ROLE OF A CELLS IN THE IMMUNOSTIMULATING ACTION OF F(ab')$_2$ FRAGMENTS

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UDC 615.375:547.962.4].015.44.007

KEY WORDS: A cells; F(ab')$_2$-fragments; immunostimulation.

It was shown previously that F(ab)$_2$-fragments of normal rabbit IgG, obtained with the aid of pepsin, nonspecifically potentiate the immune response in vivo and *in vitro* to thymus-dependent antigens [1, 4]. It has also been found that participation of A cells is essential for realization of the immunostimulating function of the F(ab')$_2$-fragment in experiments *in vitro* [1].

The aim of this investigation was to study interaction of the F(ab')$_2$-fragment of homologous IgG with A cells during realization of the immunomodulating action of F(ab')$_2$-fragments.

EXPERIMENTAL METHODS

F(ab')$_2$-fragments of rabbit immunoglobulin (from Serva, West Germany), obtained with the aid of pepsin and papain by the methods described previously [5, 6], were used in the experiments. The primary immune response to sheep's red blood cells (SRBC) in a culture of rabbit spleen cells was studied as described previously [1].

RESULTS

The experiments of series I were devoted to the study of whether products intensifying immune response and differing in their properties from the fragment used are formed during contact of F(ab')$_2$-fragments of homologous rabbit IgG with adherent cells. The F(ab')$_2$-fragment (2.5 mg in 5 ml of medium RPMI-1640) was incubated for 3 h at 37°C with rabbit spleen cells adherent (A cells) to glass. The culture fluid was passed through a PM-10 filter (from Diaflo and Amicon, USA). Such a filter retains F(ab')$_2$-fragments. The resulting filtrate was sterilized by passage through a Millipore filter with 0.22 μ mesh, and was applied in a volume of 1 ml to a culture of spleen cells from the same rabbit ($5 \cdot 10^6$ cells to 1 ml medium simultaneously with $2.5 \cdot 10^6$ SRBC). In control experiments spleen cells of the same rabbit were cultured either with antigen (SRBC) alone or with antigen and 0.5 mg of F(ab')$_2$-fragment. The number of antibody-forming cells (AFC) in the cultures to be compared was determined by Jerne's direct method [3]; this showed that the filtrate of the medium in which the F(ab')$_2$-fragment was in contact with A cells had the same stimulating action on the immune response of the spleen cells as the original F(ab')$_2$-fragment (Table 1). Since no F(ab')$_2$-fragments were present in the filtrate, it can be concluded that during interaction of these fragments with A cells a low-molecular-weight product (products) with immunostimulating properties is formed.

On further analysis of the phenomenon it was shown that the monovalent pepsin Fab'-fragment, unlike the bivalent F(ab')$_2$-fragment, does not affect the immuni response of rabbit spleen cells (Table 2). During the obtaining of monovalent Fab'-fragments, after reduction of the disulfide bond in the F(ab')$_2$-fragment the SH-groups formed were carboxymethylated with iodoacetamide. This suggested that it is this modification of the protein that leads to loss of the immunostimulating properties of the F(ab')$_2$-fragment. To test this hypothesis, a monovalent pepsin Fab'-fragment of rabbit IgG was obtained without alkylation of the hemicystine residue. So that recombination of the Fab'-fragments should not take place after reduction of the F(ab')$_2$-fragments in the presence of unblocked SH-

N. F. Gamaleya Scientific-Research Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR O. V. Baroyan.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 101, No. 5, pp. 589-591, May, 1986. Original article submitted April 24, 1985.

0007-4888/86/0005-0649$12.50

TABLE 1. Effect of Product (Products) Formed during Interaction of $F(ab')_2$-Fragments with A Cells on Immune Response

№	SRBC	Preparation	Number of AFC per 10^6 cells	P
1	—	—	1±0,34	—
2	+	—	6±1,1	$P_{1-2} \leqslant 0,5$
3	+	$F(ab')_2$-fragment	36±3,5	$P_{2-3} \leqslant 0,5$
4	+	Filtrate of medium after culture of A cells	12±0,7	—
5	+	Filtrate of medium after culture of A cells with $F(ab')_2$-fragment	51±2,1	$P_{4-5} \leqslant 0,5$

TABLE 2. Effect of Alklyation of SH Group in Fab'-Fragments on Its Immunostimulating Action

№	SRBC	Preparation	Number of AFC per 10^6 cells	P
1	—	—	3±0,38	—
2	+	—	12±1,4	$P_{1-2} \leqslant 0,5$
3	+	$F(ab')_2$-fragment	118±2,5	$P_{2-3} \leqslant 0,5$
4	+	Fab'-fragment (not alkylated)	82±4,9	$P_{2-4} \leqslant 0,5$
5	+	Fab'-fragment (alkylated)	14±2,8	$P_{2-5} > 0,5$

groups, the reduced Fab'-fragment was kept in medium in the presence of $5 \cdot 10^{-5}$ M mercaptoethanol. It follows from Table 2 that the reduced by nonalkylated Fab'-fragment stimulates the immune response of rabbit spleen cells to SRBC, whereas the reduced and alkylated Fab'-fragment had no such action. Thus alkylation of the SH-group of one hemicystine residue in the C-terminal part of the Fab'-fragment leads to complete suppression of the ability of the fragment to potentiate the immune response *in vitro*.

The results are evidence that these SH-groups participate in the formation of an effector center, responsible for the immunostimulating activity of the Fab'-fragment. This hypothesis is confirmed by the fact that Fab-fragments obtained with the aid of papain have virtually no immunostimulating activity. Papain is known [6] to attach the peptide bond between the series residue and the hemicystine residue, mentioned above, in the rabbit IgG molecule. As a result, the papain Fab-fragment contains no free SH-groups. Since the immunostimulating action of the $F(ab')_2$-fragment is impossible without their contact with A cells, it can be postulated that the SH-group of the Fab'-fragment participates in this process.

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