TABLE 1. Interlinear Differences in Manifestation of Polyclonal Activity of Copolymer NA-5 (M ± m)

Test antigen	Copolymer	Number of AFC in mouse spleen							
		BALB/c ($H\text{-}2^d$)	activation index	C57BL/6 ($H\text{-}2^b$)	activation index	CBA ($H\text{-}2^k$)	activation index	(CBA×C57BL/6) F_1 ($H\text{-}2^{kb}$)	activation index
SRBC	—	400±63 (18)		280±42 (18)		330±40 (24)		320±42 (24)	
	NA-5	800±57 (15)	2,0	1040±95 (15)	3,7	1760±124 (18)	5,3	1600±74 (30)	5,0
DRBC	—	650±71 (18)		320±61 (18)		340±38 (18)		320±63 (18)	
	NA-5	780±130 (15)	1,2	680±87 (18)	2,1	3400±365 (18)	10,0	2240±260 (30)	7,0
HRBC	—	790±80 (18)		340±38 (18)		480±61 (18)		400±63 (15)	
	NA-5	960±79 (15)	1,2	960±29 (18)	2,8	1440±143 (15)	3,0	1200±202 (21)	3,0
TNP	—	1000±141 (15)		1860±84 (20)		2000±237 (15)		2000±88 (15)	
	NA-5	2280±349 (15)	2,3	4240±420 (27)	2,3	6520±441 (18)	3,3	6800±326 (18)	3,4

Legend. Number of mice shown in parentheses.

The investigations thus showed that some of the synthetic polyelectrolytes used in the investigation possess polyclonal activity relative to B lymphocytes. This effect is independent of the presence of T cells and depends on genotype. The difference in the ability of B cells of different genotypes to respond to stimulation by polyelectrolytes is evidently genetically determined. This may perhaps be connected with differences in the number of the original pool of B lymphocytes or in their proliferative activity.

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ACTION OF POLYCLONAL MITOGENS OF THE SPLENIC LYMPHOCYTE POPULATION IN THE PRESENCE OF ANTISERUM AGAINST ISOLOGOUS AGGREGATED MOUSE IMMUNOGLOBULINS

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UDC 612.122.94:612.6.052

The role of lymphocytes in formation of the immune response is linked with the degree of differentiation of the lymphoid cells and the organization of their receptor apparatus [8, 10]. These two factors also determine the ability of lymphocytes to respond to mitogens [8, 10]. Both these processes *in vivo* are evidently largely connected with the effect of biologically active agents which appear in the blood stream when aggregated immunoglobulins and (or) antigen–antibody complexes are present in the body under normal [11, 12] or pathological conditions [2, 9]. This explains the interest in the study of the action of a biologically active factor (MAAS) in the serum of mice after injection of isologous aggregated immunoglobulin.

KEY WORDS: immunoglobulins; receptors; mitogens; inhibition of DNA synthesis; elimination of cells.

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(Presented by Academician of the Academy of Medical Sciences of the USSR P. A. Vershilova.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 31, No. 4, pp. 463-466, April, 1981. Original article submitted June 5, 1980.

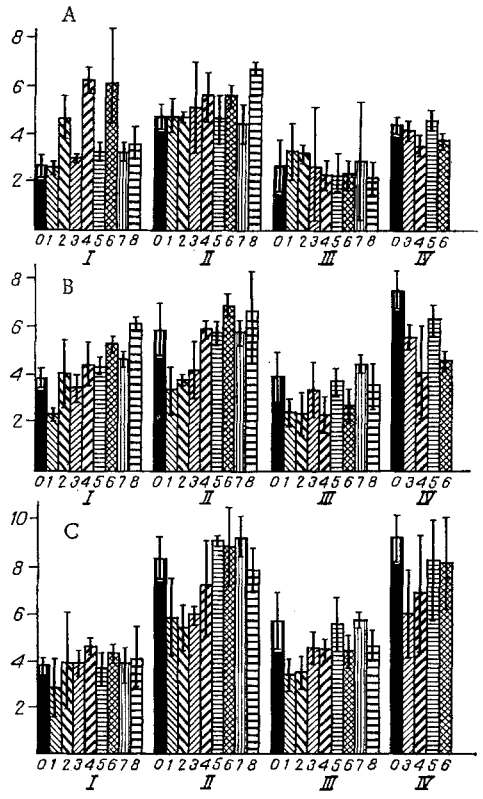

Fig. 1

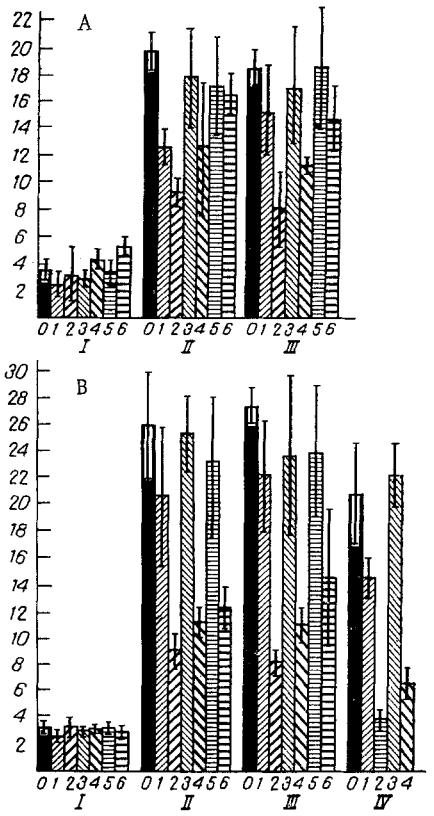

Fig. 2

Fig. 1. Action of MAAS on DNA synthesis is spleen cells. Abscissa, reagents used to treat cells before culture: centrifugation medium (I), carrageenan (II), anti-θ-serum and complement (III), anti-θ-serum, complement, and carrageenan (IV); ordinate, radioactivity (cpm × 10^3) when spleen cells are cultured for 24 h (A) 48 h (B), and 72 h (C). Culture medium (0), the same containing NMS in dilutions of 1:40 (1), 1:60 (3), 1:100 (5), and 1:120 (7); MAAS in dilutions of 1:40 (2), 1:60 (4), 1:100 (6), and 1:120 (8).

Fig. 2. DNA synthesis in spleen cells in response to LPS and DS in the presence of MAAS or NMS. Abscissa, reagents in culture medium (I): LPS in doses of 50 μg/ml (II) and 100 μg/ml (III) and DS (IV); ordinate, radioactivity, in cpm × 10^3 when cells are cultured for 48 h (A) and 72 h (B). Culture medium (0), the same containing NMS in dilutions of 1:40 (1), 1:60 (3), and 1:100 (5); MAAS in dilutions of 1:40 (2); 1:160 (4), and 1:100 (6).

In view of previous results [1, 4, 5, 7], in the present investigation the study of the action of MAAS on proliferative activity of separate subpopulations of spleen cells was continued. An attempt also was made to assess DNA synthesis in B lymphocytes at different stages of differentiation, in response to mitogens.

EXPERIMENTAL METHOD

Lipopolysaccharide (LPS) from *Salmonella typhimurium* (from Sigma) and dextran sulfate (DS) with a molecular weight of 500,000 (from Pharmacia) in quantities of 50-100 and 100 μg/ml culture medium, prepared by the method in [1], were used as mitogens. B mitogens were used in optimal concentrations after titration. Cells containing θ-antigen were eliminated with the aid of anti-θ-serum obtained and tested as described previously [6], and guinea pig complement in dilutions of 1:40 and 1:10, respectively. Carrageenan (from Serva) in a dose of 200 μg/ml [17] was used to activate macrophages in the suspension of spleen cells. After treatment of the cells with these reagents they were washed three times in centrifugation medium [4]. The viability of the cells was assessed by their ability to take up trypan blue. Spleen cells from BALB/c mice were suspended in culture medium to a concentration of $5 \cdot 10^6$/ml and cultured in microplates (Falcon Plastics No. 3040) [5] in the presence of MAAS obtained as described previously [7], normal mouse serum (NMS) in dilutions of 1:40-1:20, and B-mitogens.

Proliferative activity of the lymphocytes in the blast transformation reaction was assessed by determining incorporation of ^{3}H-thymidine (HT) into DNA, when added in a dose of

TABLE 1. Action of Antiserum against Isologous Aggregated Immunoglobulins (MAAS) on Antigen-Binding Receptors of Rosette-Forming Cells of Rapidly Dividing Splenic Lymphocytes of Mice Immunized with Sheep's Red Blood Cells in a Dose of $5 \cdot 10^7$ ($M \pm m$ and 99% confidence interval)

Number of animals	Injection of ^{3}H-thymidine	Number of cells in spleen, millions	Viability of cells, %		Number of rosette-forming cells per 10^3 splenic lymphocytes, treated with sera in dilution of 1:40			
			before fractionation	after fractionation	before fractionation		after fractionation	
					NMS	MAAS	NMS	MAAS
8	—	171,8±9,7 (123,9÷219,7)	87,3±2,7 (81,1÷93,5)	96,0±0,7 (94,4÷97,6)	18,5±1,1 (14,9÷22,1)	10,6±0,8 (7,9÷13,3)	18,9±0,7 (16,6÷21,0)	8,9±0,7 (6,7÷11,1)
7	+	94,0±9,1 (66,3÷125,8)	62,4±3,1 (55,2÷69,6)	89,7±2,4 (83,9÷95,5)	8,0±1,1 (4,3÷11,7)	7,3±0,6 (5,2÷9,4)	3,4±0,5 (1,8÷5,0)	3,0±0,6 (0,9÷5,1)

0.5 μCi per well 16 h before the end of the cultivation period, in accordance with the method described in [4].

EXPERIMENTAL RESULTS

As reported previously [5], MAAS stimulates DNA synthesis in spleen cells. Further study showed that MAAS, in dilutions of 1:40-1:120, caused a statistically significant increase in incorporation of HT into DNA of the lymphocytes only if they were cultured during the first 48 h (Fig. 1, I). To determine whether this effect is connected with activation of macrophages, as has been observed for DS [13], in the next experiments the macrophages were inactivated with carrageenan. It was found that carrageenan had a marked mitogenic action on the remaining population of spleen cells. A similar effect of carrageenan on lymphocytes was observed in other investigations [14, 17]. The mitogenic effect of carrageenan increased with an increase in the period of cultivation of the cells (Fig. 1, II). That was probably why the mitogenic action of MAAS was less marked and it was preserved in serum in dilutions of 1:40, 1:60 (48 h), and 1:120 (24 h).

In the next experiments T cells were eliminated from the cell suspension with the aid of anti-θ-serum in the presence of guinea pig complement. The results showed that elimination of cells carrying θ antigen very slightly increased DNA synthesis in the residual cell population after culture for 48 and 72 h. Unlike NMS, MAAS had no effect on DNA synthesis in this cell population at any time (Fig. 1, III). Conversely, by contrast with NMS under these conditions a tendency was observed for DNA synthesis to decline, probably in B cells.

Consequently, the mitogenic effect of MAAS is evidently mainly linked with its action on T cells. Participation of A cells cannot be completely ruled out by the evidence so far available. No satisfactory response likewise was obtained in the experiment with successive elimination of T cells and macrophages with the aid of anti-θ-serum and carrageenan. The results of these experiments confirmed that the mitogenic effect is independent of B cells (Fig. 1, IV).

To study the possible action of MAAS on B cells depending on their differentiation, experments were carried out with DS and LPS. The action of DS and LPS is known to depend on the degree of differentiation of the B cells and on the presence of appropriate receptors on their membrane [8, 10]. In the presence of MAAS in the culture medium, DNA synthesis in B cells in response to DS and LPS was inhibited (Fig. 2). The maximal inhibitory effect was observed for MAAS in dilutions of 1:40 and 1:60. In these dilutions NMS had a very weak inhibitory action.

The results of the experiments with LPS agree with the conclusion that for the reaction to this mitogen to take place, cells must be present with an Fc-receptor on their surface for immunoglobulin [8, 10], on which the MAAS factor probably exerts its action. It must be pointed out that the MAAS factor may perhaps also have reacted with the receptor for the 3rd component of complement, for the sera used were not inactivated and they contained complement. Complement could facilitate fixation of aggregate immunoglobulins, complementary for MAAS factor and liberated into the medium during culture, to the surface of the cells. MAAS evidently acts mainly on cells with a high mitotic index, as is shown by the inhibitory action of the MAAS factor on DNA synthesis in B cells in response to DS.

To test this hypothesis lymphocytes with a high level of division were eliminated *in vivo* by thymidine suicide. For this purpose, on the 3rd day after immunization of the animals with

$5 \cdot 10^7$ sheep's red blood cells, BALB/c mice were given four intraperitoneal injections, at intervals of 12 h, each of 1 μCi HT with specific activity 20 Ci/mmole [3]. On the 5th day (2 days after injection of HT) the number of rosette-forming cells (RFC) in the spleen of the immunized mice was determined before and after fractionation of the cell suspension [15].

Injection of HT reduced the number of nucleated cells in the spleen by half compared with that in immune animals not treated with HT (Table 1). The number of RFC in the experimental group fell to the levels recorded before injection of HT. The RFC in animals receiving HT were insensitive to MAAS (dilution 1:40) and they were eliminated to the extent of 83% by anti-θ-serum in the presence of complement.

Since a high percentage of nonviable cells was observed in the animals after injection of HT, making it difficult to detect RFC, the suspension of spleen cells was freed from conglomerates and disintegrated cells [16] and an enriched population of small lymphocytes with high viability was obtained in a bovine serum albumin gradient [15]. For instance, in mice not exposed to the action of HT the number of RFC in the spleen was 6 or 7 times greater than in the animals of the experimental group. Antigen-binding receptors of RFC of animals receiving HT were not sensitive to the action of MAAS, unlike in the control (Table 1). These results, like those of the experiments with B mitogens, demonstrate that populations of radiosensitive, rapidly dividing lymphocytes are the target cells for factor MAAS.

It can be concluded from the results that the mitogenic effect of MAAS is connected with the action of the serum factor mainly on T lymphocytes. It may be that this effect is also dependent to some extent on macrophages. MAAS causes inhibition of DNA synthesis in B lymphocytes in response to DS and LPS. Blockage of the Fc receptor to immunoglobulin and (or) the 3rd component of complement by MAAS may contribute to this process. The degree of inhibition of proliferative activity of the B lymphocytes depends on their differentiation and on the presence of antigen-binding receptors on the membrane.

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