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EFFECT OF MEMBRANE DESIALYLATION BY NEURAMINIDASE ON THE PHAGOCYTIC ACTIVITY OF NEUTROPHILS

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Abstract

Sialic acid or neuraminic acid, the terminal carbohydrate prosthetic group of glycoprotein component of the outer mammalian cell membrane, is believed to be the receptor site for tuftsin, the active polypeptide of leukokinin. Its removal by neuraminidase causes a reduction in the cellular electrophoretic mobility, in negative charge of the cell membrane and enhanced the phagocytic activity of phagocytes. In contrast, more recent finding indicated that bacterial neuraminidase abolished the response of polymorphonuclear neutrophils to the stimulation by tuftsin in serum. Our findings support the earlier findings that bacterial neuraminidase increased the phagocytic activity of polymorphonuclear neutrophils but not their bacterial killing capacity.

INTRODUCTION.

The outstanding role of polymorphonuclear neutrophils (PMNs) in body defence mechanism, as the microphage, is their capacity to phagocytize and degrade a variety of substances, particularly bacteria. From physical point of view, phagocytosis may be considered as consisting of two events. First, the phagocyte must made contact with the particle to be phagocytized, and then engulf it. The presence of sialic acid on the cell membrane is known to be the important factor for the maximal phagycytic activity of phagocyte. Weiss and asscoiates (1) had demonstrated

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that the phagocytic activity of monocytes were increased when they were treated with neuraminidase. The enhancement of phagocytic activity of these neuraminidase-treated cells was thought to attributed to their increased deformability related to the changes in the membrane charge due to loss of ionized sialic acid moieties (1, 2). In contrast, the most recent work by Constantopoulos and Najjar (3) indicated that treatment of PMNs with bacterial neuraminidases completely abolished stimulation of phagocytic activity by free tuftsin or by tuftsin bound to the carrier leukokinin molecule.

MATERIAL AND METHOD

The leukocyte - rich plasma samples were obtained from 5 healthy volunteers using heparin and dextran sedimentation technique (4). Fresh normal sera were used immediately or store at - 70°C no longer than 1 week. Neuraminidase from Vibrio cholerae was prepared by method described previously (5). Bacterial suspensions were prepared from the 18 hours broth culture (BHI broth) of coagglulase positive Staphylococcus aureus (6). PMN cells (approximately 1 x 10^5 viable PMNs) were incubated with or without neuraminidase (the final concentration was 42 units / ml. of the mixture) for minutes at 37°C. Phagocytosis was assayed in a total volume of 1.0 ml. of the Hank's balanced salt solution containing 1 x 10^6 viable PMNs and 2 x 10^6 Staphylococcus aureus in the presence of 10% fresh serum. Incubation was carried out at 37°C in a water - bath shaker at 15 agitation / minutes (6). Phagocytic activity was determined by examination of stained smaers of the sample removed at interval. The phagocytic index is the percentage of neutrophils ingesting bacteria. The leukocyte bactericidal activity was determined by counting the total number of viable bacteria in the samples using the pour - plate technique (6).

RESULTS

As shown in the Table I. below, there was no significant difference in the phagocytic activity between the neuraminidase-treated neutrophils and the untreated neutrophils during the first 30 minutes of incubation. When the contact time was allowed up to 120 minutes, the phagocytic index of the neuraminidase - treated neutrophils was increased significantly (p value of < 0.001). The bacterial killing capacity of both groups are the same.

TABLE I. : EFFECT OF NEURAMINIDASE ON PHAGOCYTIC INDEX OF PMNs

	30 minutes incubation		120 minutes incubation	
	+ Neuraminidase	Control	+ Neuraminidase	Control
Mean	53.00	38.00	62.60	34.00
S.D.	19.42	16.29	10.33	4.18
S.E.	8.68	7.28	4.61	1.86
p value	< 0.3		0.001	

COMMENTS.

Tuftsin, the active polypeptide cleaved from the parent plasma leukokinin molecule has recently been isolated and characterized by Najjar and associates (3, 7). It stimulates the phagocytosis, pinocytosis and motility of PMNs and macrophages. Their findings also indicated that membrane sialic acid is necessary for the maximal stimulation of phagocytic activity by tuftsin. Removal of the former by bacterial neuraminidase abolised the response of PMNs to tufisin. Membrane sialic acid may not be the ultimate receptor for tuftsin but it may simply binds the tetrapeptide (tuftsin) in order to provide a high local concentration in the vicinity of the ulimate protein receptor which then mobilizes the cell membrane for more effective phagocytosis (3). Noseworthy et al (8) have foud that simple phagocytosis of phagocytes was not affected by neuraminidase. Our results observed at 120 minutes of incubatien time indicated that neuraminidase enhanced the phagocytic activity of PMNs similar to those observed in phagocytes by Weiss and associates (1). The discrepancy of results obtained by varrious investigators may be due to the dose and purity of neuraminidase preparation, and the exposure time.

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