POLYCLONAL ACTIVATION OF B LYMPHOCYTES BY SYNTHETIC POLYELECTROLYTES

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Synthetic polyelectrolytes — the polycation poly-4-vinylpyridine (PVP) and the polyanion polyacrylic acid (PAA), if injected into animals at the time of immunization sharply intensify the immune response to various heterologous thymus-dependent antigens [4, 7]. Copolymers of acrylic acid (AA) and N-vinylpyrrolidone possess immunostimulation properties but, unlike PAA, they are nontoxic [3]. Copolymers of 4-vinylpyridine and 4-vinyl-N-alkylpyridinium bromides [1], with hydrophobic hydrocarbon radicals introduced into their molecule, can form stable complexes with proteins. Such complexes have immunogenicity 5-100 times greater than the pure protein. The copolymer itself is not an immunogen and it exhibits the same immunostimulant properties as PVP. The adjuvant action of the above-mentioned polymers is based on intensification of the proliferation and migration of hematopoietic stem cells and migration and cooperation of T and B lymphocytes in the immune response [4, 5]. These polyelectrolytes have been shown to replace the auxiliary function of T-cells to some extent in the immune response [2, 6].

In the present investigation the ability of polymers to induce transformation of B lymphocytes into antibody-forming cells (AFC) was studied.

EXPERIMENTAL METHOD

BALB/c, CBA, and C57BL/6 mice and (CBA × C57BL/6)F_1 hybrids, obtained from the **Stolbovaya nursery,** Academy of Medical Sciences of the USSR, and also nude mice with congenital absence of the thymus, from the pure-line animal unit of the Institute of Biophysics, Ministry of Health of the USSR, were used.

Aqueous solutions of the polymers PAA and PVP and copolymers of AA and N-vinylpyrrolidone with 70 mole % AA components (NA-3) and 45 mole % AA components (NA-5), and also PVP derivatives with hydrocarbon radicals of different lengths, were injected intravenously in a dose of 50 mg/kg body weight into intact (unimmunized) mice. In some experiments the animals received *E. coli* lipopolysaccharide (LPS, from Sigma, USA) in a dose of 5 mg/kg intravenously as polyclonal activator. Four days later the number of AFC in the spleen against sheep red blood (SRBC), donkey red blood cells (DRBC), horse red blood cells (HRBC), and trinitrophenyl (TNP), loaded on SRBC, was determined by Jerne's method [8]. The trinitrophenylated SRBC were obtained by the method described in [9].

The molecular weight of the copolymers of AA and N-vinylpyrrolidone, estimated viscosimetrically, was 300,000-400,000. The copolymers were readily soluble in water.

As a model of artificial T deficiency (B mice) the animals were irradiated in a dose of 800 R and protected with syngeneic bone marrow cells. Some animals received a mixture of bone marrow and lymph node cells. The dose of bone marrow cells was 10^7 and of lymph node cells $5 \cdot 10^6$.

The numerical results were subjected to statistical analysis with calculation of the arithmetic mean and the 95% confidence interval ($P \leq 0.05$).

EXPERIMENTAL RESULTS

As Fig. 1 shows, after injection of LPS, PAA, PVP, NA-3, or NA-5 into normal (nonimmunized) mice polyclonal activation of B cells took place and AFC of different types accumulated in the animals' spleens. For instance, on the 4th day AFC producing antibodies against SRBC, DRBC, HRBC, and TNP were found in the spleen of mice treated with polyelectrolytes, in significantly larger numbers than initially (Fig. 1, Table 1).

KEY WORDS: polyelectrolytes; polyclonal activators of B lymphocytes.

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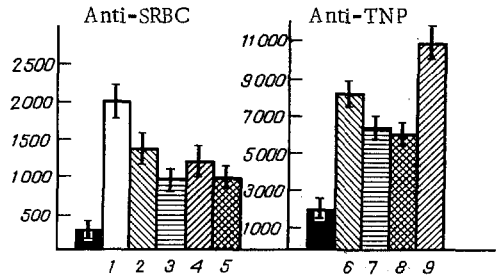

Fig. 1

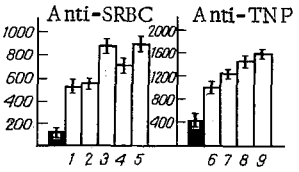

Fig. 2

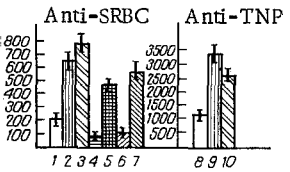

Fig. 3

Fig. 1. Number of AFC in spleen of intact mice after injection of LPS (1), PAA (2, 6), PVP (3, 7), MA-3 (4, 9), and NA-5 (5, 8). Black columns — initial level of AFC to each test antigen used. Ordinate, here and in Figs. 2 and 3 — number of AFC in spleen.

Fig. 2. Number of AFC against TNP and SRBC in mouse spleen after injection of various polycations. 1, 6) Copolymers of 4-vinylpyridine and 4-vinyl-N-alkylpyridinium bromides without hydrocarbon side radicals; 2, 7) the same copolymers with 16 carbon atoms; 3) with 10 carbon atoms in the N-alkyl fragment; 5, 8) copolymers of 4-vinylpyridine and 4-vinyl-N-alkylpyridinium bromides, completely alkylated with ethyl bromide; 4, 9) copolymer of 4-vinyl-N-ethylpyridinium and 4-vinyl-N-cetylpyridinium bromides. Black columns show initial level of AFC to each antigen used.

Fig. 3. Ability of copolymer NA-5 to induce differentiation of B lymphocytes in B mice and in mice with congenital absence of the thymus into AFC secreting antibodies against SRBC and TNP. 1, 8) Initial level of AFC in spleen of nude mice; 2, 9) number of AFC after injection of NA-5 into these mice; 3, 10) number of AFC in spleen of mice of parental line C57BL/6 with normal thymus (differences between initial levels of AFC in nude and C57BL/6 mice not statistically significant); 4) number of AFC in spleen of B mice without injection of polymer; 5) the same, with injection of copolymer NA-5; 6) number of AFC in spleen of lethally irradiated recipients protected by syngeneic bone marrow and lymph node cells without injection of copolymer; 7) the same with injection of NA-5.

The results of experiments to study the ability of PVP, with hydrocarbon radicals of different lengths introduced into its molecule, to induce transformation of B lymphocytes into AFC in the spleen of intact (nonimmunized) mice are given in Fig. 2. Copolymers of 4-vinylpyridine and 4-vinyl-N-alkylpyridinium bromides with hydrocarbon side radicals of different lengths, and also a copolymer of 4-vinyl-N-ethylpyridinium and 4-vinyl-N-cetylpyridinium bromides were used. Injection of these polymers into the animals induced a significant increase in the number of ARC against SRBC and TNP. Under these circumstances the number of AFC depended on the character of the functional groups in the composition of the polyelectrolytes. Copolymers potentially capable of forming stable complexes with proteins (with a high content of carbon atoms) stimulated transformation of B cells into AFC more strongly than copolymers without hydrocarbon radicals (Fig. 2).

Subsequent investigations showed that activation of B cells under the influence of polyelectrolytes is to a certain extent thymus-dependent (Fig. 3). It will be clear from Fig. 3 that in C57BL/6 mice with a normal thymus and in athymic nude mice, reared on the basis of the C57BL/6 line, approximately the same number of AFC accumulated against SRBC and TNP. Similar results were obtained on B mice (Fig. 3). For instance, after injection of copolymer AN-5 into lethally irradiated mice, into which syngeneic bone marrow was injected, 4 times more AFC accumulated than in the control. Transplantation of T cells (lymph node cells) into B mice did not affect accumulation of AFC in response to injection of the copolymer.

The next step was to study the effect of polyelectrolytes as polyclonal stimulators on B lymphocytes of mice belonging to several different genotypes, differing in their H-2 complex. For this purpose copolymer NA-5 was injected into CBA mice (with H-2^k haplotype), C57BL/6 (H-2^b), (CBA × C57BL/6)F_1 (H-2^{kb}), and BALB/c (H-2^d). As Table 1 shows, significant interlinear differences were observed in the ability of NA-5 to induce polyclonal activation of B cells. For instance, polyclonal activation was manifested more strongly in CBA mice and their hybrids (especially clearly when DRBC were used as the test antigen). The polyclonal effect of NA-5 was weaker in C57BL/6 and, especially, in BALB/c mice.

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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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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