Releasability of Airway Macrophages in Bronchial Asthma

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Bronchial asthma has been defined as a bronchial disease inducing a reversible airway obstruction. Reed [1] proposed to name it: eosinophilic desquamative chronic bronchitis. This term is very interesting because it outlines the importance of inflammation in the pathogenesis of the disease. It has been assumed for a long time that the narrowed airways were preferentially located centrally, that is to say in the large bronchi. However, increasing evidence indicates that the deep lung is also involved in bronchial asthma: (1) it is now well known that many aeroallergens are able to reach the alveoli [2, 3]; (2) the airway obstruction is often located in the peripheral airways, especially in the chronic stages of the disease, as assessed by flow volume curves and additional methods such as the evaluation of ventilation/perfusion abnormalities [4], and (3) extensive studies of the bronchoalveolar lavage (BAL) fluid in asthmatics have demonstrated a lot of inflammatory cells and mediators in this fluid [5, 6].

Alveolar macrophages (AM) are the principal resident phagocytes in the bronchoalveolar lumen and could be observed in the central airways, as assessed by segmental bronchial wash [7]. Therefore, it can be proposed to name them airway macrophages.

In a very elegant and important study, Patterson et al. [8] demonstrated that bronchoalveolar cells obtained by BAL in sensitized monkeys and injected into the trachea of nonsensitized syngenic monkeys were able to promote bronchial asthma after specific inhalation challenge. Since AM accounted for about 90–95% of the cells recovered by BAL, a potential role for AM can be suggested.

We would like to review the lines of evidence indicating that macrophages play an important role in the pathophysiology of human bronchial asthma. (1)

AM are able to synthesize and release chemical mediators involved in the pathophysiology of bronchial asthma [9]. They can be activated in vitro by an IgE-dependent mechanism [10], phagocytosed particles (zymosan and opsonized zymosan) or soluble stimuli such as a chemoattractant peptide (formyl-methionyl-leucyl-phenylalanine) or a potent activator of protein kinase C (phorbol myristate acetate; PMA), mechanisms which appear to be relevant to bronchial asthma. (2) AM are activated in vivo in asthmatic patients as demonstrated by their enhanced capacity to be stimulated in vitro. This activation correlated with the severity of the disease (as assessed by either the Aas clinical score [11], or airway obstruction, or nonspecific airway

reactivity [12]. (3) AM play a central role in the immune response since they act in cooperation with lymphocytes to suppress the lymphoproliferative response [13].

The concept of releasability has been proposed for basophils and could be extended to AM. It can be defined as a cellular hyperreactivity, that is to say an in vitro increased response of the cell to one or several specific or nonspecific stimuli. It has been demonstrated for peripheral-blood cells, such as basophils [14] and neutrophils [15]. In the 1980s, this concept was extended to bronchoalveolar cells, mainly mast cells. They were observed in the BAL fluid and could be activated to release histamine after incubation with specific allergens [16]. Calcium ionophore A23187-stimulated BAL cells released histamine and prostaglandin D₂; the release of both these mediators was correlated. Net maximal histamine release was significantly higher in asthmatics than in healthy subjects [17]. The majority of these cells had morphological characteristics of mucosal mast cells, since they were stained by Alcian blue-safranin after fixation in

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Carnoy but not after fixation in formalin [18]. Histamine release was induced by stimulation with an anti-IgE antibody [18]. Kirby et al. [19] showed that the number of mast cells increased in the BAL fluid in bronchial asthma and that this number was correlated with the nonspecific bronchial hyperreactivity. In To-mioka et al.'s study [20] the histamine content of bronchoalveolar mast cells was decreased in asthmatics as compared to healthy controls, suggesting that these cells were degranulated with an increased releasability. Nowadays, this concept of releasability could be extended to other bronchial or bronchoalveolar cells which are implicated in the pathophysiology of asthma: eosinophils [21], lymphocytes [22] and bronchial epithelial cells.

An indirect approach to this problem could be achieved by the evaluation of mediators in BAL fluid [19], involving at least the cooperation between alveolar cells.

The BAL technique was developed in the 1970s and allows to obtain the bronchoalveolar cells and culture them. This technique can be used for research purposes in asthmatic patients.

According to the recommendations and guidelines proposed by the NHLBI [23] and ESP task group [24], the BAL technique can be used safely in mild asthma. Even in more severely affected patients whose FEV₁ can be as low as 50% of predicted values can BAL fluid be collected without adverse effect. BAL can also be performed after inhalation challenge [25] or local endobronchial provocation test [26].

Total cell count is identical in asthmatics and in healthy subjects, but it can be higher in patients with a high eosinophil count in BAL fluid. The AM number is also identical in asthmatics and controls. However, it must be taken into account that in patients with bronchial hypersecretion the filtration of lavage fluid to remove mucus can reduce cell counts with a modest loss of macrophages [27].

AM can be studied in vitro without any purification, in the presence of other bronchoalveolar cells, enabling experiments on cellular cooperation. However, most studies are performed after purification of AM by adherence during a 2-hour culture. After removal of nonadherent cells, AM are viable (> 95%) and pure (> 95%) without contamination by eosinophils nor mast cells [10]. After an overnight culture, AM viability was reported to be good in mild asthmatics [28], but was altered in more severe asthmatics as a consequence of eosinophil toxicity [29] due to increased concentrations of major basic protein (MBP) or eosinophil cationic protein (ECP) in BAL fluid [21, 25, 30].

To prove that AM releasability is a hallmark of bronchial asthma, two theories can be postulated: (1) AM of asthmatics are different from those of healthy subjects, and (2) AM releasability parallels symptoms.

(1) As regards differences in AM between asthmatics and healthy subjects, AM from asthmatics were shown to release higher quantities of mediators. Damon et al. [31, 32] demonstrated that AM from asthmatics generate higher quantities of leukotrienes as compared to normal healthy volunteers. However, these results are in contrast to those of Baiter et al. [28], who did not find any intrinsic differences in basal or ionophore A23187-stimulated arachidonic acid release and eicosanoid synthesis by adherent AM obtained from normal and asthmatic subjects. A different severity of asthma can mainly account for the varying results. AM are phagocytic cells and a respiratory burst is observed in stimulated AM in the presence of phago-cytosed particles (zymosan, opsonized zymosan). The release of oxygen species can be studied by luminol- or lucigenin-enhanced chemiluminescence (CL). Using these methods, it was demonstrated that AM are more activated in asthmatic patients [11, 12] and they could be stimulated via an IgE-dependent mechanism [33, 34]. However, conflicting results have also been obtained [35]. Luminol-enhanced CL seems to be the consequence of phagocytosed eosinophil peroxidase, and a direct correlation between the total number of eosinophils in BAL fluid and luminol-enhanced CL has been described in asthmatics [11].

By comparison with eosinophils, it has been hypothesized that AM activation corresponds to heterogeneity. Using Percoll density fractionation, AM from asthmatics were mainly recovered in the lower-density fractions (1.03 and 1.04 g/ml), whereas AM from normal subjects were in the higher-density fractions (1.07 g/ml). Electron-microscopic studies showed that low-density AM had morphological characteristics of activated cells [36].

(2) A correlation between AM releasability and asthma symptoms has been suggested by various studies. Bronchial asthma is best defined by clinical symptoms and its severity can be correctly assessed by clinical scores. Another main characteristic of asthma is bronchial hyperreactivity as assessed by histamine or methacholine inhalation challenge test.

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Kelly et al. [12] observed an increased metabolic activity of AM, and suggested that AM activity (lucigenin-enhanced CL) was directly related to the degree of airway responsiveness. In children, Ferguson and Wong [37] showed that bronchial hyperreactivity was closely correlated with increased counts of eosinophils and macrophages in BAL fluid and with the ratio of eosinophils to macrophages; this was not reported in adults by Kirby et al. [19]. In more severe asthmatics whose disease activity was assessed by the Aas clinical score, the peak of luminol-enhanced CL induced by opsonized zymosan was directly correlated with the severity of asthma [11]. Using a specific assay for γ -glutamyltranspeptidase, Damon et al. [38] and Chavis et al. [39] showed that AM γ -glutamyltranspeptidase activity was increased according to the local endobronchial inflammation assessed by an endoscopic score; this increased activity was correlated with the ability of AM to transform leukotriene C₄ (LTC₄) into LTD₄ and LTE₄ [38, 39]. A study of phosphatidylinositol turnover in AM from allergic asthmatics showed a continuous Li⁺-sensitive production of inositol monophosphate, indicating that the cells were continuously activated [40].

AM act in cooperation with other cells present in the bronchoalveolar wall and lumen. In asthma, other cells such as lymphocytes and eosinophils can be recruited and the cellular interactions could amplify the inflammatory processes.

(1) AM and lymphocytes: During a bronchial asthma attack, there is an influx of lymphocytes in the bronchial wall, as assessed by biopsy [22], and in the deep lung, as assessed by BAL [5]. In healthy subjects, the AM/lymphocyte ratio in BAL fluid is about 10/1, and it has been extensively proved that AM suppresses the concanavalin A and phytohemagglutinin lymphoproliferative responses. In allergic asthma, this suppressive activity was significantly decreased [13].

AM could interact with lymphocytes through the release of cytokines. Some conflicting results have been reported. Gosset et al. [41] did not find any increased generation of interleukin-1 in allergic asthmatics as compared to healthy volunteers; moreover, they found that anti-IgE-stimulated AM released higher quantities of an IL-1-inhibitory factor. In contrast, Pujol et al. [42] reported that in asthmatics AM released higher quantities of IL-1, as assessed by a biological assay.

(2) AM and eosinophils: Eosinophils play an important role in the pathophysiology of bronchial asthma.

They are recruited by activated cells and mediators into the bronchoalveolar lumen and release their own mediators (LTC₄, possibly 15-HETE, cationic proteins, oxygen species and peroxidase), thus amplifying inflammatory processes.

AM recruit and activate eosinophils mainly by platelet-activating factor, but also by LTB₄ and tumor necrosis factor. Incubation of eosinophils with AM supernatants isolated from asthmatic subjects followed by stimulation with calcium ionophore A23187 resulted in an enhancement of the capacity of eosinophils to elaborate LTC₄. The enhanced activity was heat- and trypsin-sensitive and was neutralized by a specific antibody to granulocyte macrophage colony-stimulating factor (GM-CSF). GM-CSF could play a role in the amplification of the eosinophilic inflammation in asthmatic airways [43]. Tonnel et al. [44] showed by CL that supernatants of cultured AM activated non-modense eosinophils to release oxygen species.

In the same way, AM were able to transform LTC₄ released by activated eosinophils and mast cells into LTD₄ and LTE₄ [39]. This transformation depended on the activation state of AM and its intensity correlated with the local inflammatory process. Moreover, AM were also able to transform 15-HPETE and 15-HETE released by eosinophils and epithelial cells into lipoxins and related compounds [45, 46].

On the contrary, activated eosinophils appear to be hypodense and release MBP and ECP in the bronchoalveolar lumen and in the bronchial wall. In the BAL fluid, the quantities of ECP assessed by a specific radioimmunoassay correlated with the clinical severity of asthma according to the Aas score [21]. In bronchial biopsies, ECP was evidenced by specific monoclonal antibodies (EG1 and EG2); it was present in the areas of the bronchial tree where desquamation of epithelial cells was the most prominent [21]. MBP can be released in the BAL fluid and AM can phagocytize MBP granules. These observations could explain the decreased viability of AM in a 24-hour culture according to the number of eosinophils [29].

AM releasability could be an excellent model to study the effect of various drugs in order to regulate and treat asthma. However, only few studies have been performed in asthmatics. Most of them were done *in vitro*. Nedocromil Na decreased the generation of LTB₄ and 5-HETE from AM in asthmatic patients [31]. Ketotifen inhibited in a dose-dependent manner the PMA-induced CL of AM in nonasthmatic patients [47]. *In vitro* it is well known that dexametha-

sone inhibits the release of arachidonic acid metabolites; however, treatment of healthy subjects with dexamethasone resulted in no significant inhibition of the release of these metabolites from macrophages triggered *ex vivo* [48]. This could be explained by a low diffusion of the drug in the deep lung. In healthy smokers, inhaled glucocorticosteroids decrease the synthesis and release of angiotensin-converting enzyme and fibronectin, but modify neither the generation of oxygen species nor the LTB₄ production [49]. In conclusion, between the various stimuli inducing an asthma attack and the target organ, i.e. the bronchi, resident cells such as mast cells and AM, but also epithelial cells, are stimulated to release mediators and induce the inflammatory cascade which leads to the airway obstruction. An increased mediator release could explain many mechanisms which are involved in the pathophysiology of bronchial asthma and justify much attention and therapeutic consideration. As it can be assumed that AM are involved in bronchial asthma through their releasability, using their main property, i.e. phagocytosis, could enable, the development of drugs capable of reaching and inactivating them, for example via liposomes.

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