Environmental Health Perspectives
Vol. 97, pp. 85–89, 1992

Secretory and Accessory Cell Functions of the Alveolar Macrophage

by Klara Miller,[1] Barry N. Hudspith,[1] and Clive Meredith[1]

We have attempted to address the requirements necessary for alveolar macrophage accessory cell function. We have also examined the *in vitro* and *in vivo* factors that must be taken into account when interpreting results from experimental studies. Differences in phenotypic expression by rat alveolar pleural and peritoneal macrophages are noted, as well as the differing expression of major histocompatibility complex (MHC) class II molecules. Furthermore, alveolar macrophages, harvested from rat lung, do not express the interleukin (IL)-1 cytokines, and lipopolysaccharide (LPS) treatment of quiescent cells (after 24-hr *in vitro* culture) induces low levels of expression of IL-1α and IL-1β. Short-term inhalation of refractory ceramic fibers, however, results in markedly increased IL-1β expression after stimulation with LPS. We suggest that, *in vivo*, IL-1β may be involved in the initial recruitment and activation of inflammatory cells rather than in induction of immune responses. We also postulate, based on recent published evidence, that alveolar macrophages activate the dendritic cells within the respiratory epithelium. Thus alveolar macrophages would release cytokines critical for the activation of dendritic cells during the afferent limb of the immune response, and they would respond to products of sensitized T-cells such as interferon-γ and IL-4 to interact with T-helper cells in an antigen-specific MHC-restricted manner during the efferent limb of the response.

Introduction

In 1972, cells classified as mononuclear phagocyte were grouped together on the basis of having a common origin as demonstrated by kinetic studies and similar morphology, cytochemistry, and function (*1*). The concept had the advantage of simplicity and would, it was thought, help clarify the role of mononuclear phagocytes in physiological and certain pathological conditions.

Fifteen years later, however, there is ample evidence that despite their probable origin from a common bone marrow progenitor population (*2*), macrophages from different sites display considerable heterogeneity. This diversity is expressed by differences in phenotypic expression, secretory products, immunoregulation, and presentation of antigen. They also show differences in energy source. Some of these differences are discussed in the context of the secretory and accessory cell functions of the alveolar macrophage.

Phenotypic Subsets of Macrophages

Numerous reports exist of monoclonal antibodies that label macrophage/monocyte subpopulations in mouse and man, and in some instances these phenotypically defined subsets have been shown to mediate specific function. For example, the murine iC3b complement receptor Mac-1 and the human equivalent recognized by anti-OKMI (*3*) appear on few tissue macrophages. Mac-1 is absent from Kupffer cells, and OKMI is expressed on only 25% of alveolar macrophages.

The rat is the favored animal in toxicological studies, but only recently have monoclonal antibodies delineating phenotypic subsets of macrophages in the rat been developed. Two groups, one in Oxford (*4*), the other in Amsterdam (*5*), have clearly demonstrated differences between alveolar macrophages and macrophages from different sites, as well as heterogeneity among the alveolar macrophages. Our own findings are in agreement with the published reports (*4,5*) in observing that alveolar macrophages are divided into subsets; additionally, we find differences in expression between alveolar, pleural, and peritoneal macrophages (Table 1). Both MRC OX-41 and MRC OX-42 antibodies recognize cell-surface proteins that are synthesized by macrophages, although they also label granulocytes. MRC OX-42 recognizes an antigen associated with complement function (the iC3b-receptor). ED1 and ED2 recognize mononuclear phagocytes exclusively; ED1 recognizes a cytoplasmic antigen, whereas ED2 binds to membrane antigen. We found that cells recovered by bronchoalveolar lavage (BAL) from PVG rats were ED1 positive and ED2 negative; 90% were OX-41 positive and 20-30% were positive for OX-42. By contrast the majority of pleural macrophages bound all four antibodies, whereas resident peritoneal macrophages bound OX-41, OX-42, and ED1, but only 50% recognized the ED2 antibody (Table 2). Thus, peritoneal macrophages differ from pleural cells in their phenotypic appear-

[1]Immunotoxicology Department, British Industrial Biological Research Association, Woodmansterne Road, Carshalton, Surrey SM5 4DS, UK.

Address reprint requests to K. Miller, Immunotoxicology Department, British Industrial Biological Research Association, Woodmansterne Road, Carshalton, Surrey SM5 4DS, UK.

Table 1. Distribution and staining pattern of rat macrophages by ED1 and ED2.

Macrophage type	ED1 (%)	ED2 (%)
Peritoneal macrophages (resident)	+++(90)	++ (45)
Pleural macrophages	+++(80)	+++(90)
Alveolar macrophages	+++(80)	−
Lung "tissue" macrophages	+ (10)	+++(80)
Monocytes	+++(90)	−

Table 2. Measurement of mRNA specific for interleukin-1 and fibronectin in alveolar macrophages 3 days after inhalation exposure to ceramic fibers.[a]

Culture conditions		mRNA		
Preculture time	LPS (100 μg/mL) ± for 3 hr	IL-1α	IL-1β	Fibronectin
0 hr				
Control	No	−	−	+
Test	No	−	−	+
24 hr				
Control	Medium only	−	−	+
Test	Medium only	−	−	+
24 hr				
Control	LPS	−/+	−/+	+
Test	LPS	+	+++	++

Abbreviations: IL-1, interleukin-1; LPS, lipopolysaccharide.
[a]Exposure to 10 mg/m³, 6 hr/day for 5 days.

ance and both differ from alveolar macrophages. Of particular interest here is the observation that when macrophages were isolated from lung tissue, 80% were positive for ED2, but only 10% labeled with ED1. Although it is tempting to speculate that ED2 is a differentiation antigen, one study using both resident and elicited peritoneal macrophages showed a decrease in ED2-positive cells from 53 to 13% (*6*). However, it may still be that monocytes or alveolar macrophages enter the lung parenchyma and differentiate into ED2-positive pulmonary tissue macrophages under the influence of various stimuli. It is also possible that alveolar macrophages under the stimulus of cytokines produced by sensitized T-lymphocytes will mature into ED2-positive cells. Our own experiments where rats have inhaled mineral fibers demonstrate that phagocytosis per se induces no alteration with respect to these membrane antigens. All one can say at present is that all free-lying macrophages are positive for the intracellular antigen ED1 and that ED2 recognizes a membrane antigen present on most tissue macrophages. Resident pleural macrophages, anatomically located in close proximity to alveolar macrophages, appear to share the phenotypic characteristic of a subset of peritoneal macrophages.

Another important difference between rat pleural and alveolar macrophages is the expression of major histocompatibility complex (MHC) class II (Ia) molecules on the surface membrane, said to correlate with the ability to present antigen and stimulate autologous lymphocyte proliferation. We found that approximately 50% of the pleural macrophages were positive for class II when labeling with MRC OX-4 monoclonal antibody, whereas only 5% of alveolar macrophages expressed class II antigen. In our inhalation studies with ceramic fibers, class II-positive cells increased by as much as 6-fold during and immediately after fiber exposure, and this was paralleled by similar increases in OX-42-positive cells. However, although the majority of lavaged cells contained phagocytosed fibers for up to 180 days, the increase in expression of class II was transient, and macrophages obtained 30 days after exposure showed little increase in expression (Fig. 1). This upregulation of class II surface antigen expression did not appear to relate to activation by "nonantigenic" fibers and might perhaps be induced by inflammatory stimuli. In the normal state, few neutrophils were recovered by bronchoalveolar lavage, but inhalation of fibers resulted in an increase from 2 to 14.5% in test rats 3 days after exposure. It may be that proteases or other mediators released by the neutrophils activated the macrophages to enable them to play a role in regulating potentially damaging inflammatory responses in the lung, rather than endowing them with an enhanced ability to present antigen.

Antigen Presentation/Regulation of Lymphocyte Proliferation

The accessory cell functions of alveolar macrophages remains a subject of some considerable controversy in the published literature. One currently accepted postulate is that, at least in man, alveolar macrophages are capable of supporting antigen-driven blastogenic responses by T-lymphocytes in *in vitro* studies, provided a low ratio of macrophages to T-lymphocytes are used. At higher ratios of alveolar macrophages, suppressor factors would predominate, partially due to the release of prostaglandins (PGE) by the macrophages (*7*). However, experimental work on murine macrophages provide suggestive evidence that the modulation of alveolar macrophage-mediated suppression, unlike peritoneal and pleural macrophages, is independent of PGE production (*8*) (Fig. 2). In these experiments macrophages were prereated for 20 hr with either interferon (IFN)-β or lymphokine-rich supernatant before adding concanavalin A-stimulated spleen cells. Pretreatment with lymphokines, but not IFN-β, caused a significant decrease of the suppressive capacity of the alveolar macrophages without having any effect on PGE release. It must be emphasized that no attempts were made in this study to characterize the factors contained in the conditioned media; however, the suggestion is that lymphocyte products would render the alveolar macrophage less suppressive and

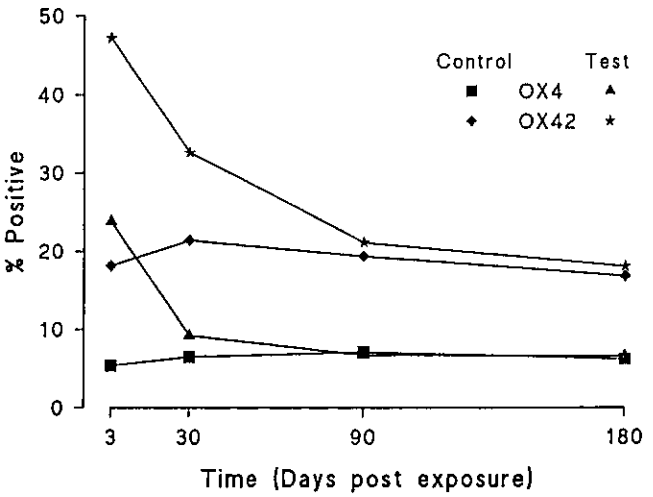

FIGURE 1. Expression of class II and complement receptor on rat alveolar macrophages after inhalation exposure to ceramic fibers (10 mg/m³; 6 hr/day for 5 days).

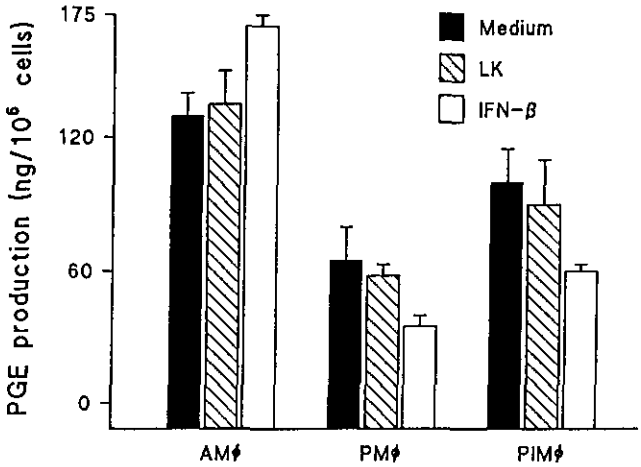

FIGURE 2. Effect of lymphokine (LK) and interferon-β (IFN-β) treatment on prostaglandin release from different macrophage populations. After Sestini et al. (*8*).

allow full expression of the immune response. No such effect, however, was found on the suppressor activity of peritoneal cells. This is very puzzling because there is evidence from other studies that rIFN can reproduce the effects of lymphokine-rich culture medium from antigen- or mitogen-activated T-cells on macrophages. IFN is a strong signal for regulation of class II MHC on antigen-presenting cells.

Returning to human alveolar macrophage for the moment, studies have also reported that even small numbers of cells can suppress lymphocyte proliferation by peripheral blood mononuclear cells when optimal concentrations of mitogen or antigen are used (*9*). These authors suggest that alveolar cells produce suppression via interactions with suppressor T-lymphocytes. This could provide some explanation as to why alveolar macrophages can act as accessory cells when responder T-lymphocytes are depleted of class II-positive cells by nylon wool adherence, but it is indeed extremely difficult to reconcile the differences in the many published studies. It therefore seems timely to examine both the requirements essential for a cell to function optimally as an accessory cell and the *in vivo* and *in vitro* factors that must be taken into account when interpreting results of experimental studies.

It is often stated that three requirements are essential for a cell to function optimally in antigen presentation: the capacity to process antigen, the capacity to synthesize and release secondary accessory factors such as interleukin 1 (IL-1), and the capacity to express class II MHC glycoproteins on its plasma membrane.

Although it is difficult to separate the scavenger role of the macrophage from its antigen-presenting role, there is little doubt that macrophages ingest and partially digest protein antigens and subsequently present the processed antigen on the surface in conjunction with an MHC antigen, generally as a peptide fragment. The next question therefore must be whether monocytes and macrophages synthesize IL-1, a co-stimulator signal said to be necessary for cell division and produced by a variety of cell types including endothelial cells, B-cells, and keratinocytes.

There can be no doubt that *in vitro* culture conditions have greatly affected our interpretation of macrophage functions in this respect. Mononuclear phagocytes are adherent cells, and *in vitro* assay to measure cytokines are carried out in flat- or round-bottomed microtiter plates. In both instances adherence to plastic occurs. We and others have found that adherence to plastic results in stimulation of IL-1 expression and secretion of the protein (*10,11*). In experiments using resident murine peritoneal macrophage, adherence to culture dishes induced expression at the level of mRNA of both IL-1α and IL-1β for at least 12 hr (the IL-β protein represents the predominant Il-1 secreted by mononuclear phagocyte); by 18 hr the cells were quiescent with regard to transcription of the cytokines examined, namely, IL-1, IL-6, and tumor necrosis factor (TNF)-α (*10*). There is little reason to believe that secreted cytokines are not present as responses to the phagocytic stimuli that would occur as a consequence of 1 or 2 hr adherence purification procedures. Such responses could sometimes be interpreted as part of the immunoregulatory or secretory functions of macrophages. Macrophages harvested from lymphoid organs or from the peritoneal cavity do not express IL-1α or IL-1β mRNA or protein (*12*), nor do freshly isolated monocytes. However, after stimulation with lipopolysaccharide (LPS) under appropriate *in vitro* conditions, significant levels of these mRNAs can be detected.

Macrophages from different sites, however, show considerable differences in the expression of cytokines. For example, studies on fresh human alveolar macrophages and blood monocyte demonstrate differences in release of cytokines (*13*). Alveolar macrophages release low amounts of IL-1β in response to LPS and high amounts or TNF-α, whereas blood monocytes release abundant amounts of IL-1β but low amounts of TNF.

Another cytokine, fibronectin, which is important in normal tissue turnover, wound repair, and inflammatory processes, is not expressed in blood monocytes but is expressed by mature mononuclear phagocytes, including alveolar and peritoneal macrophages (*14*). However, in some circumstances such as when alveolar macrophages are activated by surface stimuli, fibronectin mRNA is downregulated and the amount produced by the cells decreased. Clearly, the expression of cytokines by mononuclear phagocytes is not constitutive but may be dependent on the induction/activation signals and/or the physiological environment.

There may also be interspecies variation in the expression of cytokine genes just as there appears to be differences in the accessory activity of alveolar macrophages from guinea pig, rabbit, or rat. In agreement with others, we find that alveolar macrophages, harvested from rat lung, do not express the IL-1 cytokine genes, and LPS treatment induces low levels of expression of IL1-α or IL-β. Short-term (5 day) inhalation exposure to refractory ceramic fibers did not in itself cause an increase in IL-1 or fibronectin gene expression. After these cells became quiescent, however, and then were stimulated with LPS for 3 hr IL-1 expression was markedly increased compared with controls (*15*). It should perhaps be noted that although these cells were "primed" by the fibers to respond to a second stimulus such as LPS, there was a lower level of inducibility of the membrane-bound IL-1α (associated with cell–cell contact and antigen presentation) than IL-1β. This is in keeping with other studies which also show that IL-β is the form more readily synthesized and released by mononuclear phagocytes.

Because activated macrophages produce interleukin-1, and interleukin-1 has been shown in *in vitro* assays to induce thymocyte and lymphocyte proliferation, it has been widely accepted

that induction of a proliferative response in lymphocytes also requires accessory cell release of amplifying signals, such as those provided by interleukin-1. However, it should be remembered that IL-1 mediates a wide variety of biological responses to injury and infection including the production of the prostaglandin E series, pyrogenicity, and tissue destruction. IL-1 is also secreted in response to a wide range of antigen-independent stimuli. It is reasonable therefore to deduce that *in vivo* IL-1 is involved in the initial recruitment and activation of cells at sites of inflammation rather than in induction of immune responses. Indeed, the most potent of all accessory cells are the dendritic cells (which include the tissue dendritic cell, e.g., the Langerhans cells), and they do not produce interleukin-1 (*16,17*).

The dendritic cell lacks most conventional surface markers associated with the monocyte/macrophage lineage, and it is not clear whether the precursor is a discrete circulating cell or part of the conventional mononuclear phagocyte population (*18*). Dendritic cells possess long mobile cytoplasmic processes, are not phagocytic, have few lysosomes, and stain weakly for nonspecific esterase and ATPase. They do not proliferate in culture, do not express Fc receptors, but expression of class II antigens is very strong, and the dendritic cell plays an important role in helper T-cell activation. Dendritic cells from all species and tissues that have been examined to date have proven to be potent stimulators of the autologous mixed-leukocyte reaction (MLR) and of *in vitro* mitogen- and antigen-induced lymphocyte proliferation. Investigators have also found dendritic cells to induce both the release of IL-2 and the responsiveness of T-cells to IL-2 (*19*).

Recently, several studies on both human and rat lung have demonstrated the presence of significant numbers of dendritic cells within the respiratory epithelium as well as the ability of such nonphagocytic, nonadherant cells to act as potent accessory cells (*20–23*). The strategic location of these cells and the extensive interstitial lymphocyte proliferation that is known to occur during local immune responses in the lung suggest that the accessory cell in the lung parenchyma is the dendritic cell. This appears to leave the role of alveolar macrophages more confused than ever. However, when accessory functions involved in both primary and secondary T-immune responses are taken into account, it can be seen that pulmonary alveolar macrophages and dendritic cells would collaborate in regulating cellular immune responses. This postulate is based *a*) on the critical observations that dendritic cells can form clusters with resting T-helper cells in the absence of exogenous antigen, whereas macrophages will only form clusters with activated T-helper cells in the presence of antigen (*24*) and *b*) important developmental events that may occur during the maturation of dendritic cells.

Little is known about the events occurring during pulmonary dendritic cell maturation, so it is still unclear whether they might proliferate locally or mature within the lung tissue. Far more is known of the maturation and properties of the Langerhans cell, as it is relatively easy to isolate from skin sources. Two recent reports on murine Langerhan cells cultured *in vitro* have identified a major role for granulocyte/macrophage colony-stimulating factor (GM-CSF) in the accessory immunological maturation and function of Langerhans cells (LC) (*25,26*). Both groups made use of conditioned media from keratinocyte cultures and demonstrated that the function of cultured LC was dependent

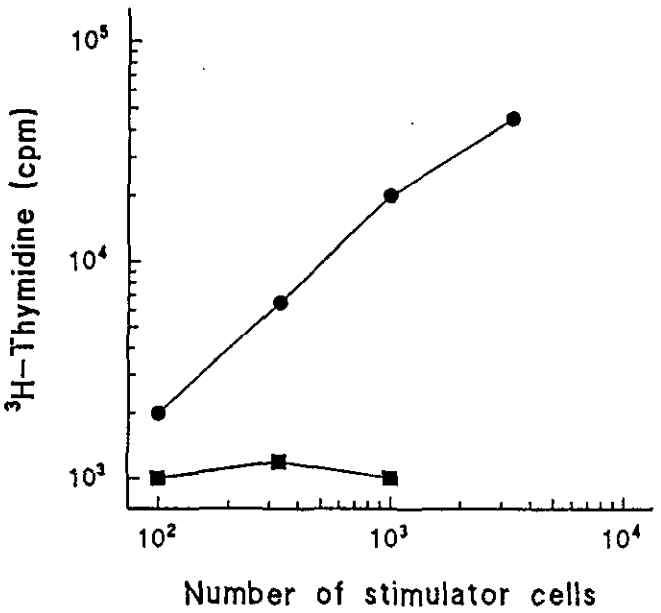

FIGURE 3. Changes in accessory function of Langerhans cells after culture with keratinocyte-conditioned media and other conditioned media. Epidermal suspensions cultured with keratinocytes (●) or L-cell (■)-conditioned media. After Witmer-Pack et al. (*25*).

on factors found in the medium of keratinocyte cultures, as well as in stimulated macrophages. The keratinocyte-conditioned medium caused LC to become larger and exhibit many cytoplasmic processes and veils, and their stimulating capacity in the primary MLR increased 20-fold or more (Fig. 3). The increase in LC stimulatory capacity was shown to be distinct from the maintenance of LC in a viable state or the level of class II products expressed. The factors in the media were identified as GM-CSF and IL-1 and further studies with rGM-CSF and rIL-1 demonstrated that IL-1 alone had no effect, but further enhanced the stimulatory activity when combined with GM-CSF. GM-CSF does not increase the function of mature LC or splenic dendritic cells and thus appears to be a differentiation signal that can mobilize dendritic cells from a weakly active, nonlymphoid tissue and turn them into potent immunostimulatory cells. These of course can be released in the absence of sensitized T-cells. A recent publication (*27*) has shown that rat peritoneal macrophages can be activated to produce GM-CSF and that macrophage-conditioned medium containing IL-1 and GM-CSF enhances the accessory activity of dendritic cells.

Although the studies described above do not specifically relate to the lung, the important point is that GM-CSF and IL-1 are cytokines produced by non-T-cells such as macrophages and keratinocytes. Because alveolar macrophages are among the first cells to encounter antigens deposited in a nonlymphoid environment, they may be critically important in modulating the function of dendritic cells. The alveolar macrophage would either migrate across the alveolar epithelial barrier or cytokines produced by the macrophage could perfuse into the interstitium, thus activating the dendritic cell population. Most studies on cytokine production by activated alveolar macrophages have measured the production and release of IL-1, TNF, and prostaglandins. There is an urgent need to study the capacity of alveolar macrophages

to express GM-CSF under different conditions of exposure. These of course can be released in the absence of sensitized T-cells.

Let us therefore test the hypothesis that the alveolar macrophage plays two important immunoregulatory roles. During the afferent limb of the immune response they release cytokines critical for the production of functionally active dendritic cells and may also pass on the processed antigen for attachment to the surface of the dendritic cell, whereas during the efferent limb of the local immune reaction they respond to products of sensitized T-cells such as INF and IL-4 to interact with T-helper cells in an antigen-specific, MHC-restricted manner or develop cytotoxic activity. The alveolar macrophage would thus control both induction and regulation of cell-mediated immunity in the lung by virtue of two different mechanisms.

The experiments performed by this laboratory were funded by the UK Health and Safety Executive to whom our thanks are due.

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