

ELEVATION OF THE LYSOZYME LEVEL IN MACROPHAGES OF GUINEA PIGS SENSITIZED BY STREPTOCOCCAL ANTIGENS FOLLOWING INJECTION OF SPECIFIC ANTIGEN

V. A. Toder

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The lysozyme content in cells of the alveolar and peritoneal exudates of animals sensitized by streptococci is increased following intradermal injection of specific antigen into them.

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Intracellular enzymes, especially lysozyme, play an active role in disintegration of the cell wall of many microorganisms. The cell wall of the group A streptococcus, which is deficient in group C polysaccharide, is also sensitive to lysozyme [7]. Investigations [6, 9] have shown an increase in the content of intracellular lysozyme in the alveolar macrophages of rabbits sensitized with BCG. Other workers [8] found no increase in lysozyme in these animals.

The object of the present investigation was to determine the lysozyme level in alveolar and peritoneal macrophages during hypersensitivity of the delayed type to streptococcal antigens. The action of intradermal injection of specific antigen on the enzyme content in the lymphocytes of the peritoneal and alveolar exudates of sensitized and control animals was also studied.

EXPERIMENTAL METHOD

White-skinned guinea pigs were sensitized by injection of 5 billion bacterial cells of group A streptococci in Freund's incomplete adjuvant into the soft tissues of the paw. The material used for intradermal injection was 1) streptococcal allergen [1, 4] in doses of 2, 4, and 5 μg and 2) purified tuberculin in doses of 4 and 8 μg . The peritoneal exudate was obtained without preliminary stimulation by irrigating the peritoneal cavity of the animals with Hanks's solution [3, 10]. Alveolar exudate was obtained by a modified Myrvik's method [9]: the trachea was dissected, its upper part was clamped, and without removing the heart - lungs complex from the thorax, the lungs were irrigated with medium, liberation of the cells being stimulated by careful massage of the lungs through the chest wall. The washed out cells were counted in a Goryaev's chamber and disintegrated by rapid freezing and thawing 6 times. Lysozyme activity in the material obtained was determined from the zone of lysis in agar using an acetone powder prepared from *Micrococcus lysodeikticus* [2, 5]. The cell composition was studied in films stained by the Romanovsky - Giemsa method.

EXPERIMENTAL RESULTS

Marked reactions of delayed type were found in all animals when streptococcal allergen was injected 17-19 days after sensitization with streptococci; no such reactions occurred in the control animals. Examination of stained films from the liquid used to wash the lungs showed that 80-85% of cells were large mononuclears, 10-15% lymphocytes, and 1-5% polymorphs. A similar cell composition was found in the peritoneal exudate. Sensitization and subsequent injection of antigen had no effect on the cell composition of the alveolar and peritoneal exudates. Estimation of intracellular lysozyme in the control group of animals showed that its level in the alveolar macrophages was from twice to three times higher than that in cells of the peritoneal exudate. The lysozyme content in the alveolar lymphocytes of control animals and of sensitized animals not receiving intradermal injection of the allergen was more than 3 $\mu\text{g}/10^6$ cells in 15% of the animals. Similar results were obtained in the control animals receiving an injection of allergen

Laboratory of Streptococcal Infections, N. F. Gamaleya Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR G. V. Vygodchikov.) Translated from *Byulleten' Éksperimental'noi Biologii i Meditsiny*, Vol. 68, No. 9, pp. 75-78, September, 1969. Original article submitted July 15, 1968.

TABLE 1. Lysozyme Content in Lymphocytes of Alveolar and Peritoneal Exudate of Control and Sensitized Animals

Content of lysozyme (in $\mu\text{g}/10^6$ cells)	Control animals				Animals sensitized with group A streptococci					
			streptococcal allergen				streptococcal allergen		Tuberculin	
	No.	%	No.	%	No.	%	No.	%	No.	%
Cells of alveolar exudate: 3 μg and more less than 3 μg	2	14,2 \pm 9,6	2	15,4 \pm 10,4	3	15 \pm 8,2	12	63,6 \pm 11	3	18,7 \pm 10
	12	85,8 \pm 9,6	11	84,6 \pm 10,4	17	85 \pm 8,2	7	36,4 \pm 11	13	81,3 \pm 10
Total number of animals	14		13		20		19		16	
Cells of peritoneal exudate: 1.3 μg and more less than 1.3 μg	5	35,5 \pm 13,2	4	30,8 \pm 13,3	5	26 \pm 10,3	14	74 \pm 10,3	5	31,5 \pm 12
	9	64,5 \pm 13,2	9	69,2 \pm 13,3	14	74 \pm 10,3	5	26 \pm 10,3	11	68,5 \pm 12
Total number of animals	14		13		19		19		16	

and in animals sensitized with streptococci following intradermal injection of tuberculin. Meanwhile, the lysozyme content in 63.6% of the sensitized animals receiving intradermal injection of streptococcal allergen was more than 3 $\mu\text{g}/10^6$ cells (Table 1). The lysozyme content in 26-35% of the control and sensitized guinea pigs not receiving injection of antigen in the lymphocytes of the peritoneal exudate was more than 1.3 $\mu\text{g}/10^6$ cells. After intradermal injection of streptococcal allergen into sensitized guinea pigs, a lysozyme content of more than 1.3 $\mu\text{g}/10^6$ cells was found in 74% of the animals. Injection of tuberculin did not increase the lysozyme content in the cells. The differences between the group of sensitized animals receiving allergen and the control groups are statistically significant, the same animals exhibiting a higher lysozyme level both in the alveolar and in the peritoneal cells.

After intradermal injection of streptococcal allergen into guinea pigs sensitized with streptococci, the lysozyme content in the lymphocytes of both pulmonary and peritoneal origin thus increased. Since the cell composition of the alveolar and peritoneal exudates was unchanged, there was no question of cells with a higher lysozyme content having come from elsewhere. In this case there was evidently an increase in the lysozyme content in the lymphocytes of the sensitized animals as a result of the action of specific antigen. Injection of tuberculin into animals sensitized with streptococci did not raise the level of the enzyme. Meanwhile Cohn [6] and Myrvik [9] reported an increase in the lysozyme content in the alveolar macrophages of a rabbit immediately after sensitization with BCG. However, it is not clear from their papers whether the animals were preliminarily tested with tuberculin before determination of the enzyme content. The increase in lysozyme content in the animals sensitized with streptococci following intradermal injection of specific antigen was evidently associated with hypersensitivity of the delayed type and not with humoral antibodies. This conclusion is confirmed by the presence of marked reactions of delayed type in the experimental animals.

LITERATURE CITED

1. N. A. Verzhikovskii et al., in: Problems in Epidemiology and Immunology [in Russian], Book 2, Moscow (1936), p. 211.

2. K. A. Kagramanova and Z. V. Ermol'eva, *Antibiotiki*, No. 10, 917 (1966).
3. T. N. Maslova, *Zh. Mikrobiol.*, No. 7, 98 (1963).
4. K. Ando, K. Kurauchi, and H. Nishimura, *J. Immunol.*, 18, 223 (1930).
5. G. Ceriotti, in: *Proceedings of the 3rd International Symposium on Lysozyme*, Session 1, Milan (1964), p. 22.
6. Z. A. Cohn and E. Wiener, *J. Exp. Med.*, 118, 991 (1963).
7. M. McCarty, in: *The Streptococcus, Rheumatic Fever, and Glomerulonephritis*. Baltimore (1964). p. 3.
8. K. Mizunoe and A. M. Dannenberg, Jr., *Proc. Soc. Exp. Biol. (N. Y.)*, 120, 284 (1965).
9. Q. N. Myrvik, E. S. Leake, and S. Oshima, *J. Immunol.*, 89, 745 (1962).
10. E. Suter, *J. Exp. Med.*, 96, 137 (1952).