ki M8 (mature macrophages), RM31 (inflammatory macrophages), RFD1, RFD7, RFD9</td>
<td></td>
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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 β-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, RFKD7, RFKD9, 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 hydroxyeicosatetraenoic acids HETEs) [68, 69]. Both kinds of metabolites play a role in the modulation of inflammatory reactions. Cyclooxygenase products secreted by alveolar macrophages include thromboxane A2, measured as its stable metabolites thromboxane B2, PGE2, PGD, and PGF2, lipooxygenase products include leukotriene B4 and 5-HETE. The largest amount of such released metabolites comprises thromboxane A2, which is released in ng·mg-1 cell protein, five times more than leukotriene B4 (LTB4), 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 LTB4 and 5-HETE than alveolar macrophages from control animals [70]. Similarly, human alveolar macrophages primed in vitro with LPS showed an enhanced production of LTB4, following stimulation with calcium-ionophore A23187 [71]. The macrophage activator, IFN-γ, 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-γ 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 LTB4, or, at higher dosages, to directly activate alveolar macrophages to enhance release mainly of cyclo-oxygenase pathway products, such as thromboxane B2 (TXB2) and prostaglandin E2 (PGE2) [73].

It was demonstrated that alveolar macrophages from rats which had been exposed to silica inhalation in vivo spontaneously produced TXB2 and PGE2, in vitro. Upon in vitro stimulation with LPS, the secretion of these cyclooxygenase products was strongly enhanced [74]. In contrast, in vivo stimulation with silica enhanced production of metabolites of the lipoxygenase pathway and decreased the production of the products of the cyclooxygenase pathway [73]. From these in vitro data, one may assume that silica can increase arachidonic acid
metabolites like LTB₄, 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 PGE₂ [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 O₂ metabolites, such as superoxide anion (O₂⁻), hydrogen peroxide (H₂O₂) and hydroxyl radical (OH⁻), 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-γ, platelet-derived growth factor (PDGF), GM-CSF, TNF, LTB₄) [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 of 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 exogeneous 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 (10⁵ organisms) can be eliminated by the alveolar macrophages alone, whereas an inoculum of 10⁷ 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 (10⁷ organisms) than macrophages alone [89]. However, this barrier can also be overcome by an exceedingly large inoculum of 10⁸ 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 *Mycobacterium 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 immunocompromised 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-α/β 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 10^6 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-β [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-α, IFN-α and IFN-β, 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], LTB4 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-1α and IL-1β [103], IL-6 [104] and TGF-β [106]. Several cytokines secreted by alveolar macrophages have fibrogenic activity: PDGF, TGF-β, FGF [114]. TNF and also fibronecin [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-α/β [97, 100, 117–121]. Viral infection of macrophages not only induces antiviral activity *via* production of IFN-α/β 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-α, 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 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-α 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].

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].

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.

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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 sarcoidosis patients [32]. 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-β, 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-α 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 optional direct "targeting" of the lung by inhalation of therapeutic agent(s), without a "co-treatment" of the whole body.

### 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-γ. 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-γ, 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 intra-tracheal application of interferon (unpublished observation).

![Figure 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. 
](image)

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)

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### References


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