C3b-MEDIATED CHEMILUMINESCENCE OF HUMAN LYMPHOCYTES

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Four principle types of receptors are known for derivatives of the C3-factor of complement: CR1, CR2, and CR3, and CR4 [5]. They differ in specificity, i.e., in the selectivity of binding C3b and its derivatives, and they are differentiated by a set of monoclonal antibodies and are unequally represented in the various blood cells. Lymphocytes, like other leukocytes, possess a complex profile of C3-receptors [3, 11]. The majority of CR-positive lymphocytes are B cells: They are CR1-labeled in 100% and CR2-labeled in about 50% of cases. CR3-rceptors are found in null lymphocytes: K- and NK-cells [4]. T lymphocytes react weakest of all with derivatives of the C3-factor [12]: CR1-receptors are represented in 15-25% of OKT-4 and OKT-8 cells [16]; in addition a small proportion of suppressor T cells express CR3 [8]. The functional role of C3-receptors of lymphocytes has not been studied in detail. There is some evidence that they regulate the sensitivity of lymphocytes to mitogens and antibodies, and participate in reactions of mixed cultures and antibody-dependent cytotoxicity [5, 10]. Besides observations on C3a- and C5a- dependent modifications of lymphocytes [6], this indicates the real importance of the component for the formation of lymphocytic reactions, and emphasizes the universality of mediator signals in the system of humoral-cellular (including opsonic) cooperation.

The aim of this investigation was to study luminol-dependent chemiluminescence (ChL) of human lymphocytes during interaction with opsonized and nonopsonized zymosan. In this case the opsonic effect is known to be achieved through the alternative complement cascade through fixation and proteolytic modification of the C3-factor [9].

EXPERIMENTAL METHOD

Mononuclears were isolated from venous blood taken from donors in a Ficoll-Verografin density gradient, washed twice, and resuspended (3×10^6/ml) in Hanks' solution (Hanks' solution without phenol red was used in all the experiments). The lymphocytes were freed from monocytes with verification by the nonspecific esterase test, and the cells were incubated in polystyrene Petri dishes for 60 min at 37°C. The number of esterase-positive forms among nonadherent cells did not exceed 0.003%, and morphologically, all the cells corresponded to lymphocytes, with viability in the trypan blue test of over 96%. Zymosan (Olaine, USSR) was heated for 30 min on a boiling water bath and suspended in phosphate buffer (0.15M, pH 7.2). For opsonization, 50 mg zymosan was incubated for 30 min at 37°C with 10 ml of a pool of normal human serum, diluted with phosphate buffer. The zymosan was thoroughly washed, suspended in 2M NaCl solution, and heated for 10 min on a boiling water bath to desorb noncovalently bound components of the serum [13]. After washing, the zymosan was suspended in Hanks' solution in a concentration of 100 mg/ml. Luminol-dependent ChL of the lymphocytes was studied by the method described previously [1]. Nonopsonized and opsonized zymesan was used in a final concentration of 10 mg/ml. Phytohemagglutinin ("Reanal," Hungary), in a concentration of 30 µg/ml was used as the positive control and Hanks' solution as the negative control. The results were subjected to statistical analysis by the usual methods, including Student's test.

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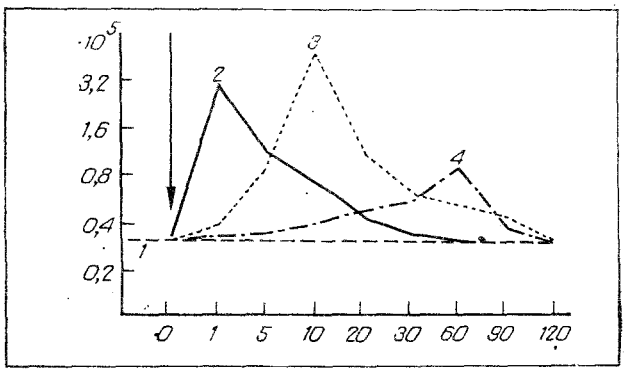

Fig. 1

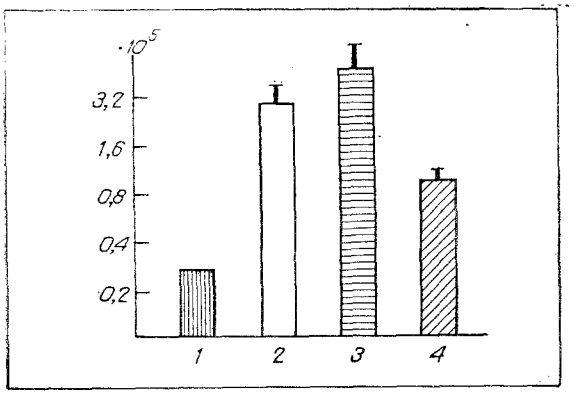

Fig. 2

Fig. 1. Kinetics of luminol-dependent ChL of lymphocytes stimulated by opsonized and nonopsonized zymosan. Abscissa, time (in min); ordinate, ChL (in cpm). 1) Negative control; 2) PHA (30 μg/ml); 3) opsonized zymosan (10 mg/ml); 4) nonopsonized zymosan (10 mg/ml).

Fig. 2. Maximal parameters of ChL of lymphocytes in experiments with opsonized and nonopsoniz i zymosan. 1) Negative control; 2) PHA (30 μg/ml, 1st minute; 3) opsonized zymosan (10 mg/ml, 10th minute); 4) nonopsonized zymosan (10 mg/ml, 60th minute). Results of negative control taken as unity.

EXPERIMENTAL RESULTS

The lymphocytes reacted (Fig. 1) to addition of both opsonized and nonopsonized zymosan. The reaction appeared after the end of the first minute and its kinetics differed. In the experiments with opsonized zymosan the peak of ChL was observed at the 10th minute; parameters of the reaction were 14-58 times greater (33.2 ± 4.9) that the negative control. Nonopsonized zymosan induced a slower rise of ChL with a maximum toward the 60 th minute; at the peak of the response ChL was 4-17 times more intensive (10.7 ± 3.4) than the negative control (Fig. 2), i.e., on average it was about 3.1 times lower ($p < 0.001$) than with opsonized zymosan.

In the reactions with phytohemagglutinin (PHA) the peak of ChL was observed toward the end of the first minute, in agreement with previous observations [1].

The majority of present-day methods of studying C3-receptors of lymphocytes and other cells are based on the principle of rosette formation with opsonized corpuscular objects. In our experiments we used a different, functional approach, based on assessment of metabolic stimulation of lymphocytes during interaction with opsonized zymosan.

It has been shown that C3b-factor binds covalently to objects activating complement by the alternative pathway, and on proteolysis, it is transformed successively into iC3b, C3b, g, C3d. Collectively these components determine the molecular profile of opsonization, by creating a basis for interaction with cells possessing CR-receptors; the remaining subcomponents (C3a, C3c, C3e, C3d) pass into solution and do not take part in the opsonic effect [9]. Under ordinary conditions of opsonization, the C3b- and iC3b-factors and, correspondingly, the Cr1- and Cr3-receptors of the cells are the most important.

Incubation with zymosan, treated with normal human serum under conditions of selective fixation of C3b and its derivatives, was accompanied by a burst of luminol-dependent ChL of the lymphocytes. The parameters reached their maximum toward the 10th minute, when they were 3-11 times greater than those in the experiments with native zymosan. Incidentally, potentiation of the lymphocytic reactions was observed without monocytes (adherent esterase-positive cells). The kinetics of the rise of ChL differed from that of PHA (maximum at the 1st minute) and also from that for nonopsonized zymosan (60th minute). Whereas in the first case, the effect of physicochemical characteristics of the stimulating agents (solubility of the PHA and the corpuscular nature of the zymosan) cannot be ruled out, in the second case, there are definite grounds for speaking of differences in the character of interaction

of the lymphocytes with the different ligand structures. It has been shown that β-glucan (one of the two main components of zymosan) binds with the α-peptide chain of CR3, mediating uptake of zymosan particles in experiments with polynuclear and mononuclear phagocytes [7, 10]. The second polymer, mannan, differs from glucan in not mediating the ingestive reaction, but stimulating luminol-dependent ChL of neutrophils [14]. It is difficult to extrapolate these data to experiments with lymphocytes, but, noting the analogies, it may be assumed that lymphocytes, utilizing CR3-receptors, receive β-glucans as a functionally meaningful signal. Reception of mannans through lectin-like structures of the plasma membrane may play the same role. It will be recalled that the possibility of lectin-mediated junctions in lymphocytes is supported by the evidence of their sensitivity to polyclonal mitogens of the lectin series, and also the binding of nonopsonized bacteria: the two types of reactions are unequally expressed for different lymphocyte subpopulations [14]. On the whole, if allowance is made for differences in the distribution of CR-receptors among different lymphocytes, it must be assumed that the luminol-dependent ChL which we recorded in the experiments with opsonized zymosan determined principally B lymphocytes (stimulation through Cr1). K/NK-cells, suppressor T cells expressing CR3 (reaction on account of β-glucan), or lymphocytes possessing mannan-specific lectins, could be involved in reactions with nonopsonized zymosan. Interaction with PHA, which binds with D-galactose radicals of membrane glycoproteins [12], may also take place to an even greater degree on a definite lectin-specific basis. This means that it is possible, with the aid of luminol-dependent ChL, to differentiate between variants of reactions connected with different categories of lymphocytes, and developing on a different molecular basis.

Considering that opsonization in the alternative cascade system is universal in character, and is found in many objects of microbial nature, and on malignantly transformed and injured tissue components [9], stimulation of lymphocytes by C3-opsonized substrates can be taken as a real reconnaissance and, possibly, effector mechanism in the immunity system. This is all the more likely if it is recalled that interactions of this kind can potentiate target destruction and can also induce the secretion of lymphokines, which potentiate phagocytic cells [2].

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