ABILITY OF Fab-FRAGMENTS OF NORMAL RABBIT AND HUMAN γ-GLOBULINS TO INHIBIT BLAST TRANSFORMATION OF HUMAN LYMPHOCYTES INDUCED BY PHYTOMITOGENS

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UDC 615.373.6:547.962.4.015.4:
616.155.321.02

Monovalent and bivalent Fab fragments from normal human and rabbit γ-globulins were shown to suppress blast transformation of human lymphocytes induced by phytohemagglutinin and concanavalin A. The pepsin $F(ab')_2$ fragments obtained from highly purified rabbit antidinitrophenyl antibodies possess similar properties. An inhibitory action of the fragments was observed when they were added to the culture both simultaneously with and 24 and 48 h after the mitogen. The results may mean that suppression of lymphocyte transformation by fragments of γ-globulins is not due to their composition with the mitogens for receptors on the target cells; activity of the Fab fragment is evidently determined by structures located outside the combining site of the antibody.

KEY WORDS: γ-globulin; blast transformation; phytomitogens; Fab fragment.

A previous investigation showed that $F(ab)_1$ fragments obtained from normal homologous IgG with the aid of pepsin can potentiate the immune response [2]. This effect has been shown not to be due to the ability of these fragments to interact specifically with the antigen used for immunization. On the basis of these findings, and also of the fact that the adjuvant effect of the Fab fragments was observed during the immune response to a thymus-dependent antigen, it might be supposed that these fragments can directly or indirectly affect the activity of T lymphocytes. One method of testing this hypothesis in experiments in vitro is to assess the action of Fab fragments on blast transformation of lymphocytes induced by phytohemagglutinin (PHA) and concanavalin A (con A), specific mitogens for T cells.

In the investigation described below the effect of fragments of human and rabbit γ-globulins, obtained with the aid of pepsin and papain, on blast transformation of human blood lymphocytes induced with the above mitogens was studied.

EXPERIMENTAL METHOD

Rabbit γ-globulin (Serva), freed from aggregated protein by gel chromatography on Sephadex G-200, and a commercial preparation of human measles γ-globulin (from the Moscow Scientific-Research Institute of Epidemiology and Microbiology) were used.

Rabbit antibodies against the dinitrophenyl group (anti-DNP antibodies) were obtained in the purified form from a hyperimmune rabbit serum by the method of Eisen et al. [5]. The degree of purity of the antibodies, determined by hapten–DNP-lysin binding [6], was 90%.

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(Presented by Academician of the Academy of Medical Sciences of the USSR P. A. Vershilova.) Translated from Byulleten' Eksperimental'noi Biologii i Meditsiny, Vol. 84, No. 8, pp. 178-181, August, 1977. Original article submitted December 13, 1976.

TABLE 1. Effect of Fab Fragments of Normal Rabbit and Human γ-Globulins on Blast Transformation of Human Lymphocytes Induced by PHA

Fragment	Concentration, μg/ml	Mitogen	Number of donors tested	Number of cases of significant inhibition ($P < 0.05$)	Degree of inhibition, % (mean value)
$F(ab')_2$ rabbit	200	PHA	25	21	61
Fab' ditto	200	PHA	3	2	60
Fab "	200	PHA	7	5	54
$F(ab')_2$ human	100	PHA	6	5	60
$F(ab')_2$ "	200	PHA	4	3	47
$F(ab')_2$ rabbit anti-DNP	200	PHA	3	3	74

To obtain $F(ab')_2$ fragments the human and rabbit γ-globulins were hydrolyzed by pepsin [8]. The $F(ab')_2$ fragments were isolated from the digest on Sephadex G-200 [4]. $F(ab')_2$ fragments were obtained in the same way from rabbit anti-DNP antibodies.

The monovalent pepsin Fab fragment of rabbit γ-globulin was obtained by reducing bivalent $F(ab')_2$ fragments with 0.1 M 2-mercaptoethanol followed by alkylation with sodium iodoacetate. The resulting preparation was further purified by chromatography on Sephadex G-200. The papain Fab fragment of rabbit γ-globulin was obtained by Porter's method [9]. Lymphocytes were isolated from donors' blood by centrifugation of a leukocyte suspension in a Hipac–Ficoll density gradient (d = 1.072) for 20 min at 800g [3]. Next, $2.5 \cdot 10^5$ cells were cultured in 1 ml medium No. 199 containing 5% inactivated calf serum or human group AB serum for 72 h at 37°C. To stimulate lymphocytes optimal concentrations of PHA (Reanal) and con A (Serva) in a concentration of 10 μg/ml were used. The preparations of the Fab fragments were added to the lymphocyte culture simultaneously with or at various times after the mitogen. Three hours before the end of cultivation 1 μCi of [^{3}H]thymidine was added to each tube. The cells were transferred to Synpore membrane filters (0.6-1.5 μ) and then treated successively with physiological saline and a 5% solution of TCA. The radioactivity of the dry samples was measured on a Mark II liquid scintillation radiospectrometer. The results were calculated on the basis of measurement of the radioactivity of samples from five parallel cultures.

EXPERIMENTAL RESULTS

After addition of bivalent and monovalent Fab fragments of rabbit and human γ-globulins obtained with the aid of pepsin and papain to a culture of human lymphocytes stimulated by PHA, a decrease in the uptake of [^{3}H]thymidine into DNA was observed compared with the corresponding controls. A similar effect was observed in the case of lymphocytes stimulated by con A. As Table 1 shows, this effect was significant for cells of most donors tested when the concentration of fragments was 200 μg. The degree of inhibition did not increase with an increase in the concentration of the fragment to 300 μg; with a decrease in its concentration to 100 μg no significant effect on the fragment on the level of label incorporation was observed. None of the preparations of Fab fragments used had a cytotoxic action, as could be judged from the test to assess the viability of the cells with the aid of Trypan Blue.

The phenomenon described above was not due to the effect of the fragments on transport of the labeled base into the cells. For instance, after addition of the $F(ab')_2$ fragment of rabbit γ-globulin to the lymphocyte culture immediately before [^{3}H]thymidine, no decrease in incorporation of the label was observed (Table 2). It was concluded from these results that Fab fragments from normal rabbit and human γ-globulin can inhibit blast transformation of human lymphocytes induced by PHA and con A.

Besides Fab fragments from normal γ-globulin, the effect of $F(ab')_2$ fragments obtained from highly purified rabbit anti-DNP antibodies on blast transformation also was studied. As Table 1 shows, fragments of these antibodies effectively blocked blast transformation of lymphocytes induced by PHA. The preparations of antibody fragments had no cytotoxic action on the lymphocytes.

The results of the experiments with fragments of anti-DNP antibodies mean that: 1) inhibition of blast transformation by Fab fragments is not due to the presence of impurities that could be present in the preparation of fragments from normal rabbit and human γ-globulin used; 2) the inhibitory action of the Fab fragment is not due to the presence of fragments of antilymphocytic or any other type of antitissue antibodies in the preparation used; 3) the property of the Fab fragments of inhibiting blast transformation is determined by structures located outside the region of the combining sites of the molecule.

TABLE 2. Dependence of Degree of Inhibition of Transformation of Human Lymphocytes on Time of Addition of $F(ab')_2$ Fragments of Rabbit γ-Globulin

Time of addition	Counts per minute (M ± m)	Degree of inhibition, %
Simultaneously with PHA	30 541±184	65
24 h after PHA	44 570±3834	49
48 h after PHA	44 891±5970	50
69 h after PHA		
Simultaneously with [^{3}H]-thymidine	75 962±3841	13
Control (PHA only)	87 404±7487	

A fact pertinent to the explanation of the mode of action of Fab fragments is that they can block blast transformation when added to the culture 24 and 48 h after PHA (Table 2). Under those conditions the $F(ab')_2$ fragment inhibited blast transformation just as effectively as when added simultaneously with the mitogen. Consequently, the inhibitory action of Fab fragments can be manifested even after binding of the mitogen and activation of the target cells by them. It can accordingly be postulated that inhibition of blast transformation by Fab fragments is due to their effect on metabolic processes in lymphocytes stimulated by the mitogen.

As has recently been demonstrated [11], the immobilized complex of antigen with $F(ab')_2$ fragments of rabbit antibodies partially inhibits blast transformation of mouse lymphocytes induced by PHA. These observations agree with the results of the present investigation and they confirm the conclusion that the structures responsible for the inhibitory properties of the Fab fragment are spatially separate from the combining site of the antibody molecule.

As has already been mentioned, Fab fragments of homologous IgG can potentiate the immune response to T-dependent antigens [2]. In the writers' view, there is a definite connection between this property of the fragments and their ability to inhibit blast transformation of T cells. When activated by antigens or by phytomitogens, T cells are known to produce several factors inhibiting antibody biosynthesis [7, 10, 12]. It may be that, by depressing the activation of T suppressors by antigen and thereby preventing the production of inhibitors of antibody formation by these cells, the Fab fragments are able to potentiate the response to T-dependent antigens.

The further study of the mechanism of action of Fab fragments on lymphocytes is of considerable interest because it has been reliably shown that such fragments can serve as intermediate products in the catabolism of γ-globulin [1].

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