EFFECT OF Fab-FRAGMENTS OF ANTIBODIES AGAINST SPLEEN CELL SURFACE ANTIGENS ON ANTIBODY FORMATION IN CULTURES OF VARIED DENSITY

A. A. Korukova, A. B. Kim,
O. S. Grigor'eva, and A. E. Gurvich

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Induction and subsequent development of antibody formation are known to be linked with intercellular interaction [1, 7]. Intercellular interaction also determines inhibition of proliferation in monolayer and high-density suspension cultures [4]. The writers showed previously that the primary immune response in high-density cultures is depressed, and this is evidently due to the presence of local intercellular interactions [2, 3, 5].

The aim of this investigation was to study how these interactions are affected by blocking of the largest possible number of surface receptors of lymphoid cells by antibodies.

EXPERIMENTAL METHOD

Experiments were carried out on splenocyte suspensions from C57BL/6 mice cultured for 4 days [2, 8] in the presence of water-soluble antigen from sheep's red blood cells (SRBC) [14]. The number of antibody-forming cells was determined by Jerne's method [11].

Antiserum against splenocyte surface antigens (SSA) was obtained by intravenous immunization of rabbits with a suspension of C57BL/6 mouse splenocytes. Five injections of 300×10^6 cells were given at intervals of 1 week. Blood was taken on the 7th, 9th, and 11th days after the last immunization. The resulting antisera (SSA) were pooled. The immunoglobulin fraction was isolated [6] from SSA, and hydrolyzed with pepsin [12, 15] to obtain Fab-fragments (Fab-SSA). The immunoglobulin fraction from donkey antiserum against rabbit IgG, supplied by the N. F. Gamaleya Research Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR, was used as the source of antibodies interacting with Fab-SSA (anti-Fab).

Attachment of Fab-SSA to the surface of mouse splenocytes was tested by direct or indirect radioactive methods [13]. For the direct method the Fab-SSA was labeled with ^{125}I [10]. In the indirect test ^{125}I-labeled donkey antibodies against rabbit IgG (obtained from the N. F. Gamaleya Research Institute) were used.

EXPERIMENTAL RESULTS

Direct (^{125}I-Fab-SSA) and indirect (^{125}I-anti-Fab) radioimmunoassay showed the presence of specific antibody activity in the Fab-SSA preparation. One of the 100-300 molecules present in the Fab-SSA preparation was found to bind specifically with the mouse splenocytes. The number of Fab-SSA molecules attached to the surface of one cell increased with an increase in concentration and reached $500 \cdot 10^3$.

After the properties of the Fab-SSA preparation had been determined its action was studied on antibody formation induced in cultures with different densities of suspension. Attempts were made to use Fab-SSA to block the surface of the cells in culture at different stages of the immune process.

Table 1 gives the results of an experiment in which Fab-SSA were added twice to the cultures — on the 2nd and 3rd days of incubation. A fourfold increase in density of the suspen-

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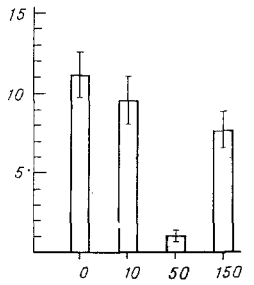

Fig. 1. Dependence of inhibition of antibody formation in dense cultures on dose of Fab-SSA on successive treatment of cells with Fab-SSA and Anti-Fab. Abscissa, Fab-SSA concentration (in μg/ml); ordinate, number of AFC per 10^6 living cells in dense cultures (in % of number of AFC per 10^6 living cells in optimal cultures with the same treatment). Concentration of anti-Fab 250 μg/ml. Results expressed in $M_a \pm m$.

TABLE 1. Antibody Formation in Cultures with Optimal and Increased Density on Addition of Fab-SSA

Group No.	Culture medium (2-4 days)	Addition of Fab-SSA (in μg)		Number of AFC per 10^6 living cells on 4th day in cultures ($M_g \pm mt$)	
		on 2nd day	on 3rd day	with optimal density	with fourfold increase of density on 2nd day
1, 2	Previous	—	—	1622 (1371—1921)	347 (326—369)
3, 4	»	15	15	1876 (1595—2206)	378 (296—483)
5, 6	Fresh	—	—	1867 (1282—1720)	324 (237—441)
7, 8	»	15	15	1645 (1341—2019)	310 (230—416)

Legend. Mouse splenocytes were cultured with antigen for 2 days in optimal initial density ($5 \cdot 10^6$ cells/ml). The cultures were then pooled, centrifuged, and resuspended in the same volume of medium (previous or fresh), so that the density of the suspension either was unchanged or was increased fourfold. Fab-SSA was added in a volume of 100 μl to the cultures (1 ml) after treatment on the 2nd day and again on the 3rd day. At the same times 100 μl of medium was added to the control cultures.

sion in the control cultures on the 2nd day of incubation led to marked inhibition of the increase in number of antibody-forming cells (AFC) (groups 1 and 2). The second addition of Fab-SSA did not affect the level of antibody formation either in optimal or in high-density cultures (groups 3 and 4). Absence of action of Fab-SSA also was observed when the medium was changed on the 2nd day for fresh medium in order to remove cell breakdown products, which could neutralize the Fab-SSA molecules (groups 7 and 8). Treatment of Fab-SSA cells at the beginning of incubation also as a rule did not affect the level of antibody formation in cultures of different density.

Attachment of monovalent Fab-fragments to cell receptors is known not to cause displacement of complexes already formed nor to induce "cap" formation. This could explain why Fab-SSA had no action in the present experiments. However, such displacement could be induced by antibodies against attached Fab-fragments [9].

TABLE 2. Strengthening Inhibition of Antibody Formation in High-Density Cultures by Consecutive Treatment of Cells with Fab-SSA and Anti-Fab

Group No.	Treatment with Fab-SSA (μg/ml)	Treatment with anti-Fab (μg/ml)	Initial number of cells in culture, 10^6 cells/ml	Number of AFC per 10^6 living cells on 4th day ($M_g \pm mt$)	K	%
1	—	—	5	906 (732—1121)		
2			20	142 (114—176)	6,4	15,7
3	50	250	5	919 (757—1117)		
4			20	9,3 (6,5—13,3)	99	1,0
5	50	—	5	1147 (843—1560)		
6			20	178 (122—260)	6,4	15,5
7	—	250	5	1339 (954—1879)		
8			20	215 (177—292)	6,2	16,0

Legend. K) Coefficient of inhibition of antibody formation in high-density cultures ($20 \cdot 10^6$ cells/ml) relative to optimal cultures ($5 \cdot 10^6$ cells/ml) for the same method of cell treatment; %) level of antibody formation (in %) in high-density cultures ($20 \cdot 10^6$ cells/ml) relative to optimal cultures ($5 \cdot 10^6$ cells/ml), cells being treated by the same method.

It was accordingly decided to carry out a series of experiments in which mouse splenocytes, after binding rabbit Fab-SSA, were treated with donkey antibodies against rabbit IgG (anti-Fab). Cells in a concentration of $20 \cdot 10^6$/ml were treated successively in the cold with Fab-SSA and anti-Fab (30 min in each case) and after each stage they were washed to remove unattached components, transferred to culture medium with antigen, and incubated for 4 days in optimal or high density. The experiments showed (Table 2) that consecutive treatment in this way (with Fab-SSA and anti-Fab) did not affect the formation of AFC in cultures with optimal density (groups 1 and 3). However, it sharply intensified inhibition of antibody formation in dense cultures (group 4) compared with the untreated control (group 2). Treatment of the cells with only one of these components (Fab-SSA or anti-Fab) did not intensify inhibition of antibody formation in dense cultures (groups 6 and 8) and did not affect antibody formation in optimal cultures (groups 5 and 7) compared with the untreated control (groups 1 and 2).

Potentiation of density-dependent inhibition of antibody formation in the cultures as a result of successive treatment of the splenocytes with Fab-SSA and anti-Fab depended on the dose of Fab-SSA (Fig. 1).

The experiments thus showed that consecutive treatment of splenocytes, initially with Fab-fragments of antibodies against the surface receptors of these cells, and later by antibodies against Fab-fragments, potentiates density-dependent inhibition of antibody formation dramatically. The effect described above cannot be explained by agglutination of the cells under the influence of anti-Fab. Evidence against this explanation is given by absence of agglutination in cultures in which potentiation of density-dependent inhibition of antibody formation was observed. To explain the nature of the receptors with which this effect is linked, and its mechanism, research must be continued in the following directions: 1) the study of the time during which complexes remain on the cells and determination of the phase of action during the period of culture; 2) adsorption of Fab-SSA preparation by cells of different types combined with treatment with agents removing various surface receptors from cells.

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pAP20 PLASMID CONTROLLING HEMOLYTIC ACTIVITY OF *Escherichia coli*

I. N. Sharova, N. A. Medvedkova, and A. P. Pekhov

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Most plasmids determining hemolytic (Hly) activity of *E. coli* have been found in bacteria of this species isolated from animals [5]. The F-like plasmids have been found to be separate from these Hly-plasmids and have been classified among Inc FIII-FIV and FVI groups [3]. Plasmid Hly pAP20 was identified for the first time in cells of a strain of *E. coli* isolated from man [1]. However, it has not been studied.

The aim of this investigation was to study the principal physicochemical, biological, and genetic properties of this plasmid.

EXPERIMENTAL METHOD

Strains of *E. coli* AP115 met thi lac Nal^r, AP106 trp his lac str and C600 thr leu thi lac str were used.

Hemolytic properties of bacteria with plasmid pAP20 were determined by seeding them on nutrient agar (NA) containing washed human erythrocytes, and incubating the seedings for 18 h at 37°C. α-Hemolysin production was determined by estimating hemolysis of a 2% erythrocyte suspension to which supernatant after centrifugation of 2.5-h broth cultures of bacteria containing plasmid pAP20 was added.

DNA of the test plasmid pAP20 was isolated by bacterial lysates, clarified with Triton X-100, followed by gradient (CsCl — ethidium bromide) centrifugation [4]. To determine the molecular weight of the plasmid, restriction analysis of its DNA was carried out with the aid of EcoRI enzyme and electrophoresis in 0.8% agarose gel. The buffer used for EcoRI enzyme contained 100 mM Tris-HCl buffer (pH 7.5) and 10 mM $MgSO_4$. The restriction reaction was stopped by heating the samples for 5 min at 65°C. The molecular weight of the plasmid was determined by adding together the molecular weights of its restriction fragments. *E. coli*-fragments of DNA of phage λ served as standards for molecular weight.

Transmissibility of the test plasmid was studied by the use of *E. coli* AP115, AP106, and C600 as recipients.

Compatibility of plasmid pAP20 was determined by Datta's scheme [2], using reference plasmids of all incompatibility (Inc) F groups. Data on surface exclusion were obtained by

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