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FcμR ON NEUTROPHILS

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Fixation of an antigen on cells involved in protection against infection (and in other immunologic processes) is of decisive importance for the outcome of its interaction with the host. If on contact with a lymphocyte that cell can receive information, which is followed by a phase of specific immune response, it is only close contact between antigen and phagocyte which can lead to ingestion of the former without which its degradation cannot take place.

Micorbial antigens can bind with the surface of a phagocyte through various receptors. An important fraction of these receptors consists of those for Fc-fragments of immunoglobulines (FcR) and for complement (CR), through which the antigen–antibody or antigen–antibody–complement complex (EAC) is attached to the surface of the phagocyte (opsonization of antigen). Although these receptors themselves are without microbial specificity and are directed either against the corresponding class of immunoglobulins or against complement (C), the phase of immune complex formation (i.e., interaction between antigen and antibody) is specific, and this determines the orientation of interaction between immune complex and a phagocyte which has lost the ability to distinguish between "its own" and "another's." The property of the phagocyte of binding immune complexes creates unity of the cellular and humoral factors of antimicrobial defense.

The most active phagocytic cell is the polymorphonuclear neutrophil (PMN). It is this cell which first encounters the microbe in the blood stream and migrates quickly into a focus of inflammation. The presence of FcγR and CR on PMN of man and animals [3, 4, 8] and the absence of FcμR on them [5, 9] are generally accepted. However, it has been shown in a microbial system that IgM can also play the role of opsonins [7, 11, 14], i.e., they can bind antigen with the surface of PMN, and in the absence of C this is possible only through FcμR.

The aim of this investigation was to detect FcμR on PMN of man and animals.

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TABLE 1. FcμR on Human and Guinea Pig PMN (M ± m)

Object	Number of observations	Time of testing after beginning of isolation of cells, h	Hypotonic shock	Treatment with acetate buffer	Reactions			
					intact erythrocytes	EA_μ		
						no treatment	treatment with 2-mercaptoethanol	incubation with myeloma IgM
Man	10	18	— or +	—	4,7±0,43	17,5±1,1	6,6±1,1	5,7±0,8
Guinea pig	3	18	»	»	4,4±0,3	14,6±1,9	4,3±0,8	4,0±0,8
Man	7	2	+	+	5,1±0,5	22,3±2,1	5,7±0,6	3,6±0,5
	3	2	—	—	3,0±0,6	5,3±0,7	4,2±0,2	4,5±0,4
	3	2	Four times	—	1,0±0,4	5,3±0,7	3,3±0,7	3,0±0,8
	3	2	—	+	2,2±0,6	4,2±1,3	8,2±0,2	3,2±0,7
Guinea pig	3	2	+	+	1,2±0,4	13,1±0,7	1,5±0,2	1,7±0,6

EXPERIMENTAL METHOD

Altogether 17 patients with various suppurative conditions and six intact guinea pigs were investigated. The supernatant from heparinized human venous blood, after standing, was layered above a Ficoll—Verografin density gradient with a density of 1.077 g/ml and centrifuged at 400g for 40 min, after which mainly PMN were concentrated in the residue. Guinea pig PMN were obtained by precipitation of the cells from heparinized blood in 3% solution. To remove erythrocytes, the suspension of PMN was subjected to hypotonic shock in distilled water, followed by addition of hypertonic physiological saline.

FcμR was determined by a modified method in [5]: centrifugation at 100g and 0°C for 10 min, followed by incubation at 0°C for 15 min. Bovine erythrocytes, loaded with rabbit IgM against them, isolated by gel-filtration on a column with Sephadex G-200 [12], were used as diagnostic agent (EA_μ). The hemagglutinin titer was 1:16-1:64; a direct subagglutinating dose was used for sensitization (i.e., 1:18 or 1:70).

The reaction was carried out in triplicate, using two modifications: preliminary culture of PMN for 18 h in medium 199 with the addition of 10% absorbed calf embryonic serum (heated to 56°C) in an atmosphere of 5% CO_2 (hypotonic shock was not always used in this case), and treatment of freshly isolated PMN after hypotonic shock with acetate buffer solution, pH 4.0, followed by rinsing with phosphate buffer (the method of removing cytophilic antibodies was described in [16]). In the latter case the reaction was carried out on the same day (about 2 h after isolation of PMN). Intact bovine erythrocytes and erythrocytes sensitized with IgM, treated beforehand with 0.1 M 2-mercaptoethanol [14], were used as the controls; blockade of FcμR also was carried out by incubation of PMN with 200 μg/ml of myeloma IgM [14], provided by the Laboratory of Genetics of Bacterial Virulence, N. F. Gamaleya Research Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR. Viability of PMN after treatment on the same day was 90-96%, and after culture 80-90%.

After the test the system was fixed with glutaraldehyde and stained by Romanovsky's method. The significance of differences was estimated by Student's test.

EXPERIMENTAL RESULTS

The data given in Table 1 show that with both methods of conducting the reaction, on average 13-22% of cells with FcμR (up to 39%) may be present among PMN. Destruction of IgM by means of 2-mercaptoethanol or blockade of FcμR by myeloma IgM completely suppressed rosette formation. Culture of PMN for 18 h in medium without IgM gave the same results as their treatment with distilled water and removal of cytophilic antibodies followed by testing on the same day. In the last case both types of treatment were necessary (Table 1). The same results were obtained by culturing PMN in 10% AB serum (the content of PMN with FcμR for man and the guinea pig was 16 ± 1.2 and 18 ± 1.5% respectively).

The results agree with those for detection of FcμR on eosinophils after culture for 12-18 h [14], when it was found that up to 30% of these cells in man carry such receptors.

Failure of the standard methods to detect FcμR on PMN [5, 15] or weak results of reactions [1] may be due both to blockade of these receptors by IgM in mammalian sera to the high agglutinating capacity of IgM used to prepare the diagnostic agents, when dilution to subagglutinating doses, with titration in the usual way, deprives the sera of their activity

[15]. Removal of both these factors, just as culturing PMN in medium with a reduced IgM concentration, revealed the above-mentioned receptors, and this is confirmed by controls with 2-mercaptoethanol and myeloma IgM. It may be that the combined action of hypotonic shock and acetate buffer solution was suitable to detect T_{μ} immediately after isolation of the pure population of T cells.

The results are evidence that early antibodies can play an opsonizing role. This is essential for our understanding of the mechanism of defence against ubiquitous microbes because of their ability to stimulate formations predominatly of IgM under ordinary conditions [2, 6, 10].

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NONIDENTITY OF Lyt PHENOTYPES AND RADIOSENSITIVITY OF "EARLY" AND "LATE" MIF PRODUCERS RESPONDING TO H-2 ANTIGENS

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Macrophage migration inhibition factor (MIF) is a lymphokine produced by T lymphocytes in the course of the delayed-type hyerpsensitivity reaction and responsible for concentration and activation of macrophages in the zone of the immune response, which has not been studied in detail. Nevertheless, T cells which produce MIF, unlike other T subpopulations, have not yet been adequately characterized. These cells, induced by microbial antigens or soluble proteins [10], and also by mitogens [8], carry the T lymphocyte phenotype Lyt-$1^{+}2^{-}$. However, MIF producers are in fact heterogeneous. Their different variants differ in size and affinity for lymphoid organs [9], immunologic specificity [1], and sensitivity to drugs [2] and irradiation [11]. In all cases MIF producers have been investigated 1 week or more later. However, as early as 16-18 h after intravenous immunization with allogeneic cells T lymphocytes producing MIF and γ-interferon on repeated contact *in vitro* with the same alloantigen, appear in the recipient's spleen [3]. Activity of "early" MIF producers has been

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