PROCEEDING OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 151. 376-379 (1976)

Effects of Synthetic Single- and Multistranded Polynucleotides on Human Monocyte IgG Receptor Activity *In Vitro* (39214)

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Synthetic polynucleotides have been shown to influence cell-mediated immune reactions. It is well established that they can enhance or increase the functional activities of different cell types involved in delayed type hypersensitivity, thus exerting effects on T lymphocytes (5, 9, 10, 12, 13), B lymphocytes (21, 23), and mononuclear phagocytes (1, 11, 20). The findings that defined polyribonucleotides, namely polyadenylic acid, complexed with polyuridylic acid (poly(A:U)), increased antibody production to different antigens (4, 25), and that this effect was more pronounced after preincubation of normal peritoneal exudate cells with poly(A:U) (11), led to further studies on the morphologic, biochemical, and functional alterations of mononuclear phagocytes induced by polynucleotides. Ultrastrutural studies revealed an increase in lysosomal size and number of lysosomes (A. F. Johnson, personal communication); biochemical investigations detected an elevation in DNA-dependent RNA polymerases, an increase in DNA and RNA and protein synthesis (20), and activiation of nonspecific cytotoxicity (1). In other investigations, depressed phagocytic capacity was reported (11, 29), and studies on bactericidal capacity described either increased activity (8) or no effect (27).

Since cytotoxic reactions may require direct membrane contact between the killer mononuclear phagocyte and the tumor cells, an increased cytotoxic potential may suggest changes in membrane structure. Fc receptors may be directly involved in antibody-dependent cell-mediated cytotoxicity and may be requisite for the binding of the killer cell to the target (14). The following experiments have been designed to determine the effect of synthetic single- and multistranded homoribopolynucleotides on human monocyte IgG receptor activity *in vitro*. The binding of polynucleotides at cell surfaces is the initial event in the interaction of polynucleotides with mammalian cells (18) and leads to changes in electrophoretic mobility. It is therefore of interest to investigate whether these substances cause alterations in membrane structure which influence IgG receptor activity.

Materials and methods. Monocytes were isolated from heparinized venous blood by the attachment of total white cells (TWC) to coverslips in Leighton tubes. Blood donors were normal healthy individuals. These methods are described in detail elsewhere (24).

Polyinosinic acid (poly(I)), polycytidylic acid (poly(C)), or complexes of polyinosinic and polycytidylic acid (poly(I:C)), or multistranded complexes of polyinosinic, polycytidylic, and polyuridylic acids (poly(I:C:U)) (Serva, Feinbiochemica, Heidelberg, West Germany; and P. L. Biochemicals, Inc., Milwaukee, Wis.) were dissolved in PBS (pH 7.2) before use.

Six × 10^6 TWC were incubated in Leighton tubes for 60 min in TC-199 medium (Gibco, Grand Island, New York) containing 15% heat-inactivated fetal calf serum (FCS). Nonadherent cells were removed by washing twice with serum-free medium. The slides were then incubated with different concentrations of polynucleotides (vide infra) for different time periods from 10 min to 16 hr. Coverslip attached cells were washed three times with Hank's solution without serum, and receptor activity was assayed using sheep red blood cells (SRBC) coated with different dilutions of IgG rabbit anti-Forssman antibody (EA) at dilutions from 1:1600 to 1:6400. The adherent cells were incubated for 45 min at 37°. The bound sheep red blood cells were removed by several washings and coverslips were stained by panoptic Pappenheim stain and

mounted. Two hundred monocytes per slide were counted. Monocytes exhibiting specific interaction with coated SRBC expressed as binding and/or phagocytosis were recorded as "active." Nonsensitized SRBC's served as controls in all experiments. In each experiment, cell preparations without polynucleotides and cells incubated in the presence of single-stranded poly(I) and/or poly(C) were run in parallel.

Results. The initial experiments to investigate the effect of poly(I:C) on human coverslip-attached monocytes (after incubation periods of 10 min to 2 hr using concentrations of 10 and 100 μg/ml) failed to demonstrate any effect on specific interaction of coated SRBC in comparison to controls. Longer incubation periods, however, with poly(I:C) or poly(I:C:U) resulted in an increase of "activie" monocytes, which was evident after 4 hr (Fig. 1). The differences in the number of active monocytes between cultures preincubated with polynucleotides and controls were most pronounced at lower antibody concentrations and were detectable throughout the culture periods. After culture for longer than 6 hr, a decrease in the overall activity occurred both in the control and in the test group, suggesting a decrease in cell viability under these conditions.

Further experiments were thus limited to 6-hr incubation periods with concentrations of polynucleotides of 50 μg/ml for poly(I:C) and poly(I:C:U). There was no significant difference between 10 or 50 μg/ml amounts, and different polynucleotide preparations obtained from different sources showed the same *in vitro* effects (Table I).

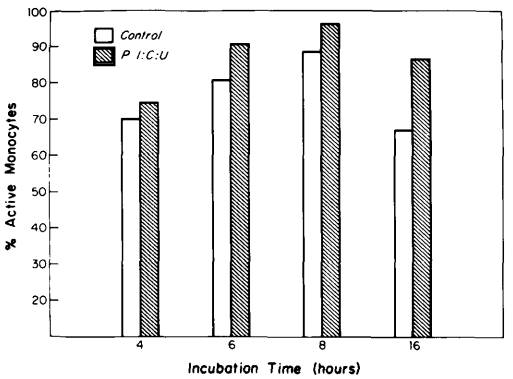

FIG. 1. The bars represent the percentage of "active" monocytes (cells showing attachment and/or phagocytosis of sheep erythrocytes coated with EA 1:3200) at the different incubation periods indicated.

In studies using SRBC coated with different dilutions of EA, an increase in the total percentage of "active" monocytes was observed after preincubation with poly(I:C) at all concentrations tested, and this difference was most significant at EA 1:6400 dilution (Table II). Comparable data were obtained when coverslip-attached monocytes were preincubated with triple-stranded complexes of poly(I:C:U), indicating an effect of complexed polynucleotides on receptor activity (Table III). With all antibody dilutions tested, there was a distinct difference in the number of specifically reacting monocytes with significance at EA concentration of 1:1600 and 1:3200. No interaction was observed with uncoated SRBC, thus excluding nonspecific erythrocyte binding to polynucleotide preincubated monocytes.

Single-stranded polyinosinic acid (poly(I)) had no effect on monocyte IgG receptor activity at the concentrations tested (Table IV). Single-stranded polycytidylic acid (poly(C)) at concentrations of 50 μg/ml resulted in slight, but not significant, increase in the number of active monocytes (five experiments; data not shown).

Discussion. The data presented indicate that multistranded polynucleotides (poly(I:C) and poly(I:C:U) result in a significant increase in human monocyte IgG receptor activity after incubation periods of longer than 4 hr. Thus, a higher percentage of coverslip-attached monocytes specifically interacted with IgG coated SRBC's. This effect is most pronounced at lower antibody concentrations (namely, 1:3200 and 1:6400). Single-stranded polynucleotides (poly(I) and poly(C)) failed to exert these effects.

The increase in detectable IgG receptor sites may be related to either changes in monocyte membrane architecture, unmasking more receptors, perhaps through steric changes, or by increased (re)synthesis of IgG binding sites, which would be consistent with the assumption of an accelerated turnover of membrane constituents. Experimental data which would differentiate between

TABLE I. THE EFFECT OF DIFFERENT PREPARATIONS AND CONCENTRATIONS OF POLYNUCLEOTIDES ON HUMAN MONOCYTE IgG RECEPTOR ACTIVITY.[a]

		Polynucleotide concentration	
Control		10 μg/ml	50 μg/ml
69			
	poly(I):poly(C)[b]	88.5	85.5
	poly(I):poly(C)[c]	82.5	79.5

[a] Numbers represent percentage of "active" monocytes with specific interaction with SRBC-coated with EA 1:3200.

[b] Polynucleotide from Serva, Feinbiochemica, Heidelberg, W. Germany, incubation time, 6 hr.

[c] Polynucleotide from P.L. Biochemicals, Inc., Milwaukee, Wis., incubation time, 6 hr.

TABLE II. THE EFFECT OF POLYINOSINIC-POLYCYTIDYLIC ACID (POLY(I):POLY(C)) ON HUMAN MONOCYTE RECEPTOR ACTIVITY USING VARIOUS DILUTIONS OF RABBIT ANTI-FORSSMAN ANTIBODY (EA).[a]

EA	Number of experiments	Controls	Poly(I):Poly(C)[b]	*P* value
1:1600	4	82.5 ± 8.0	89.6 ± 9.5	<0.1
1:3200	3	62.3 ± 33.0	76.5 ± 19.5	<0.1
1:6400	4	45.6 ± 28.4	69.5 ± 31.7	<0.005

[a] Numbers represent percentage of "active" monocytes (binding or phagocytosis of sensitized SRBC) ± SD.

[b] Fifty micrograms per milliliter; incubation for 6 hr.

TABLE III. THE EFFECT OF POLYINOSINIC-POLYCYTIDYLIC-POLYURIDYLIC ACID (POLY(I):POLY(C):POLY(U)) ON HUMAN MONOCYTE RECEPTOR ACTIVITY USING SRBC COATED WITH VARIOUS DILUTIONS OF RABBIT ANTI-FORSSMAN ANTIBODY (EA).[a]

EA	Number of experiments	Controls	Poly(I):poly(C):poly(U)[b]	*P* value
1:1600	3	89.6 ± 8.9	93.5 ± 6.5	<0.05
1:3200	5	75.5 ± 10.3	84.3 ± 9.8	<0.01
1:6400	5	60.8 ± 20.7	82.6 ± 12.9	<0.1

[a] Numbers represent percentage of "active monocytes with specific binding or phagocytosis of coated SRBC ± SD.

[b] Fifty micrograms per milliliter; 6 hr of incubation. (P.L. Biochemicals, Inc., Milwaukee, Wis.)

these possibilities are not yet available. The observations of increased monocyte receptor activity, however, provide further evidence that coverslip attached cells may be activated in the presence of polynucleotides. Increased receptor activity and avidity is expressed in peritoneal and alveolar macrophages induced by adjuvants (2, 3) and in circulating human monocytes derived from patients with granulomatous diseases (sarcoidosis, Crohn's disease), which, in addition to higher receptor activity, also show increased functional capacity (7). The findings of increased monocyte-macrophage IgG receptor activity in granulomatous diseases (7, Schmidt and Douglas, in preparation) and induced by adjuvant *in vivo* (1) and multistranded polynucleotides *in vitro* may have important implications for investigations of plasma membrane dynamics as well as for monocyte-macrophage function.

It remains to be determined whether the increase in monocyte receptor activity following preincubation with different multistranded polynucleotides reflects a specific process, or is merely due to binding and uptake of these polycomplexes (6, 15, 17, 22) resulting in monocyte activation via a phagocytic event. Our findings differ from data on the effect of poly(A:U) on the dynamics of membrane immunoglobulin receptors in spleen cells of sensitized animals which lose and then regain binding capacity during continous exposure to the adjuvant (26). At no time did receptor activity disappear or become decreased in our experiments. We have no explanation for these differences; however, different cell types were studied and in our experiments the influence of poly(I:C), but not poly(A:U), has been investigated. The antitumor activity of polynucleotides observed in different animal systems has been considered to be due in part to interferon induction (16, 19, 28). Our findings, however, support concepts that a major effect of homoribopolynucleotides may be direct macrophage activation which is perhaps followed by en-

TABLE IV. THE INFLUENCE OF SINGLE-STRANDED POLYINOSINIC ACID (POLY(I)) ON HUMAN MONOCYTE RECEPTOR ACTIVITY.[a]

Number of experiments	Controls	Poly(I) (50 μg/ml)	Poly(I) (100 μg/ml)
11	73.0 ± 13.3	68.0 ± 26.9	–
15	71.8 ± 21.3	–	73.1 ± 19.8

[a] Percentage of "active" monocytes with binding and/or phagocytosis of SRBC coated with rabbit anti-Forssman antibody; incubation for 6 h.

hancement of immune mechanisms which are involved in the processing and/or elimination of foreign antigens.

Summary. Human peripheral monocytes demonstrated a higher IgG receptor activity *in vitro* after incubation with multistranded polynucleotides (poly(I:C) and poly(I:C:U)) after 6-hr cultivation periods. These changes were most pronounced using SRBC coated with low anti-Forssman antibody concentrations (EA, 1:1600 and 1:6400). Polyinosinic and polycytidylic acids failed to show changes in receptor activity. These findings suggest monocyte activation *in vitro* by multistranded polynucleotides and may in part explain their antitumor activity.

This research was supported in part by the National Insititutes of Health USPHS Grant No. AI-12478-02 and the National Leukemia Association, Inc., and in part by Deutsche Forschungsgemeinschaft.

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Received September 22, 1975. P.S.E.B.M. 1976, Vol. 151.