PRELIMINARY ACTIVATION OF LYMPHOCYTES WITH MITOGEN AS THE CONDITION FOR MANIFESTATION OF ABILITY OF Fab-FRAGMENTS OF NORMAL IgG TO INHIBIT LYMPHOCYTE TRANSFORMATION

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Inhibition of blast-transformation of human lymphocytes, induced by phytohemagglutinin (PHA), with the aid of $F(ab')_2$-fragment of rabbit IgG was studied. Preincubation of lymphocytes with $F(ab')_2$-fragments for 24 h at 37 and 4°C followed by change of medium and the addition of PHA did not affect the intensity of blast transformation. However, the $F(ab')_2$-fragment could depress blast-transformation of lymphocytes if removed from the medium 24 and 48 h after the beginning of stimulation. It can be concluded from these results that the $F(ab')_2$-fragment interacts only with cells activated by the phytomitogen and exhibits its inhibitory action on cells already bound with PHA.

KEY WORDS: γ-globulin; blast-transformation; lymphocyte; phytomitogens; $F(ab')_2$-fragment.

It was shown previously that Fab-fragments of homologous IgG have the ability to potentiate the immune response to thymus-dependent antigens nonspecifically [6]. Experiments in vitro also have shown that human and rabbit Fab- and $F(ab')_2$-fragments inhibit the blast-transformation reaction of human peripheral blood lymphocytes induced by phytomitogens and that, in this case, the action of the fragments was not due to their antigen-binding properties [2]. These observations suggested that these fragments of the IgG molecule can regulate the immune response through their influence, in particular, on the activity of thymus-dependent lymphocytes (T-cells). Since fragments of the $F(ab')_2$ type serve as intermediate products of immunoglobulin catabolism [1], the study of the mechanism by means of which such fragments influence activation of T-cells by antigen or mitogen could be of great importance for the elucidation of relations between humoral and cellular immunity.

The object of this investigation was to determine the stages of activation of the lymphocyte by phytomitogen which are influenced by the $F(ab')_2$-fragment.

EXPERIMENTAL METHOD

Rabbit γ-globulin was obtained from Serva. The $F(ab')_2$-fragments were obtained by the method in [5, 7].

All procedures used in isolating lymphocytes from donors' blood, culturing them in the presence of phytohemagglutinin (PHA) and $F(ab')_2$-fragment and determining the incorporation of thymidine-^{3}H into DNA were carried out as described previously [2,4].

In the experiments with a change of medium the cells were centrifuged for 20 min at 400g, the supernatant was poured off, and 1 ml medium 199 containing 5% inactivated human group AB serum and, in some cases, 12 μg PHA was added to the cells.

EXPERIMENTAL RESULTS

Preincubation of the lymphocytes with $F(ab')_2$-fragments for 24 h at 37 and 4°C followed by replacement with medium containing PHA did not affect the intensity of blast-transformation of the human lymphocytes. These findings indicate that firm interaction between the $F(ab')_2$-fragment and target cell does not take place

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TABLE 1. Effectiveness of Inhibition of Blast-Transformation Reaction by Fragments Depending on Duration of Contact between Fragments and Cells, M ± m

Expt. No.	Reagent	Radioactivity, cpm	Degree of inhibition, %	P
1	PHA (control)	47 665±3 002	—	—
2	PHA+F(ab')$_2$-fragment	24 318±2 836	49	$P_{1-2} \leqslant 0,05$
3	PHA with replacement of medium after 2 h by medium without PHA (control)	34 222±1 398	—	$P_{1-3} \leqslant 0,05$
4	PHA+F(ab')$_2$-fragment with replacement of medium after 24 h by medium without PHA and fragment	25 534±861	25	$P_{3-4} \leqslant 0,05$
5	PHA with replacement of medium after 48 h by medium without			
	PHA (control)	43 535±4 714	—	$P_{1-5} > 0,05$
6	PHA+F(ab')$_2$-fragment with replacement of medium after 48 h by medium without PHA and fragment	26 913±3 697	38	$P_{5-6} \leqslant 0,05$

and that the F(ab')$_2$-fragment does not change the ability of the intact lymphocyte to respond to PHA. It might be supposed that the F(ab')$_2$-fragment interacts only with cells activated by the mitogen. To test this hypothesis experiments were carried out in which the lymphocytes were kept in contact with the F(ab')$_2$-fragment for different time intervals after the beginning of stimulation, after which the F(ab')$_2$-fragment was removed and the lymphocytes subsequently cultured in medium not containing PHA or F(ab')$_2$-fragment. Altogether seven experiments were carried out on cells from different donors. The results obtained were similar. Those of one experiment are given in Table 1.

Analysis of the results shows, first, that changing the medium 24 and 48 h after the beginning of culture of the cells, with simultaneous removal of the excess of mitogen, had no significant effect on PHA-induced blast-transformation. These results are in agreement with those described in [3]. It also follows from Table 1 that the F(ab')$_2$-fragment could depress PHA-induced blast-transformation of lymphocytes if it was removed 24 and 48 h after the beginning of stimulation.

The experiments also showed that the inhibitory action of the F(ab')$_2$-fragment was the same whether the 300 μg of F(ab')$_2$-fragment was added simultaneously with the PHA or whether it was added in three separate fractions, each of 100 μg: the first with PHA, the rest 24 and 48 h after PHA (Table 2). The writers showed previously [2] that if the F(ab')$_2$-fragment is added 48 h after PHA, the same inhibitory effect is observed as after incubation of the cells with F(ab')$_2$-fragment for 72 h. It can be concluded on the basis of these observations that F(ab')$_2$-fragment interacts only with cells activated by phytomitogen and has an inhibitory action on cells which have already bound PHA even if its contact with these cells is limited to 24 h at any period of culture of the cells from 0 to 72 h.

In an attempt to explain this phenomenon it must first be remembered that as a result of activation of

TABLE 2. Effect of F(ab')$_2$-Fragment on Blast-Transformation Reaction when Added Simultaneously or in Repeated Small Doses to Culture of Peripheral Blood Lymphocytes, M ± m

Reagent	Radioactivity, cpm	Degree of inhibition, %
PHA (control)	67 957±4 870	—
PHA with simultaneous addition of 0.3 mg of F(ab')$_2$-fragment	31 559±5 371	54
PHA with addition of three doses of F(ab')$_2$-fragment, each of 0.1 mg: simultaneously with PHA and 24 and 48 h after PHA	25 513±3 239	63

lymphocytes, including T-lymphocytes, by antigen or mitogen, receptors may appear on the cell membrane which either are completely absent on resting cells or are expressed only in individual cells. These include receptors for the Fe-fragments of IgG [9] and also immunoglobulin receptors [8]. Although receptors for Fab-fragments have not yet been found on B- and T-lymphocytes by the use of traditional methods (for example, the indirect rosette-formation test), the possibility cannot be ruled out that receptors with this specificity do nevertheless appear on activated lymphocytes, but their affinity for the ligand is low. In that case visual observation could be insufficient for the detection of binding of Fab-fragments by the cell. One suggestion which the writers are currently testing is that the function of receptor for the Fab-fragment is performed by cytophilic antiglobulin-homoreactants [1], with low affinity for specific ligands. In particular, homoreactant activity may be a feature of immunoglobulin receptors which, like serum homoreactants, may also have low affinity for Fab-fragments [1].

LITERATURE CITED

1. A. Ya. Kul'berg, Immunoglobulins as Biological Regulators [in Russian], Moscow (1975).
2. A. Ya. Kul'berg, F. S. Baranova, G. U. Margulis, et al., Byull. Éksp. Biol. Med., No. 8, 178 (1977).
3. N. R. Ling, Stimulation of Lymphocytes [Russian translation], Moscow (1971).
4. A. Boyum, Tissue Antigens, 4, 269 (1974).
5. J. C. Cerottini, J. Immunol., 101, 433 (1968).
6. A. Ya. Kulberg (A. Ya. Kul'berg) et al., Immunology, 30, 715 (1976).
7. A. Nisonoff, F. C. Wissler, L. N. Lipman, et al., Arch. Biochem., 89, 230 (1960).
8. B. Pernis, J. F. A. P. Miller, L. Forni, et al., Cell. Immunol., 10, 476 (1974).
9. J. A. Van Boxed and D. L. Rosenstreich, J. Exp. Med., 139, 1002 (1974).