EFFECTS OF IMMUNOPOTENTIATING AGENTS ON ALVEOLAR MACROPHAGE PROPERTIES

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Abstract—Infectious respiratory diseases in man and in domestic animals are characterized by the presence of a large number of different microorganisms: viruses, bacterias, mycoplasmas. It is therefore necessary to stimulate non-specific defense mechanisms in the lung and especially alveolar macrophages (AM). These cells, located in the alveolar air-spaces, play a major role in the lung clearance mechanisms and exert antibacterial, antiviral and antitumoral activities. Activation of alveolar macrophages was studied *in vitro* with lipopolysaccharide (LPS), lymphokines or mycobacterial derivatives (MDP). Rodent alveolar macrophages were rendered cytotoxic by *in vitro* exposure to LPS, free MDP or liposome-encapsulated MDP derivatives. *In vivo*, intravenously administered liposomes containing lipophilic MDP derivatives induced cytotoxic alveolar macrophages and protected mice against the development of pulmonary metastases.

Key words: Alveolar macrophages, lung, macrophage activation, LPS, MDP, liposomes

Résumé—L'étiologie infectieuse des maladies respiratoires de l'Homme et des animaux domestiques se caractérise par la présence simultanée de nombreux agents pathogènes: virus, bactéries, mycoplasmes. De ce fait, il convient de rechercher les moyens susceptibles de stimuler les défenses *non spécifiques* du tractus pulmonaire et, parmi celles-ci, les fonctions du macrophage alvéolaire. Cette population cellulaire, majoritaire dans l'espace bronchoalvéolaire, participe, en effet, à l'épuration du poumon et à la résistance aux infections pneumotropes, tant par ses capacités phagocytaires et bactéricides, par ses fonctions cytotoxiques antivirales que par son aptitude à produire de l'interféron. L'activation du macrophage alvéolaire a été recherchée *in vitro* à l'aide de lipopolysaccharides (LPS), de lymphokines, ou de dérivés mycobactériens, tels que les muramylpeptides (MDP...). Chez les rongeurs, les fonctions cytotoxiques du macrophage alvéolaire sont activées *in vitro* par le LPS, par du MDP libre ou encapsulé dans des liposomes. L'injection intraveineuse d'un dérivé lipophile du MDP encapsulé dans des liposomes entraîne l'activation des fonctions cytotoxiques des macrophages alvéolaires et protège les souris contre le développement de métastases pulmonaires.

Mots-clefs: Macrophages alvéolaires, poumon, activation macrophagique, LPS, MDP, liposomes

INTRODUCTION

In man as well as in domestic animals, there is a high incidence of respiratory diseases, including infectious and tumoral processes. If we consider lung infections, it is striking to notice that a large number of different microorganisms are encountered. For instance, several pathogens are associated with pneumoniae observed in 30–65% of pigs in France: pseudorabies and influenza viruses [1], *Pasteurella multocida, Bordetella bronchiseptica, Haemophilus parasuis* and *Mycoplasma hyopneumoniae* [2]. The lung is also the most frequent site for growth of metastatic tumor cells and its is therefore reasonable to assume that non-specific immune mechanisms are to play a major role in lung defense against multiple microorganisms and neoplastic cells. Among the various parameters which contribute to protect the lower respiratory tract, alveolar macrophages (AM) are

recognized as the major cellular defense mechanism. However, although numerous data exist concerning the effects of immunomodulators on peritoneal macrophages and on blood monocytes there is a more limited literature devoted to studies on AM activation by immunopotentiating compounds.

In the present paper, we will describe briefly some particular features of alveolar macrophages and thereafter review several recent contributions about the *in vitro* and *in vivo* effects of immunomodulators on AM activities.

ORIGIN, POST-NATAL DEVELOPMENT AND MAIN FUNCTIONS OF ALVEOLAR MACROPHAGES

AM are the resident mononuclear phagocytes of the lung. The study of these cells became possible when lung washing techniques were developed in man [3] and in animals [4, 5, 6]. AM are located in the alveolar-air spaces, at the air-tissue interface, and are therefore in direct exposure to inhaled microorganisms, particles and air pollutants.

AM derive from lung capillary blood monocytes [7] which themselves arise from bone-marrow precursor cells. Direct evidence for a bone-marrow origin of human AM was obtained in studies of bone-marrow transplantations: after engraftment of male bone-marrow into female patients, the presence of Y body in AM collected from the patients indicated that AM were of donor (male) origin [8].

Several experiments indicate that AM population appears just before or shortly after birth: by lung washing at sequential times during animal development, the total number of rabbit AM recovered was found to increase markedly before birth [9]. This apparent influx of lung macrophages continued during the first week of life and stopped by 4 weeks after birth, a time at which the total AM number was identical to that of adults. During the same early period of life, a progressive maturation of rabbit AM was described, including morphological differentiation [9] and increased production of superoxide [10]. Similar results were obtained in the porcine species in which AM gradually appear during the first week of life [6]. This influx is under environmental influences since the total number of cells recovered by lung washings is much lower in germ-free piglets than in specific-pathogen free animals [11].

Many other aspects of alveolar macrophages were recently reviewed [5, 6, 12, 13] and some of the functions involved in non-specific immune mechanisms in the lower respiratory tract are summarized in Table 1. The major role of AM in the clearance of bacteria from

Table 1. Functional properties of alveolar macrophages

(1) *Cell interactions with bronchoalveolar lymphocytes:*
—Stimulatory or inhibitory effects on lymphocyte proliferation [14]
— Production of Interleukine 1 [15, 16].

(2) *Antibacterial properties:* phagocytosis, bactericidal effects, superoxide production [13]

(3) *Antiviral properties:*
—Interferon production following infection by paramyxoviruses [17] Coronaviruses [18] or herpesviruses [19]
—Lysis of virus-infected cells [20]

(4) *Antitumoral properties: cytostasis and cytotoxicity* [21]

the lung has been clearly defined by Green and Kass [22] who exposed mice to aerosols of ^{32}P-labeled bacteria. They found that the number of viable bacteria declined much more rapidly that the total lung-associated radioactivity and that inhaled bacteria were localized within AM implying that the bactericidal action of macrophages predominated largely over the mechanical removal process of the mucociliary apparatus.

IN VITRO EFFECTS OF IMMUNOPOTENTIATING AGENTS

The possible *in vitro* activation of AM was studied with three main groups of immunomodulators: lymphokines, endotoxins and mycobacterial compounds.

Rat AM were rendered tumoricidal by incubation with supernatants rich in Macrophage-Activating-Factor (MAF). Moreover, when MAF was encapsulated in liposomes, it was 16,000 times more active that soluble (free) MAF [23].

Incubation with lipopolysaccharides (LPS) increased antitumor cytotoxic activities of rat AM [21, 23] but decreased human AM phagocytic properties [24]. LPS increased the production of Interleukin 1 by porcine AM but was without significant effect on Interferon production, phagocytic and cytostatic activities of porcine AM [16].

Muramyldipeptide (MDP), a synthetic adjuvant which mimics mycobacterial cell wall components, is also able to induce cytotoxic rat AM. MDP-activated AM could lyse syngeneic, allogeneic and xenogeneic tumor cells but not normal cells [25]. Dose–response experiments showed that liposome-encapsulated MDP rendered AM tumoricidal at concentrations of approx 4000 times lower than free MDP. A lipophilic MDP derivative, muramyl dipeptide–glyceryl dipalmitate (MDP–GDP) was recently shown to induce mouse AM-cytotoxic activity [26]. MDP–GDP incorporated into liposomes was 7000-fold more effective than free MDP. Moreover, MDP–GDP incorporated into freeze-dried liposomes, which enable identical batches to be prepared and kept for long-term storage, were 50,000-fold more effective than soluble MDP [26]. Finally, free MDP was also shown to induce Interleukin 1 production by porcine AM without effects on other activities tested [16].

IN VIVO EFFECTS OF IMMUNOPOTENTIATING AGENTS

Murine AM collected 24 h after intravenous injection of lymphokines (MAF) encapsulated within liposomes were shown to be tumoricidal *in vitro* [27]. Interestingly, the degree of macrophage activation depended upon the route of liposome administration since AM were activated by intravenous injection but not by intraperitoneal injection of encapsulated MAF [27].

Mycobacterial preparations were also used *in vivo* to activate AM. Intratracheal injection of BCG could activate mouse AM [28] and protect mice against influenza virus intranasal challenge [29]. Intravenous administration of BCG or Complete Freund Adjuvant (CFA) induced, in 2–4 weeks, an influx of rabbit [30] and porcine [31] AM. CFA treatment increases lung clearance mechanisms [32] but the phagocytic capacity of collected AM after treatment was not modified [30, 31, 33]. CFA-treated AM have an increased lysozymal content [34] and Fc receptors with a higher affinity [35]. However, intravenously administered mycobacteria induce interstitial pneumonia and granulomatous reactions [31, 36, 37] which obviously hampers their use.

In fact, a recent break-through in this area was obtained with the demonstration that intravenously administered liposome-incorporated MDP derivatives could activate AM and reduce the pulmonary metastatic burden of mice carrying tumors [26, 38, 39]. The use of liposomes to deliver MDP has been shown to result in a considerable enhancement of AM tumoricidal activity compared to free MDP and the main advantage for liposomal MDP is that it could act within macrophages, following its release from internalized liposomes [27]. One day following intravenous injection of freeze-dried liposomes containing 10 μg of lipophilic MDP–GDP, harvested mouse AM exhibited a markedly enhanced cytotoxicity. Freeze-dried liposomes were localized in the lungs of normal mice and the number of pulmonary metastases following injection of melanoma cells was significantly reduced whereas free MDP had no effects [26].

CONCLUSION

Alveolar macrophages play a major role in pulmonary defenses against infections and neoplastic cells. It is possible to activate AM *in vitro* as well as *in vivo*, especially when immunopotentiating agents such as MDP derivatives are encapsulated into liposomes. Following AM activation under similar conditions, some AM functions appear to be significantly enhanced (antitumor cytotoxicity, Interleukin 1 production) whereas some others are unaltered (phagocytosis). This could imply that AM can be activated only for a limited range of functions and that AM activation is a multistage process, whose effects could depend on the number or sequence of stimulating signals [40].

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