ALLERGOLOGY

On the Mechanism of Specific Inhibition of Stimulated Chemiluminescence of Polymorphonuclear Leukocytes in Allergic Processes

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Luminol-dependent chemiluminescence of polymorphonuclear leukocytes stimulated by barium sulfate is studied in blood samples preincubated with a specific allergen in experimental allergic processes. Specific inhibition of chemiluminescence is observed in blood samples obtained from sensitized animals. The degree of inhibition depends on the nature of sensitization and the stage of the allergic process and is related to myeloperoxidase inhibition.

Key Words: *luminol; chemiluminescence; myeloperoxidase; Arthus phenomenon; anaphylactic shock*

It is known that polymorphonuclear leukocytes (PML) are involved as effector cells in an allergic response, which may be accompanied by the secretion of active forms of oxygen (AFO), myeloperoxidase, and lactoferrin [5,6]. Measurement fo the chemiluminescence (CL) of leukocytes is an accurate method of assessing the secretion of AOF [7,8].

Previously we studied the effect of specific allergens on stimulated luminol-dependent CL of PML in allergic diseases and experimentally induced allergic processes [2,3]. We were the first to report the specific inhibition of stimulated luminol-dependent CL after the addition of allergen to blood obtained from allergic patients [1] and in the Arthus phenomenon in rabbits [2]. However, it was not clear what enzyme system is suppressed to cause the specific inhibition of CL.

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The aim of this study was to find the answer to this question using a guinea-pig model of anaphylactic shock and a rabbits model of the Arthus phenomenon.

MATERIALS AND METHODS

Leukocytes were isolated by precipitation in the presence of Dextran. Chemiluminescence was measured in a chemiluminometer equipped with a FEU-127 photomultiplier. The cell suspension (1 mln.) was introduced into the cuvette, luminol (0.25 ml) was added, and then Hanks balanced salt solution was added to a final volume of 3.25 ml. The spontaneous CL was measured. Then stimulator (0.25 ml of 2 mg/ml $BaSO_4$) was added to the suspension, and the induced chemiluminescence was recorded. Stimulated CL was calculated as the difference between induced and spontaneous CL and expressed in relative units. The activity of NADPH-dependent oxidase was determined by the

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method of Wymann *et al.* [9]. For this purpose 1 mln. PML, luminol (9 μM), sodium azide (90 μM), and horseradish peroxidase (9 U/ml) were added to the cuvette. Chemiluminescence reflected the amount of hydrogen peroxide formed due to activation of NADPH-dependent oxidase.

The experiments were performed on rabbits and guinea pigs. Chinchilla rabbits weighing 3-4 kg were divided into two groups: the Arthus phenomenon was induced in group I (n=12), while group II (n=37) served as a control. Group I rabbits were injected with normal horse serum (NHS) during a 1-month period (2 ml intracutaneously on the back, every 5 days). Control rabbits were injected with the same volume of Ringer's solution. All the experimental rabbits developed an inflammatory response at the site of injection by the 10th-15th day, and by the 25th-30th day ulcerations and hemorrhages appeared. There were no changes at the injection site in group II rabbits. On the 30th day blood was obtained from the marginal ear vein in both groups of animals, leukocytes were isolated, and CL was measured.

Guinea pigs weighing 200-300 g were divided into 2 groups: group I (n=26, experiment) and group II (n=20, control). Group I animals were sensitized by an intramuscular injection of NHS (0.2 ml), while control animals were injected with the same volume of normal saline. Two weeks later, half the animals of each group were injected with the resolving dose of NHS (0.5 ml), after which the guinea pigs of group I died from anaphylactic shock. Acute emphysema was identified post mortem. This dose induced no changes in the control animals. In the other half of animals of each group blood was taken from the heart, leukocytes were isolated, and stimulated CL was measured.

RESULTS

Blood obtained from group I guinea pigs was incubated with NHS (1:3000, experimental animals) or with Hanks balanced salts (control). Leukocytes were then isolated, and CL was measured. In the experimental samples stimulated CL was 27.7±6.85 arb. units, while in the control animals it was 64.6±11.91 arb. units (p<0.05). In the second control group under the same conditions CL was 58.4±14.88 and 60.1±13.22 arb. units. Luminol-dependent CL was thus inhibited after blood had been incubated with the specific allergen. This has been observed in pollinoses, atopic bronchial asthma, and drug-induced allergy in humans and in type III allergy in rabbits [1-3], suggesting that

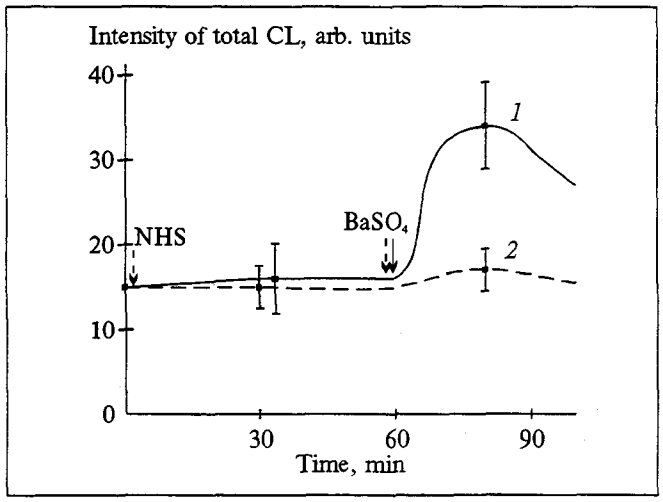

Fig. 1. Effect of preincubation of PML from a sensitized rabbit with NHS (1:3000) on chemiluminescence. *1*) CL of PML from sensitized rabbit after incubation with Hanks solution (control); *2*) CL of PML from a sensitized rabbit after incubation with NHS (experiment).

it is a versatile response of leukocytes to an allergic alteration.

It is known that luminol-dependent CL reflects the total amount AFO formed due to simultaneous activation of NADPH-dependent oxidase and myeloperoxidase. In order pinpoint the PML enzyme system that is inhibited by an allergen, we studied the activity of NADPH-dependent oxidase. The method was based on the ability of sodium azide to inhibit enzymes that metabolize hydrogen peroxidase (myeloperoxidase). At the same time, the intensity of luminol-dependent CL, reflecting the release of H_2O_2 by the NADPH-oxidase system, can be measured with the use of horseradish peroxidase, which is resistant to high concentrations of sodium azide. The activity of NADPH-oxidase was assayed in guinea pigs 2 weeks after their sensitization with NHS and in rabbits on day 30 of induction of the Arthus phenomenon. Blood was preincubated with NHS diluted 1:3000 or with Hanks balanced salts. The results are given in Table 1.

There was no inhibition of NADPH-oxidase after blood incubation with the specific allergen

TABLE 1. Determination of the Amount of Hydrogen Peroxide Released from PML in Rabbit and Guinea Pig Blood (M±m)

Animals	Stimulated intensity of CL in PML suspension, arb. units	
	incubation with NHC	incubation with Hanks solution
Rabbits (Arthus phenomenon)	40.3±10.5	41.3±9.7
Sensitized guinea pigs	90.7±12.0	100.3±14.3

(Table 1). If we take into account that superoxide radical formed due to NADPH-oxidase activity is rapidly dismutated (spontaneously and enzymatically) to hydrogen peroxide, which serves as a substrate for the myeloperoxidase reaction, it can be assumed that the specific inhibition of luminol-dependent CL is a consequence of myeloperoxidase inhibition or release of this enzyme from PML during incubation.

In order to find out whether CL inhibition is associated with the release of myeloperoxidase from PML during blood incubation with an allergen, we incubated PML (10^6 cells/ml) of rabbits (Arthus phenomenon, day 30) with NHS (1:3000) directly in the cuvette with constant stirring, and CL was measured in the presence of luminol. The stimulator ($BaSO_4$) was added after 1 h of incubation. Control cells were incubated in an equal volume of Hanks solution.

As seen from Fig. 1, the addition of NHS induced no peak of CL emission, while after 1-h incubation the stimulator-induced peak of CL was markedly inhibited (in comparison with the control). In intact rabbits the CL peak appeared after incubation with either Hanks solution or NHS. These results indicate that CL inhibition is not related to PML degranulation or AFO production at the moment of contact with an allergen followed by PML depletion, but rather to suppression of the ability of PML to secrete AFO. This finding may account for the reduction in completed phagocytosis in allergy to penicillin [4].

Thus, the specific inhibition of CL in a PML suspension in types I and III allergic reactions is propbably associated with the inhibition of PML peroxidase caused by the allergen.

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