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Functional Peculiarities of Monocytes Isolated from Patients with Bronchial Asthma: Respiratory Burst and Lipid Peroxidation

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UDC 616.248-07:[616.155.33+616.153.915-39

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 118, № 12, pp. 585-588, December, 1994
Original article submitted May 20, 1994

The capacity of monocytes obtained from patients with bronchial asthma for a "respiratory burst" in response to phorbol myristate acetate and arachidonic acid was studied, the level of lipid peroxidation in the monocytes of asthmatic patients during the disease onset in comparison with healthy donors was determined, and lipid peroxidation activation in the plasma membranes in response to phorbol myristate acetate and arachidonic acid was studied. The generation of $O_2^{\cdot-}$ by the bronchial asthma patients' and healthy donors' monocytes after stimulation with phorbol myristate acetate was similar. Arachidonic acid induced no generation of $O_2^{\cdot-}$ by donor monocytes; however, upon incubation of patient monocytes with arachidonic acid the respiratory burst was markedly enhanced, and the quantity of $O_2^{\cdot-}$ generated during 15 min was almost 6-fold greater than upon stimulation with phorbol myristate acetate. The lipid peroxidation level in donor and patient monocytes differed significantly. A similar potentiation of lipid peroxidation in response to phorbol myristate acetate was observed in both groups; incubation of cells with arachidonic acid induced a greater increase in lipid peroxidation, which was similar for both groups, the increase in lipid peroxidation products in response to arachidonic acid being significantly higher than in response to phorbol myristate acetate. It can be assumed that the considerable enhancement of the oxygen response of the monocytes of the bronchial asthma patients indicates the activation of mononuclear macrophages and their participation in the pathogenesis of the disease.

Key Words: *bronchial asthma; monocyte; lipid peroxidation*

Different cell types are involved in the pathogenesis of bronchial asthma (BA), and the eventual manifestations of this pathology depend on their interaction [11,13]. Numerous studies have shown that local inflammation in asthma is accompanied by activation of the alveolar macrophages [5], infiltration of the inflammation focus by granulocytes, and their activation [11], which leads to the development of hypersensitivity of the respiratory pathways. The role of mononuclear phagocytes in the pathogenesis of BA has received much less attention. An increase in the aggregation activity of monocytes obtained from BA patients in response to arachidonic acid (AA) was demonstrated in our

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0007-4888/94/0012-1276$12.50

laboratory and a correlation between the severity of the allergic reaction and the aggregation parameters was also established [1]. The objective of this work was to study other parameters of the functional activity of monocytes from BA patients. The capacity of monocytes for a respiratory burst in response to phorbol myristate acetate (PMA) and AA - soluble activators of protein kinase C (PKC) - was investigated. Since there is a relationship between the functional activity of phagocytizing cells and the lipid peroxidation (LPO) level in their plasma membrane [15], the LPO level was determined in the monocytes of patients during the disease onset in comparison with healthy donors, and LPO activation in the plasma membrane in response to PMA and AA was also studied.

MATERIALS AND METHODS

Five male patients with BA aged 28-47 years were included in the study. The disease was exacerbated in all the patients. Three of them had infectious-allergic BA and two had atopic BA with hypersensitivity to common allergens and positive skin tests. Seven healthy volunteers (men aged 20-40 years) served as the control.

Monocytes were isolated by the method of Boyum [3]. The respiratory burst was assessed by the generation of the superoxide anion radial $O_2^{\cdot-}$, which was measured by the superoxide dismutase-inhibited reduction of ferrocytochrome C [8]. The cells were stimulated with PMA (Sigma) in a final concentration of 1 μg/ml and AA (40 μM, Fluka). Monocytes were incubated in Hanks' solution with the stimulators for 15 min at 37°C, and the reaction was then stopped in an ice-cold bath. The cytochrome absorbance (550 nm) with (50 μg) and without superoxide dismutase (Sigma) was measured after centrifugation (10 min at 800 *g*). The amount of generated superoxide anion was calculated from the amount of reduced cytochrome C, using the extinction coefficient 21.0 $M^{-1} \cdot sec^{-1}$.

The baseline LPO in the monocytes was determined in a cell suspension (300 μl) which was frozen at -20°C immediately after cell isolation until use. For the measurements of induced LPO 15 μl of the stimulator (PMA or AA to the same final concentration as in the previous experiment) was added to 300 μl of the cell suspension, incubated for 15 min at 37°C, and rapidly frozen. LPO was assessed by the amount of TBA-active products (interaction with 2-thiobarbituric acid) by the modified spectrofluorimetric method of Yagi [19]. The LPO level was expressed in arbitrary fluorescence units calculated per 10^6 monocytes.

RESULTS

The generation of $O_2^{\cdot-}$ by monocytes of BA patients and donors was similar after stimulation with PMA. We did not detect $O_2^{\cdot-}$ generation by donor monocytes in response to AA, but when patient monocytes were incubated with AA, the respiratory burst rapidly manifested itself: the quantity of $O_2^{\cdot-}$ generated over 15 min was almost 6-fold greater than that during stimulation with PMA (Table 1). There were no statistically significant differences in the LPO levels in patients and donors. A similar LPO enhancement in response to PMA was observed in the monocytes of both groups: 64% in donor and 56% in patient monocytes ($p<0.05$). Incubation of cells with AA induced a still greater increase in the LPO intensity, which was similar in both groups: 179% in donors and 144% in patients, the increase in LPO products in response to AA being significantly higher than in response to PMA (1.5-fold, $p<0.05$, Table 1).

The respiratory burst in phagocytizing cells results from activation of the electron transport chain termed NADPH-oxidase. Although NADPH-oxidase has been intensely studied in many laboratories, the regulation of its activity is not understood completely. Undoubtedly, the stimulus-mediated conversion of phosphoinositides with the formation of diacylglycerol, a physiological activator of PKC, and inositol triphosphate, which stimulates the mobilization of intracellular Ca^{2+} from the endoplasmic reticulum, is involved in the

TABLE 1. Production of $O_2^{\cdot-}$ and LPO Products by Monocytes of Patients with BA

Parameter, experimental conditions		Patients with BA (n=5)	Control group (n=7)
$O_2^{\cdot-}$ (nmol/15 min/10^6), inducer PMA (1 μg/ml)		50.34±2.23	50.28±5.30
$O_2^{\cdot-}$ (nmol/15 min/10^6), inducer AA acid (40 μM)		289.05±1.91	0
LPO, rel. units:	baseline level	7.97±2.73	14.80±4.31
	inducer PMA	12.43±2.05*	24.40±6.34*
	inducer AA	19.46±2.89*°	41.35±7.05**°

Note. One asterisk indicates $p<0.05$, two asterisks $p<0.01$ compared with the baseline level of LPO in this group. A circle indicates $p<0.05$ compared with the PMA-induced LPO.

regulation of NADPH-oxidase [2]. PKC participates in the phosphorylation of the cytosolic component of NADPH-oxidase and translocation of cytochrome B_{558} from specific granules in the plasma membrane, where the association of the components of the electron transport chain and its activation occur [2,10]. However, the activation of PKC is only part of the process of signal transduction from the receptor with subsequent activation of NADPH-oxidase. There exists a Ca^{2+}-independent pathway of signal transduction and activation of the respiratory burst [18]. It is believed that activation of NADPH-oxidase requires both pathways of signal transduction, which are initiated by the agonists of the corresponding receptors. In both cases G-proteins are necessary, these subsequently dividing into Ca^{2+}-dependent and Ca^{2+}-independent pathways. The second pathway is instantaneous, which may be due to the changes occurring in the properties of the plasma membrane [7].

As a PKC ligand, PMA activates the first pathway of signal transduction from the receptor in the zone below the diacylglycerol and inositol triphosphate formation. The absence of differences between the intensity of the respiratory burst in the monocytes of donors and patients in response to PMA suggests that the first PKC-dependent pathway of NADPH activation does not change in BA patients. However, the manifold increase in the respiratory burst in response to AA attests to marked activation of NADPH-oxidase of patient monocytes. The mechanism of activation of NADPH-oxidase is not fully understood, but it is commonly believed that AA (like all other fatty acids and the detergent sodium dodecyl sulfate, SDS) induces structural transformations of the membrane-bound components of NADPH-oxidase and can interact with its hydrophobic zone, activating the enzyme "assembled" on the plasma membrane [4,6,17]. In contrast to other activators, AA has no induction period. Therefore, it is thought that AA acts at the most distal point, probably by affecting the lipid-protein interactions, changing the conformation or charge of the NADPH-oxidase components, and reducing the microviscosity of the microenvironment, which facilitates the entry of $NADPH_2$ into the electron transport chain of NADPH-oxidase [4]; the detergent-mediated activation occurs without the participation of PKC [17]. There is evidence that AA and other unsaturated fatty acids can directly activate PKC in a dose- and Ca^{2+}-dependent manner, though, in much higher concentrations (100-200 μM) than those used in our experiments. The investigation of the PKC subtypes showed that type II enzyme is activated by diacylglycerol even in the absence of Ca^{2+} but is weakly activated by AA, type I enzyme is activated by micromolar concentrations of AA, and type III respond to high concentrations of AA but only at an increased intracellular Ca^{2+} concentration [12]. Most of the above-mentioned data were obtained in experiments with leukocytes, but they can be used to interpret the processes occurring in monocytes, since the generation of $O_2^{\cdot-}$ by monocytes is similar to, though sometimes higher than, that in leukocytes. NADPH-oxidase of monocytes has the same structure but a lower specific activity [16].

In BA, the products secreted by activated alveolar macrophages and granulocytes (leukotriene B4 and platelet activation factor) activate monocytes [10,13]. Leukotriene B_4 and platelet activation factor, being the most important activators in BA [13], induce an increase in the intracellular Ca^{2+} in phagocytes. The increase in the Ca^{2+} concentration cannot by itself induce a functional response of the cells to these simulators; however, it is a promotor of subsequent activation. An increase in the intracellular Ca^{2+} in the monocytes of BA patients has been demonstrated [8]. It can be assumed that translocation of the cytosolic component of NADPH-oxidase and "assembly" of the electron transport chain in the plasma membrane occur upon activation in the monocytes of BA patients. Subsequent addition of AA *in vitro* induces activation of NADPH-oxidase and the generation of considerable quantities of $O_2^{\cdot-}$. In donor monocytes a concentration of 40 μM AA does not induce an oxidative response, since it is 2-2.5-fold lower than the optimal concentration required for respiratory burst activation in resting monocytes [4,6]. It can be assumed that at an increased concentration of the intracellular Ca^{2+} type I PKC in monocytes can be activated by the Ca^{2+}-dependent neutral protease calpain.

Changes of the membrane properties during the activation of phagocytizing cells have been reported by numerous researchers [11]. It cannot be ruled out that these changes reflect the second Ca^{2+}-independent pathway of intracellular signal transduction. We did not find any changes in the LPO level in patient and donor monocytes, which can affect plasma membrane fluidity. Moreover, stimulation of the functional activity of the cells by different inducers leads to an equal augmentation of the LPO products in donor and patient monocytes. The enhancement of LPO in response to PMA may be associated with the activation of phospholipase A_2. This enzyme is activated by

PKC [2,12], and the subsequent synthesis of AA metabolites is lipid peroxidation of AA catalyzed by lipoxygenase. The considerable augmentation of the TBA-active products upon stimulation with AA indicates that AA induces greater changes in the plasma membrane of phagocytizing cells than does PMA. This may confirm the detergent-like role of AA in the activation of NADPH-oxidase. The membrane transformations induced by exogenous AA occur to an equal degree in the monocytes of donors and BA patients, whereas a potent respiratory burst was observed only in patient monocytes. This finding confirms the assumption that the "assembly" of the NADPH-oxidase electron transport chain components occurs in the plasma membrane upon activation in patient monocytes and refutes the hypothesis of the activation of AA-sensitive PKC. In the latter case, the increase of LPO products would have been greater, since activation of PKC is accompanied by LPO enhancement in an activated cell. In addition, it should be noted that the LPO potentiation in the membrane of phagocytizing cells dose not result from the influence of generated free radicals, as it has been assumed by some researchers [9], but rather reflects the final step of intracellular signal transduction, the PKC-dependent pathway inducing 1.5- to 2-fold lesser changes in the membrane structure than the detergent effect of exogenous AA.

Undoubtedly, such a marked potentiation of the oxidative response in the monocytes of BA patients indicates activation of mononuclear macrophages and their involvement in the pathogenesis of BA.

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