ALLERGOLOGY

Evaluation of Changes in Secretion of Active Oxygen Forms by Polymorphonuclear Lymphocytes

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The increased NBT activity of leukocytes in immunized rabbits is found to be dependent on the antigen concentration and is probably related to the direct effect of antigen on the cells.

Key Words: *active oxygen forms and their secretion; leukocytes; allergens; Arthus phenomenon*

Polymorphonuclear leukocytes (PML) are known to take an active part in the development of immune complex injuries [3]. The generation of active oxygen forms (AOF), such as superoxide anion radical (SAR), singlet oxygen, etc., is one of the manifestations of activated PML phagocytosis [4,7]. The process of reduction of nitroblue tetrazolium (NBT) to dark-blue formazan (FR) is thought to correlate with the generation of AOF [5,12]. The reaction proceeds with the participation of the NADPH-oxidase enzyme system [10] and yields SAR, which reduces soluble NBT to insoluble intracellular grains of FR [6,9].

However, at present there are very contradictory data on AOF secretion by PML in the course of allergic processes [8,13]. In this connection, the aim of the present study was to assess by means of the NBT test the changes in AOF secretion in the course of immune complex reaction, such as the Arthus phenomenon in rabbits.

MATERIALS AND METHODS

Chinchilla rabbits were divided into two groups: control (10 animals) and experimental (14 animals). The experimental rabbits were subcutaneously injected with 2 ml normal horse serum (NHS) every 5 days at the same site on the back, while control rabbits received injections of isotonic physiological saline. An inflammatory reaction at the site of injection developed 12 to 16 days after the first injection of NHS to experimental rabbits and was the transformed after 25-30 days into necrosis with ulceration (4×4×1 cm) in the middle of the inflammatory focus. No changes were observed in the control group. In both groups of rabbits blood was drawn from the marginal ear vein on day 30 of the experiment, a leukocyte suspension was isolated, and the parameters of the NBT test were determined.

For preparation of the leukocyte mass the blood was mixed 5:1 with 6% Dextran-500 solution and incubated at 37°C for 30 min, after which the supernatant was centrifuged at 1000 g for 15 min. The supernatant was removed and 3-4 drops of distilled water were added to the cell pellet for hypotonic lysis of erythrocytes, and the remaining cells were resuspended in 5 ml Hanks medium (pH 7.4), recentrifuged, and resuspended in Hanks solution. The number of isolated PML was estimated with allowance for their viability.

The reaction of the reduction of nitroblue tetrazolium (NBT test) was performed after Okamura

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[11] with modifications developed by Gordienko [2]. Induced and spontaneous activity of PML was assessed using $BaSO_4$ [1] and Hanks medium, respectively. Photometry was carried out at 710 nm using a Spektromom 361 spectrophotometer. The results were expressed in optical density units per million PML.

RESULTS

The first experimental series was aimed at assessing O_2^- production during incubation of whole rabbit blood with a specific allergen (NHS) using the NBT test. To this end before the NBT test, 3 ml whole blood were incubated with 1 ml NHS diluted from 1:1 to 1:10,000 at 37°C during 1 hour. Blood samples incubated with Hanks medium served as a control. After the incubation the PML were isolated from each sample and their NBT reducing capacity was examined. Both the spontaneous and the induced NBT activity in the experimental group exceeded these in the control group, both without antigen and in the presence of various titers, the maximal difference being observed at 1:10,000. The data on O_2^- production in the course of phagocytosis, determined as the difference between the induced and spontaneous NBT activity, are presented in Fig. 1. No reliable differences between the control and experimental groups were found after incubation of the blood with high titers of NHS (1:1 to 1:1000), whereas at low titers (1:10,000) phagocytic NBT activity of PML in the experimental group differed reliably from that in the control group ($p<0.01$), suggesting a sharp increase of O_2^- production.

However, it remains unclear, whether this is a result of a direct action of antigen on the cells or caused by circulating immune complexes. In order to clarify this, we carried out the second experimental series. The NBT activity of PML preincubated with various dilutions of NHS was determined. The difference between the induced and spontaneous NBT activity is shown in Fig. 2. It was demonstrated that the increase in phagocytic activity of PML persisted also at low NHS titers. The effect was independent on whether a leukocyte mass or whole blood was incubated with NHS. The increase of O_2^- generation during phagocytosis after incubation of the PML with low concentrations of specific antigen apparently resulted from the direct effect of the antigen on PML.

The obtained results suggest that either PML directly interact specifically with antigen, or, due to the action of antigen on mononuclear cells, they produce a factor modulating the oxygen metabolism of PML. The data make it possible to propose the NBT test together with incubation of PML with either whole blood or low antigen titers as a method for determination of the sensitization antigen in patients with an immune complex type allergy.

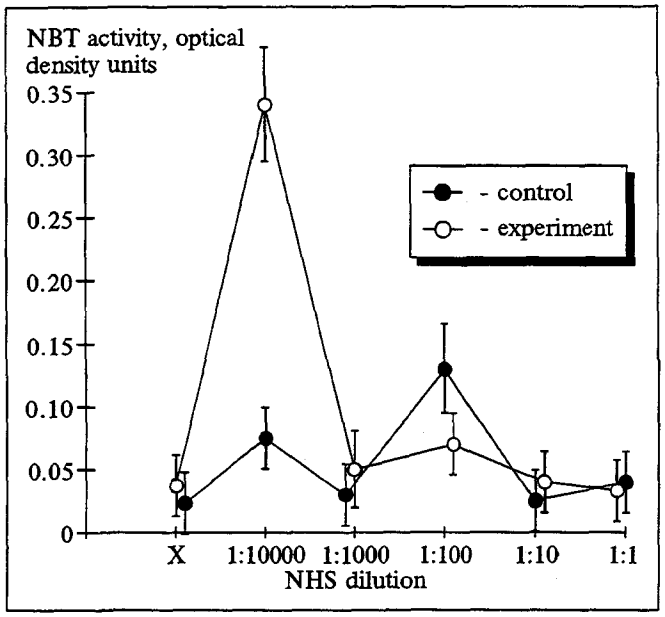

Fig. 1. NBT activity as a function of the dilution of NHS preincubated with whole blood. Here and in Fig. 2: dilution of NHS obtained in X incubation of the blood with Hanks medium: *1*) control; *2*) experiment.

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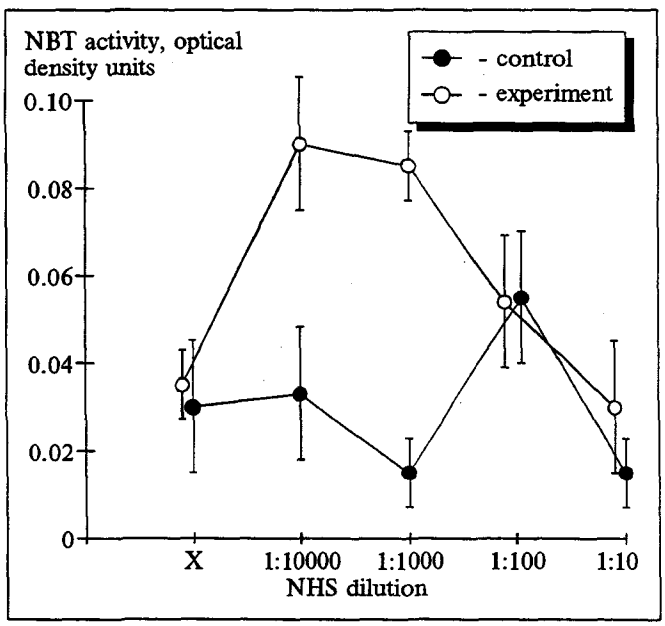

Fig.2. NBT activity as a function of the dilution of NHS preincubated with PML.

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EXPERIMENTAL GENETICS

Expression of the Human α-1-Antitrypsin Gene in Heterologous Mammalian Cells

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A genetic-engineering construction is developed containing the full-size cDNA of human α-1-antitrypsin, controlled by the promotor and enhancer elements from cytomegalovirus. It is shown that, after transfection with this recombinant DNA, it is properly expressed in heterologous animal cells.

Key Words: *α-1-antitrypsin; cell cultures; gene transformation*

Alpha-1-antitrypsin (AAT) is a protein inhibitor of serine proteases, the main function of which is the controlled suppression of elastase released by neutrophilic leukocytes in inflammatory foci [1]. A hereditary deficiency of AAT, which is one of the most widespread autosomal recessive diseases in man, most frequently results from mutations in the AAT gene, leading to disrupted secretion of this protein into the circulation and to its accumulation in the parenchymatous cells of the liver [1,2]. Specific methods of treatment of this disease should be based on substitutive introduction of normal AAT into the organism or on the radical genetic correction of the primary defect of the AAT gene. Either approach requires the development of genetic-engineering constructions providing for proper and effective expression of the AAT gene in heterologous cells. In this connection we constructed a recombinant DNA containing the full-size cDNA of AAT, controlled by the promotor

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