

EFFECT OF SYNTHETIC DOUBLE-STRANDED POLYNUCLEOTIDES ON LEUKOCYTE MIGRATION

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The effect of synthetic double-stranded polynucleotides (polyI:polyC) on migration of peripheral blood leukocytes from donors and patients with rheumatic fever was investigated. PolyI:polyC in large quantities was found not to inhibit migration of the donors' leukocytes, but it inhibited migration of leukocytes from patients with rheumatic fever, in whom antibodies against double-stranded RNA were found more often than in donors.

KEY WORDS: synthetic polynucleotides; leukocyte migration; rheumatic fever.

Synthetic double-stranded polynucleotides prolong the survival of animals with experimental tumors [3], induce interferon formation [2], and have been tested in the treatment of human malignant diseases [5]. Meanwhile reports have been published of their toxicity toward karyocytes and colony-forming units (CFUs) of bone marrow and spleen [6].

However, polyanions, which include double-stranded polynucleotides, immediately after their entry into the body, promote active liberation of karyocytes into the blood stream [9]; this suggests that the decrease in the number of cells in the hematopoietic organs caused by administration of polyinosinic and polycytidylic acids (polyI:polyC) [6] is due to a redistribution of the cells and not to the toxic properties of the polynucleotide.

With these observations in mind, experiments were carried out to study the effect of polyI:polyC on the migration of peripheral blood leukocytes [4, 8] of patients with rheumatic fever and clinically healthy donors.

EXPERIMENTAL METHOD

PolyI:polyC (Calbiochem, USA) was dissolved in standard salt solution (0.15 M NaCl, 0.015 M tri-substituted sodium citrate). Samples of blood cells from 36 clinically healthy donors aged from 18 to 40 years and from 47 patients with rheumatic fever, admitted to the Department of Internal Medicine of Volgograd Medical Institute for treatment, were investigated. Tests were carried out at intervals, before the beginning and in the course of treatment. Antibodies against polyI:polyC were detected by the passive hemagglutination test, using the method described previously [1]. Inhibition of leukocyte migration by polyI:polyC was determined by a modification of George's method [8]. During the investigation of each blood sample, dilutions of antigen in concentrations of 10 and 50 $\mu\text{g/ml}$ were made up in medium No. 199 with 20% bovine serum, and a parallel series of controls was set up without the addition of polyI:polyC. Pairs of capillary tubes containing cells washed with medium No. 199 were placed in glass chambers. The chambers were hermetically sealed with coverslips and incubated for 24 h. After incubation, the zones of migration were drawn and projected onto x-ray film by means of a photographic enlarger (1:4), cut out, and weighed on torsion scales. All the experiments were duplicated, so that 6 to 8 areas of migration were

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TABLE 1. Inhibition of Migration of Leukocytes from Donors and Patients with Rheumatic Fever in the Presence of PolyI : polyC

Group of subjects	Conc. of polynucleotides (in $\mu\text{g/ml}$)	Inhibition of migration index ($M \pm m$)
Donors	10	$1,02 \pm 0,205$
	50	$1,10 \pm 0,13$
Patients with rheumatic fever:	100	$1,09 \pm 0,13$
before treatment	10	$0,724 \pm 0,25$
	50	$0,512 \pm 0,18^*$
after treatment	10	$1,186 \pm 0,16$
	50	$1,007 \pm 0,21$

* Difference significant compared with donors ($P < 0.05$).

changes in the composition of the bone marrow cells (a reduction of more than 50% in their number), reduced the number of CFUs by 4-6 times, the number of formed elements by 3 times, and the number of lymphocytes by 20 times [6].

The results thus demonstrate that synthetic double-helical polynucleotides, administered in vitro in quantities much higher than those existing in vivo, do not disturb leukocyte migration and do not cause the liberation of a factor inhibiting leukocyte migration from the lymphocytes of healthy individuals.

If this is so, the phenomena developing in animals after administration of double-stranded polynucleotide are probably not connected with its direct toxic action on leukocytes. The pattern observed can be supposed to be due to the redistribution of cells under the influence of the polyanion, or to be the result of the action of substances formed in the body under the influence of polyI : polyC on the cells. Moreover, polyI : polyC is a powerful interferonogen [2], and interferon has an activating effect on various cell populations of hematopoietic tissue [7].

As Table 1 shows, synthetic double-stranded polynucleotides can induce inhibition of migration of leukocytes from patients with rheumatic fever. These effects are probably due to the fact that antibodies against double-stranded RNA are found more often in patients with rheumatic fever than in healthy subjects (Table 2).

High polyI : polyC concentrations in vitro thus have no significant effect on the viability of leukocytes (their ability to migrate) and do not induce the liberation of a factor inhibiting migration from lymphocytes. All these factors suggest that changes observed in the hematopoietic system under the influence of polyI : polyC in vivo are not attributable to the direct action of the double-stranded polynucleotide on the cells, but are the result of the redistribution of cells or the result of action of a substance (possibly interferon), formed in vivo after administration of polyI : polyC.

TABLE 2. Antibodies against Double-Stranded RNA in Donors and Patients with Rheumatic Fever

Group of subjects	Number tested	No. tested with antibodies present in undermentioned titers					
		total	1:5	1:10	1:20	1:40	1:80 and over
Donors	50	7	7	—	—	—	—
Patients with rheumatic fever	47	19	—	5	8	5	1

LITERATURE CITED

1. V. K. Podgorodnichenko, A. M. Poverennyi, V. A. Nasonova, et al., *Vopr. Med. Khim.*, No. 6, 578 (1973).
2. A. Field, A. Tytell, and G. Lampson, *Proc. Nat. Acad. Sci. USA*, **58**, 1004 (1967).
3. H. Gelboin and H. Levy, *Science*, **167**, 205 (1970).

4. M. George, *Proc. Soc. Exp. Biol. (New York)*, 111, 514 (1962).
5. M. Ailleman, *J. Infect. Dis.*, 121, 196 (1970).
6. P. Jullien and J. Maeger-Guignard, *Brit. J. Cancer*, 7, 468 (1971).
7. T. McNeill and J. Grosser, *Nature*, 244, 173 (1973).
8. M. Söborg and G. Bendixen, *Acta Med. Scand.*, 181, 247 (1967).
9. T. Suchi, *Nature*, 216, 1013 (1967).