The Inability of RNA from Lymph Node Cells from Immunized Strain 2 Animals to Confer Immune Competence To Strain 13 Lymph Node Cells in the DNP-PLL Immune System (35007)

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In the past few years a variety of experimental models have been presented in which RNA obtained from specifically sensitized lymphoid cells have been able to confer on lymphoid cells obtained from normal animals immune properties originally present in the lymphoid cells from the actively immunized animals.

Some of the immunological reactivities allegedly transferred were: the ability to produce specific antibody (1, 2), the ability to mediate specific skin and tumor allograft rejection (3, 4), as well as graft versus host disease (5), the ability to release migration inhibitory factor in the presence of antigen (6), and finally, the ability of lymphoid cells to synthesize DNA *in vitro* upon exposure to antigen (7).

It has been reported that RNA extracted from lymph node cells of responder Strain 2 guinea pigs immunized with dinitrophenylated oligolysines, when added to lymph node cells from nonresponder Strain 13 guinea pigs, would confer upon the latter the capacity to be transformed *in vitro* by the antigen (7). Since the immune response in guinea pigs to oligolysines, poly-L-lysine, copolymers of L-glutamic acid and L-lysine, and hapten conjugates of these materials is under the control of a dominant autosomal gene (8), we felt that further studies of the possible role of RNA as an immunologically relevant mediator in this immune system would be particularly interesting.

Materials and Methods. Reagents. Poly-L-lysine (PLL) hydrobromide (av mol wt 110,000) was obtained from Pilot Chemical Co., Watertown, Mass. 1-Fluoro-2,4-dinitrobenzene (DNFB) was obtained from Eastman Organic Chemical Co., Rochester, N.Y. ^{3}H-Thymidine was obtained from New England Nuclear Corp., Boston, Mass. Biosolv BBS-3 was obtained from Beckman Instruments, Fullerton, California. Diethylaminoethyl (DEAE)-dextran, molecular weight about 2 million, was obtained from Pharmacia Corp., Upsala, Sweden. Bentonite was a gift from Dr. S. Wolff of the Laboratory of Clinical Investigation, NIH.

Preparation of conjugates. DNP-PLL was prepared by reacting PLL with DNFB under alkaline conditions as previously described (9). The conjugates were freed from DNPOH and unreacted DNFB by exhaustive dialysis. DNP_{62}-PLL_{550} was prepared. The subscripts refer respectively to the average number of groups of hapten and the average number of amino acid residues.

Polypeptide concentrations were calculated from micro-Kjeldahl nitrogen determinations. The degree of DNP substitution was calculated from the absorbency at 360 mμ on the basis of the molar extinction coefficient of ϵ-DNP-L-lysine ($\epsilon = 17{,}400$).

Animals and immunization. Strain 2, strain 13, and NIH Hartley guinea pigs were obtained from the Animal Production Section, National Institutes of Health. The basic immunization procedure consisted of the injection of 0.4 ml of an emulsion, composed of equal parts of DNP-PLL at 0.5 mg/ml in 0.15 *M* NaCl and of complete Freund's adjuvant (Difco) which was distributed into four footpads. Thus each animal received 100 μg of the antigen. Strain 13 animals received

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0.4 ml of an emulsion of adjuvant with saline alone.

Assay of the immune response to DNP-PLL. The intensity of delayed hypersensitivity reactions to DNP-PLL was evaluated 24 hr after intradermal injection of 10 μg of these antigens in 0.1 ml of 0.15 *M* NaCl. All strain 2 animals used as donors for lymphocyte DNA had strong delayed reaction to DNP-PLL.

Extraction of RNA. At 10–21 days after immunization the draining lymph nodes were removed surgically from strain 2 guinea pigs immunized with DNP-PLL and trimmed of fat. The nodes were teased apart in Eagles minimal essential medium (MEM) containing 10% heat-inactivated guinea pig serum. The resulting cell suspension was filtered through 4-ply surgical gauze and centrifuged for 10 min at 1000 rpm in an IEC PR-2 centrifuge. The cell pellet was resuspended in 16 ml of 0.01 *M* acetate buffer, pH 5.0, containing 0.1 *M* NaCl, 0.001 *M* $MgCl_2$, and 1% Bentonite [washed as described by Frankel-Conrat *et al.* (10)] and divided into four aliquots. RNA was extracted from these suspensions by the hot phenol–detergent method described by Cooper and Kay (11). The procedure we used was identical with that described by these authors with the following modifications: (i) after each phenol extraction the aqueous and phenol phases were separated by high speed centrifugation, 24,000*g* for 5 min in a Sorval RC-2B centrifuge; (ii) after the final phenol extraction, the aqueous phase was extracted four times with 3 vol of ether to remove any phenol which may have been present and nitrogen was bubbled through the aqueous phase to remove the ether; (iii) the RNA was precipitated from the aqueous phase without the addition of carrier RNA. The RNA obtained from one animal was dissolved in 1.0 ml of EM and quantitated by its absorption in the UV at 260 mμ. The RNA extinction coefficient was assumed to be $E^{1\%}_{260} = 200$. This procedure does not differ significantly from that of Thor and Schlossman (7, 12).

Assay of RNA activity. The extracted RNA was incubated in culture with lymph node cells from nonresponder strain 13 animals. The ability of specific antigen (DNP-PLL) to stimulate DNA synthesis in these cultures was investigated by measuring the incorporation of ^{3}H-thymidine into DNA in the cultures incubated with the RNA as compared to cultures of strain 13 lymph node cells not incubated with RNA.

Two different culture conditions were used in the attempt to demonstrate the effect of added RNA. (i) Ten million cells, 80% strain 13 lymph node cells and 20% strain 13 peritoneal exudate cells (obtained 4 days after ip injection of 30 ml of light mineral oil/guinea pig) were incubated with 120 μg of RNA in 1.5 ml of MEM containing 100 units/ml of penicillin, 100 μg/ml of streptomycin, and 10% heat-inactivated guinea pig serum. After 30 min at 37°, 4.6 μg of DNP-PLL in 0.1 ml of MEM was added/tube and incubation at 37° in a 5% CO_2, 95% air atmosphere. (ii) Ten million strain 13 lymph node cells were incubated with 120 μg of RNA and 37.5 μg of DEAE-dextran in 1.5 ml of MEM containing 100 units/ml of penicillin, 100 μg/ml of streptomycin, and 10% guinea pig serum for 30 min at 37° in a 5% CO_2, 95% air atmosphere. Then 4.6 μg of DNP-PLL in 0.1 ml of MEM was added to each tube and incubation was continued. After 24 hr with either culture condition, 1 μCi of ^{3}H-thymidine was added to each tube in 0.1 ml of MEM and incubation was continued. After another 18 hr, a 0.5-ml aliquot from each tube was filtered under negative pressure through a 25-mm Millipore filter (type HAWP 025) which had been prewetted with 5 ml of phosphate buffered saline, pH 7.6, containing 0.001 *M* thymidine (PBS + thymidine). The filters were then washed 2 times with 5 ml of PBS + thymidine, 2 times with 5 ml of 5% TCA containing 0.001 *M* thymidine and one time with ml of 95% ethanol. The filters were placed in 10 ml of scintillation fluid (2365 ml of toluene: 500 ml of Bio Solv 3:100 ml of Liquifluour) and counted in a Beckman liquid scintillation counter.

Results. The RNA extracted from lymph node cells obtained from strain 2 guinea pigs immunized with DNP-PLL and incubated with the mixture of strain 13 lymph node

TABLE I. The Response of Lymph Node Cells from Normal Strain 13 and Immunized Hartley Guinea Pigs to DNP-PLL in the Presence or Absence of RNA Extracted from the Lymph Node Cells of DNP-PLL Immunized Strain 2 Guinea Pigs.

	Lymph node cells from normal strain 13 animals						Lymph node cells from immunized Hartley responder animals			
	−Antigen −RNA	+Antigen −RNA		−Antigen +RNA		+Antigen +RNA	−Antigen −RNA	+Antigen −RNA		+Antigen +RNA	
Exp. no.	(cpm)	(cpm)	(*E/C*)[a]	(cpm)	(*E/C*)[a]	(cpm)	(*E/C*)[a]	(cpm)	(cpm)	(*E/C*)[a]	(cpm)	(*E/C*)[a]
1	269	229	(0.85)	156	(0.58)	200	(0.74)	237[c]	11,313[c]	(47.7)	2378	(10.0)
2	199	148	(0.74)	190	(0.95)	171	(0.86)	237[c]	11,313[c]	(47.7)	2583	(10.9)
3	343	307	(0.90)	307	(0.90)	249	(0.73)	143	722	(5.0)	387	(2.7)
4[b]	261	194	(0.74)	185	(0.71)	205	(0.79)	143	722	(5.0)	749	(5.2)
5[b]	631	382	(0.60)	585	(0.93)	271	(0.43)	445	991	(2.2)	1344	(3.0)

[a] *E/C* is the ratio: (counts in DNA from experimental culture)/(counts in DNA from control culture without antigen and RNA).
[b] Cultured in the presence of DEAE-dextran 25 μg/ml (see Results).
[c] Here the same cultures served as the control for the effect of adding two different RNA preparations.

cells and PE cells could not confer upon the strain 13 cells the ability to respond to antigenic stimulation with increased DNA synthesis. The results of three such experiments are shown in the first 3 experiments of Table I. There was no significant difference in the tritiated thymidine uptake of the strain 13 cells when incubated with no additions, with DNP-PLL alone, with RNA alone, or with RNA and antigen. It is also shown in Table I that the RNA, when incubated with lymph node cells obtained from Hartley guinea pigs immunized to DNP-PLL, does not prevent antigen stimulation of increased DNA synthesis in these cells. The RNA did, however, decrease the magnitude of this response.

Since the lack of success in transferring immune competence with the RNA in this system may have been due to inadequate uptake of the RNA by the strain 13 cells, an attempt was made to increase the uptake of RNA by the nonimmune lymph node cells. DEAE-dextran causes a significant increase in the uptake of RNA by strain 13 lymph node cells. This was demonstrated in the following experiment. ^{3}H-RNA extracted from strain 2 lymph node cells which had been PHA stimulated and pulse labeled with ^{3}H-uridine as described by Cooper and Kay (11) was incubated with strain 13 lymph node cells with and without DEAE-dextran at 25 μg/ml. After a 30-min incubation at 37°, the cells were trapped on Millipore filters and washed with PBS, 5% TCA, and 95% ethanol successively as indicated in the Methods section. In the absence of DEAE-dextran there were 1001 ^{3}H cpm cell bound, in the presence of DEAE-dextran (25 μg/ml) there were 4727 ^{3}H cpm cell bound. After 24 hr of incubation, there was no significant difference in the amount of cell bound ^{3}H-RNA, 1595 ^{3}H cpm in the absence of DEAE-dextran, 1841 ^{3}H cpm in its presence.

The effect of DEAE-dextran at several concentrations on the antigen-stimulated tritiated thymidine uptake by lymph node cells obtained from immunized Hartley responder animals is shown in Table II. In the dose range tested, DEAE-dextran was not toxic to the cells. Maximal stimulation by antigen was seen with DEAE-dextran at 25 μg/ml.

TABLE II. The Effect of DEAE-Dextran on DNP-PLL Stimulated DNA Synthesis in Lymph Node Cells from a Hartley Responder Guinea Pig Immunized to DNP-PLL.

DEAE-dextran (μg/ml)	DNP-PLL (μg/ml)	^{3}H (cpm)	(E/C)[a]
0	0	644	—
	1.7	6361	(9.9)
10	1.7	7117	(11.1)
25	1.7	11,005	(17.1)
50	1.7	6060	(9.4)
	0	752	(1.2)

[a] E/C is the ratio: (counts in DNA from experimental cultures with antigen)/(counts in DNA from control cultures without antigen).

RNA extracted from lymph node cells obtained from strain 2 animals immunized with DNP-PLL and incubated with strain 13 lymph node cells in the presence of DEAE-dextran at 25 μg/ml also could not confer upon the strain 13 cells the ability to respond to antigenic stimulation with increased DNA synthesis. The data from 2 experiments of this type are shown on lines 4 and 5 of Table I. Here, too, it is demonstrated that the RNA in the presence of DEAE-dextran does not interfere with the response of sensitized lymph node cells to antigenic stimulation.

Discussion. It has previously been reported that RNA extracted from lymph node cells of guinea pigs immunized to DNP-oligolysine and incubated *in vitro* with a lymph node cell peritoneal exudate cell mixture from strain 13 guinea pigs was able to confer upon the nonresponder cells the ability to respond to the antigen with increased DNA synthesis (7). In the present experiments, despite near duplication of the experimental conditions, we are unable to confirm these results. The experiments differed only in the antigen used. In our experiments DNP-PLL having a chain length of about 550 lysine residues, 62 of which have DNP groups attached to their ϵ-amino group, was used instead of the much shorter (8 or 9 residues) oligolysine. However, previous experiments have shown that both molecules behave similarly as antigens in responder guinea pigs (13).

In the present experiments we have demonstrated that DEAE-dextran increases the uptake of RNA by lymph node cells. Its use in these experiments was predicated on the previously demonstrated increased infectivity of viral RNA in mammalian cells in the presence of DEAE-dextran (14, 15). Its mechanism of action is not clear. The alteration of the three dimensional configuration of nucleic acids by polyamines, presumably mediated by the interaction of the amino groups with the phosphate of the nucleic acid backbone, has been demonstrated (16). Bluestein *et al.* (17) have demonstrated a polyamine mediated conversion of an inactive form of transfer RNA to a fully active configuration. The effect of DEAE-dextran, which is a macromolecular polyamine, may be related to a similar interaction with RNA forming a much larger and more easily pinocytosed complex.

Despite the increased RNA uptake by stain 13 lymph node cells in the presence of DEAE-dextran, RNA extracted from lymph node cells of immunized animals could not transfer to the strain 13 cells the ability to respond to the antigen with increased DNA synthesis.

The present study lends no support to the idea that RNA extracted from lymphoid cells of an immunized donor can transfer immune competence to the lymphoid cells of a nonimmunized animal.

Summary. Ribonucleic acid (RNA) obtained from the lymph nodes of responder strain 2 guinea pigs immunized with DNP-PLL was added *in vitro* to the lymph node cells from normal nonresponder strain 13 guinea pigs. These strain 13 lymph node cells were then tested for their ability to be stimulated to proliferate by the addition of DNP-PLL. Under two different culture conditions normal strain 13 cells exposed to the RNA failed to respond to antigen. These experiments do not lend support to the idea that RNA obtained from immunized lymphoid cells can transfer immunological information to naive lymphoid cells.

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