

## STUDIES ON MEMBRANE-BOUND RECEPTORS FOR ANTIGEN

### PREPARATION OF POPULATIONS OF RECEPTOR-DEPLETED LYMPHOCYTES\*

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While the presence of immunoglobulin receptor molecules on immunocompetent cells had been detected by a variety of procedures (1), much remains to be learned as to the dynamics of their synthesis and function. The adjuvant action on the immune response of complexed synthetic polynucleotides has been described (2) and characterized (3). The cellular targets for this adjuvant seem to include the three cell populations implicated in humoral immunity: the macrophage (4), the thymus-influenced cell (5, 6), and the bone-marrow derived cell (7). Recently, it was reported that complexes of polyadenylic acid-polyuridylic acid (poly A:U)<sup>1</sup> could inhibit both rosette formation and the immune responsiveness of primed spleen cells, presumably by depressing the antigen-binding capacity of the cells (8). The present report confirms the hypothesis that poly A:U depresses the antigen-binding capacity of lymphoid cell populations and demonstrates that the depression of antigen-binding is associated with polynucleotide-mediated stimulation of cell metabolism and release of membrane proteins, resulting in a loss of membrane-bound receptors for antigen.

#### *Materials and Methods*

*Immunizations.*— $\beta$ -Galactosidase ( $\beta$ GZ) was obtained from Worthington Biochemicals, Freehold, N.J. 6-wk old female Balb/c mice (Charles River Breeding Laboratories, Wilmington, Mass.) were inoculated intraperitoneally with 50  $\mu$ g  $\beta$ GZ in Freund's incomplete adjuvant. Studies were initiated 6–10 wk later.

*Cortisone Treatment.*—6 wk after priming with  $\beta$ GZ, the mice received 5.0 mg cortisone

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<sup>1</sup> *Abbreviations used in this paper:*  $\beta$ GZ,  $\beta$ -galactosidase; BSA, bovine serum albumin; cAMP, 3', 5'-cyclic monophosphoric acid; FCS, fetal calf serum; FD $\beta$ G, fluorescein di- $\beta$ -galactopyranoside; FITC-RAMiG, fluorescein conjugated rabbit antimouse immunoglobulin serum; HBSS, Hanks' balanced salt solution; MEM, minimum essential medium; PBS, phosphate-buffered saline; poly A, polyadenylic acid; poly A:U, polyadenylic acid-polyuridylic acid; poly U, polyuridylic acid; TCA, trichloroacetic acid.

(Cortone; Merck, Sharp, and Dohme, Philadelphia, Pa.) intraperitoneally. Spleens were removed for experimentation 72 h after the cortisone injection.

*Antimetabolites.*—Puromycin dihydrochloride (Nutritional Biochemicals Corp., Cleveland, Ohio) was suspended in 0.15 M NaCl to 1 mM. Iodoacetic acid (Mann Research Labs, Inc., New York) was suspended to 1 M in 0.15 M NaCl and the pH adjusted to 7.0 with 0.5 N NaOH. Sodium azide (Alfa Inorganics, Inc., Beverly, Mass.) was suspended to 10 M in 0.15 M NaCl. All antimetabolites were diluted to desired concentration in tissue culture medium.

*Homoribopolynucleotides.*—Polyadenylic acid (poly A), potassium salt (lot no. 73), and polyuridylic acid (poly U), ammonium salt (lot no. 81), were obtained from Miles Laboratories Inc., Miles Research Div., Kankakee, Ill. Each polymer was stored at  $-20^{\circ}\text{C}$  at a concentration of 4 mg/ml in sterile 0.15 M phosphate-buffered saline (PBS), pH 7.2 (0.073 M NaCl, 0.018 M  $\text{KH}_2\text{PO}_4$ , 0.057 M  $\text{Na}_2\text{HPO}_4$ ). Polymers were complexed before use by mixing equal volumes of the two stock solutions for 2 min at room temperature. The polymer complex was diluted to desired concentration in tissue culture medium.

Dibutyl adenosine-3',5'-cyclic monophosphoric acid (cAMP) and theophylline were obtained from Sigma Chemical Corp., St. Louis, Mo. They were suspended in sterile 0.15 M NaCl and diluted to desired concentration in tissue culture medium.

*Cell Suspensions.*—Mice were killed by cervical dislocation. The spleens or thymuses were removed and dispersed in cold Hanks' balanced salt solution (HBSS, Microbiological Associates, Inc., Bethesda, Md.) using a loose-fitting Ten-Broeck tissue homogenizer (Fisher Scientific Co., Springfield, N.J.). The cells were washed by centrifugation at  $450 \times g$  three times in HBSS containing 5% fetal calf serum (FCS, Grand Island Biological Co., Grand Island, N.Y.). After the first wash, red blood cells were lysed by suspending the cells in a hypotonic solution composed of 0.83%  $\text{NH}_4\text{Cl}$  and 0.2% dextran, pH 7.0, for 8 min at  $0^{\circ}\text{C}$ . Adherent cells were removed, when necessary, by incubating the cells at  $37^{\circ}\text{C}$  with glass wool for 15 min. The cells were resuspended to  $1.5 \times 10^7$  cells/ml in either HBSS or Eagle's minimum essential medium (MEM, Hyland Div., Travenol Laboratories, Inc., Costa Mesa, Calif.).

*Polynucleotide Treatment.*—The cell suspensions were warmed to  $37^{\circ}\text{C}$ . Poly A:U was added to the appropriate concentration and the suspension was incubated at  $37^{\circ}\text{C}$  for 10 min. The cells were centrifuged to remove excess poly A:U, resuspended in warm HBSS containing 5% FCS, and incubated for an additional 30 min at  $37^{\circ}\text{C}$ . The cells were washed two times in cold HBSS + 5% FCS before exposure to antigen. The viability of the poly A:U-treated cells, as measured by Trypan blue dye exclusion, was not significantly different than the viability of control cells, both of which were greater than 85%.

*Fluorescent Anti-Ig Assay.*—Fluorescein conjugated rabbit antimouse immunoglobulin serum (FITC-RAM Ig) was kindly provided by Dr. Emil Unanue (9). Spleen cells were suspended in HBSS to  $5 \times 10^7$  cells/ml.  $10 \mu\text{l}$  of the FITC-RAM Ig reagent was added to  $100 \mu\text{l}$  of the cell suspension. The mixture was incubated 30 min at  $0^{\circ}\text{C}$ , washed four times with HBSS + 5% FCS, and resuspended to  $1 \times 10^7$  cells/ml. Smears were fixed in methanol and scanned for fluorescein label.

*$\beta$ -Galactosidase-Binding Assay.*—Antigen ( $\beta\text{GZ}$ ) binding activity of cell suspensions was determined by a method which is based on the ability of  $\beta\text{GZ}$  to hydrolyze a fluorogenic substrate (10). Briefly, spleen cells to be assayed were suspended to  $5 \times 10^7$  cells/ml in HBSS + 5% FCS.  $\beta\text{GZ}$  was added to a final concentration of  $50 \mu\text{g/ml}$ . The suspensions were incubated at  $0^{\circ}\text{C}$  for 60 min and then washed by centrifugation four times in HBSS. Replicate 0.2 ml samples (containing approximately  $3 \times 10^6$  cells) were dispensed to fluorometer tubes. An equal volume of  $4 \times 10^{-5}$  M fluorescein di- $\beta$ -galactopyranoside (FD $\beta\text{G}$ , Schwarz/Mann Div., Becton, Dickinson, and Co., Orangeburg, N.Y.), a fluorogenic substrate for  $\beta\text{GZ}$ , was then added to the tubes. Controls for the determination of specific activity of the enzyme ( $\beta\text{GZ}$ ) consisted of triplicate tubes containing 1 ng  $\beta\text{GZ}$  in 0.2 ml HBSS and 0.2 ml  $4 \times 10^{-5}$  M FD $\beta\text{G}$ . The assay tubes were incubated at  $37^{\circ}\text{C}$  for 10–90 min. At regular intervals (10–20

min), the tubes were read in a fluorometer and the rate of fluorescein release (FD $\beta$ G breakdown) determined. The amount of  $\beta$ GZ bound to the cells was calculated as follows:

$$\text{pg } \beta\text{GZ bound}/10^6 \text{ cells} = \frac{\Delta\text{FU}/\text{min}}{N \times S_a}$$

where  $\Delta$ FU equals increase in fluorometer units, N equals number of cells ( $\times 10^{-6}$ ), and  $S_a$  equals specific activity of  $\beta$ GZ ( $\Delta$ FU/min per pg).

*Radioiodination of Membrane Proteins.*—The membrane proteins of spleen cells were radioiodinated by the technique described by Marchalonis et al. (11). Carrier-free  $^{125}\text{I}$  (50 mCi/ml,  $\text{Na}^{125}\text{I}$  in 0.1 N NaOH) was obtained from New England Nuclear, Boston, Mass. Lactoperoxidase (B-grade, lyophilized) was obtained from Calbiochem, San Diego, Calif. Spleen cells were suspended in PBS, pH 7.2, to  $1.5 \times 10^7$  cells/ml. The cells were dispensed in 10 ml aliquots to 50 ml plastic screw-capped centrifuge tubes. To each 10 ml aliquot of cells was added 0.4 ml lactoperoxidase (0.3 mg/ml), 0.05 ml  $^{125}\text{I}$  (diluted in PBS to 5 mCi/ml), and 0.6 ml of 10  $\mu\text{M}$   $\text{H}_2\text{O}_2$ . After incubation for 5 min at 30°C, 30 ml of cold PBS containing 5 mM cysteine (L-cysteine HCl, Nutritional Biochemicals Corp.) was added. The cells were washed one time with PBS + 5 mM cysteine, two times with HBSS, and resuspended to  $1.5 \times 10^7$  cells/ml in HBSS. Poly A:U was added to half the cells to a final concentration of 100 ng/ml. Both poly A:U-treated and untreated (control) cells were incubated for 10 min at 37°C, centrifuged, resuspended to  $1.5 \times 10^7$  cells/ml in Eagle's MEM, and returned to the 37°C water bath. At regular intervals, 0.5 ml samples were removed. The cells were eliminated by centrifugation at  $500 \times g$  and the supernatant was held at 0°C until all samples were collected. To each sample was added 50  $\mu\text{l}$  of 10% bovine serum albumin (BSA, Armour Pharmaceutical Co., Chicago, Ill.) and 0.5 ml cold 10% trichloroacetic acid (TCA) and the mixture was held at 0°C for 15 min. The precipitate was collected on a 25 mm Millipore filter (Millipore Corp., Bedford, Mass.) (0.2  $\mu\text{m}$ ), washed two times with 5 ml of 5% TCA and one time with 5 ml of 1 N HCl. The filters were dried 1 h at 80°C and counted in a Packard Gamma Scintillation Spectrometer (Packard Instrument Co., Inc., Downers Grove, Ill.).

*Determination of Protein and RNA Synthesis.*—Tritiated uridine (1 mCi/ml, 20 mCi/mg) and [ $^{14}\text{C}$ ]L-amino acids (0.1 mCi/ml containing ala, asp, arg, glu, gly, his, ile, leu, lys, phe, pro, ser, thr, tyr, and val) were obtained from New England Nuclear. Spleen cells were suspended in HBSS to  $1.5 \times 10^7$  cells/ml. Poly A:U was added to half the cells to a final concentration of 100 ng/ml. Both poly A:U-treated and untreated (control) cells were incubated at 37°C for 10 min, centrifuged, and resuspended to  $1.5 \times 10^7$  cells/ml in Eagle's MEM. To each 2 ml culture was added 2  $\mu\text{Ci}$  [ $^{14}\text{C}$ ]L-amino acid mixture and 10  $\mu\text{Ci}$  [ $^3\text{H}$ ]uridine. The cells were incubated at 37°C. At regular intervals, 0.2 ml samples were withdrawn, frozen in dry ice-ethanol, and held at  $-70^\circ\text{C}$  until all samples had been collected. The samples were thawed and dispersed in 2.0 ml 5% TCA for 15 min at 0°C. The precipitate was collected on a 25 mm Millipore filter (0.2  $\mu\text{m}$ ), washed two times with 5 ml of 5% TCA and one time with 5 ml of 1 N HCl. The filters were dried for 1 h at 80°C, dispersed in 5 ml of toluene-Omnifluor (New England Nuclear) and counted in a Packard Tri-Carb liquid scintillation counter (Packard Instrument Co.).

## RESULTS

Approximately  $\frac{1}{2}$  of the nucleated cells in mouse spleen carry immunoglobulin determinants on their surface membrane as indicated by the observation that >40% become labeled when exposed to FITC-RAMiG serum (Table I). However, if, before exposure to the FITC-RAMiG serum, the spleen cells were

incubated for 30 min at 37°C after a 10 min exposure to 100 ng/ml poly A:U, less than 25% of the cells could be scored positive for fluorescein label (Table I).

Concomitant with the apparent loss of surface Ig determinants upon treatment with poly A:U was a loss of antigen-binding capacity. When spleen cells primed with  $\beta$ GZ were exposed to poly A:U, there was a time-dependent decrease in the amount of  $\beta$ GZ subsequently bound per  $10^6$  cells (Fig. 1). After a 40 min incubation period, the poly A:U-treated cells bound only 93 pg  $\beta$ GZ/ $10^6$  cells, which was only 40% of the 253 pg  $\beta$ GZ-bound per  $10^6$  untreated control cells (Fig. 1).

The depression of antigen-binding capacity was seen only within a limited

TABLE I  
*Poly A:U-Mediated Depression of Numbers of Cells Labeling with FITC-RAM Ig*

Spleen cell pretreatment	No. of cells positive/no. examined	Fluorescent positive cells	
		%	
HBSS	351/832	42.2	
Poly A:U	169/813	20.8	

Spleen cells from mice primed with  $\beta$ GZ were incubated 30 min at 37°C after a 10 min exposure to 100 ng/ml poly A:U. The cells were washed three times before exposure to FITC-RAM Ig.

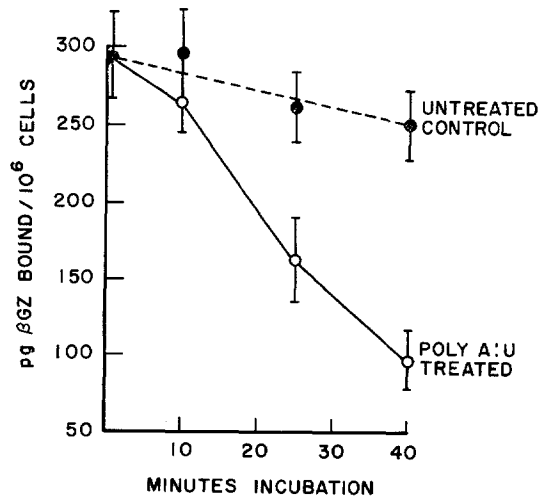


FIG. 1. Poly A:U-mediated depression of specific antigen-binding by primed spleen cell populations. Spleen cells obtained from mice primed with  $\beta$ GZ were exposed to 100 ng/ml poly A:U for 10 min, centrifuged, resuspended in HBSS + 5% FCS, and returned to the 37°C water bath. Samples were removed before exposure to poly A:U, after the 10 min exposure, and at 15 min intervals thereafter, and assayed for  $\beta$ GZ-binding capacity. Vertical bars represent the 95% confidence limits around the arithmetic average of three samples.

concentration range of poly A:U (Fig. 2). Maximum depression (approximately 60%) of  $\beta$ GZ-binding was observed when the cells were exposed to 0.01–1.0  $\mu$ g/ml poly A:U. Concentrations below or above this range were less effective (Fig. 2).

To determine if the poly A:U-mediated depression of antigen-binding capacity of primed spleen cells involved an active metabolic process, two experimental approaches were followed: the effect of poly A:U on the incorporation of radiolabeled metabolic precursors into TCA insoluble cell fractions and the

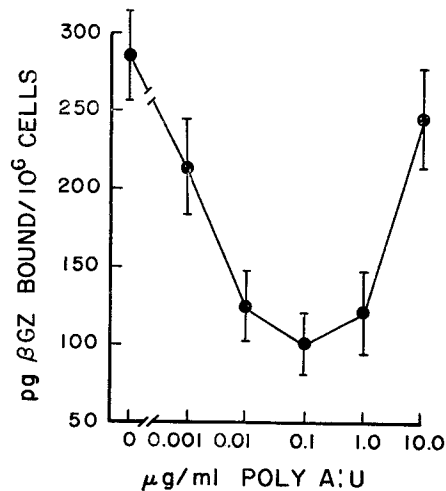


FIG. 2. Concentration dependency of poly A:U effect on primed spleen cells. Spleen cells obtained from mice primed with  $\beta$ GZ were exposed to 0.001–10  $\mu$ g/ml poly A:U for 10 min at 37°C, centrifuged, resuspended in HBSS + 5% FCS, and incubated for 30 min at 37°C. The cells were then washed and assayed for  $\beta$ GZ-binding capacity. Each point represents the arithmetic average of three samples. Vertical bars represent the 95% confidence limits.

effect of metabolic poisons on the poly A:U-mediated depression of antigen-binding capacity.

To determine the effect of poly A:U on RNA and protein synthesis by primed spleen cells, spleen cells were exposed to 100 ng/ml poly A:U for 10 min at 37°C, centrifuged, and resuspended in Eagle's MEM containing [ $^3$ H]uridine and [ $^{14}$ C]amino acids. Aliquots of the cells were removed at regular intervals over a 4 h period and assayed for TCA-precipitable  $^{14}$ C and  $^3$ H. The poly A:U treatment caused a significant increase in [ $^3$ H]uridine and [ $^{14}$ C]amino acid incorporation after 2–3 h incubation (Fig. 3).

To determine the effect of metabolic poisons on the poly A:U effect, spleen cells primed with  $\beta$ GZ were incubated at 37°C with 0.1 M azide or  $10^{-3}$  M iodoacetate for 1 h, or with  $10^{-5}$  M puromycin for 2 h, and then treated with poly A:U in the presence of these metabolic poisons. As can be seen in Table II,

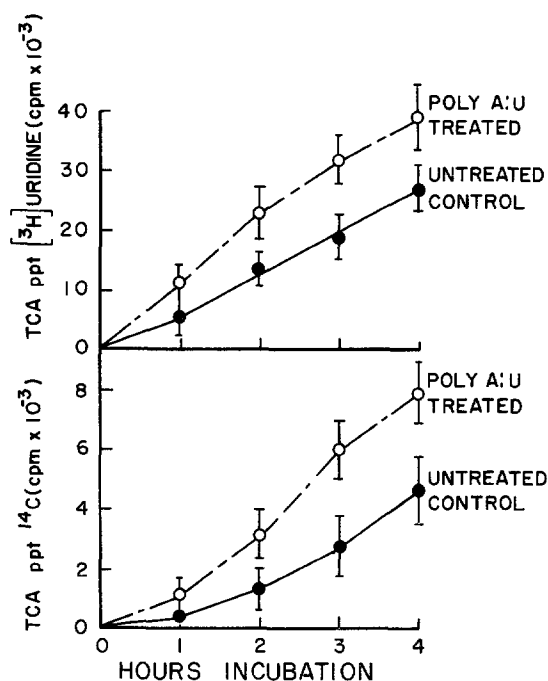


FIG. 3. Effect of poly A:U on RNA and protein synthesis by primed spleen cells. Spleen cells from mice primed with  $\beta$ GZ were exposed to 100 ng/ml poly A:U for 10 min at 37°C, centrifuged, and resuspended in Eagle's MEM containing [<sup>3</sup>H]uridine and [<sup>14</sup>C]amino acids. Samples were removed after various intervals at 37°C and assayed for TCA precipitable <sup>3</sup>H and <sup>14</sup>C. Each point represents the arithmetic average of three samples. Vertical bars represent the 95% confidence limits.

TABLE II

*Effect of Antimetabolites on the Poly A:U-Mediated Depression of Antigen-Binding Capacity of Primed Spleen Cells*

Antimetabolite	pg $\beta$ GZ bound/10 <sup>6</sup> cells	
	Control cells	Poly A:U-treated cells
None	322 ± 79	128 ± 42
0°C, 40 min	291 ± 71	317 ± 87
0.1 M azide, 1 h, 37°C	267 ± 91	302 ± 106
10 <sup>-3</sup> M iodoacetate, 1 h, 37°C	316 ± 80	383 ± 98
10 <sup>-5</sup> M puromycin, 2 h, 37°C	241 ± 83	96 ± 29

Spleen cells from mice primed with  $\beta$ GZ were incubated with an antimetabolite before, during, and after exposure to poly A:U. They were then tested for their ability to bind  $\beta$ GZ. It can be seen that the poly A:U effect took place only on cells whose metabolism was not arrested by cooling, by azide, or by iodoacetate. Puromycin did not prevent the loss of antibody activity.

treatment of the cells with poly A:U at 0°C or in the presence of iodoacetate or azide at 37°C did not result in a reduction of antigen-binding capacity of the cells, whereas treatment of the cells with poly A:U at 37°C in the absence of these poisons or with puromycin caused a 60% decrease in antigen-binding.

Due to the increased metabolic activity of the poly A:U-treated cells and the sensitivity of the suppressive effect to respiratory poisons, it was considered possible that the decreased levels of  $\beta$ GZ bound to the cells might be due to an increased rate of endocytosis of the receptor-antigen complex during the short 37°C incubation required for assay of the enzyme. To exclude this possibility, poly A:U-treated spleen cells were fixed with glutaraldehyde before exposure to  $\beta$ GZ. Glutaraldehyde fixation did not affect the level of  $\beta$ GZ bound by control cells, nor did it affect the level of  $\beta$ GZ bound by the poly A:U-treated cells, which remained at levels less than 40% of the control cells (Table III).

To investigate the hypothesis that the poly A:U treatment resulted in an

TABLE III  
*Effect of Glutaraldehyde Fixation on  $\beta$ GZ-Binding by Poly A:U-Treated Spleen Cells*

Spleen cell pretreatment	pg $\beta$ GZ bound/10 <sup>6</sup> cells	
	Nonfixed	Fixed
HBSS control	318 $\pm$ 81	356 $\pm$ 77
Poly A:U-treated	112 $\pm$ 49	131 $\pm$ 38

Spleen cells from mice primed with  $\beta$ GZ were treated with poly A:U, washed, and divided into two groups: one was treated with 1% glutaraldehyde for 15 min at 4°C (fixed), the other was held at 0°C without treatment (nonfixed). The cells were washed and assayed for  $\beta$ GZ-binding capacity.  $\pm$  values represent the 95% confidence limits.

“elution” of receptors for antigen from the membrane, the membrane proteins of spleen cells were labeled with <sup>125</sup>I by the technique of Marchalonis et al. (11). The cells were exposed to 100 ng/ml poly A:U for 10 min at 37°C, centrifuged, resuspended in Eagle’s MEM, and incubated at 37°C. At regular intervals over a 3 h period, aliquots were removed and the supernatants assayed for TCA precipitable <sup>125</sup>I. As can be seen in Fig. 4, poly A:U treatment of the spleen cells resulted in an increased (> two times) rate of release of membrane proteins from the cells within the first hours of incubation.

As has been reported by others regarding the immunoenhancing effects of poly A:U (12), the “stripping” effect of poly A:U seems to involve the adenyl cyclase system. Thus, treatment of spleen cells with dibutyryl cAMP reduced the levels of  $\beta$ GZ-binding to the same extent (60%) as did treatment with poly A:U (Table IV). Theophylline reduced binding only to a small degree (28%) but acted synergistically with suboptimal concentrations of poly A:U (Table IV).

Finally, to gain some insight into the cell targets of poly A:U, four cell

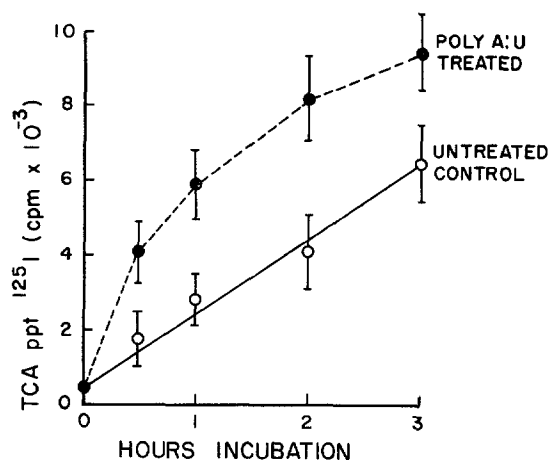


FIG. 4. Effect of poly A:U on the release of  $^{125}\text{I}$ -labeled membrane proteins. Membrane proteins of spleen cells from mice primed with  $\beta\text{GZ}$  were labeled with  $^{125}\text{I}$ . The cells were then exposed to 100 ng/ml poly A:U for 10 min at  $37^\circ\text{C}$ , centrifuged, and resuspended in Eagle's MEM. Samples were removed at regular intervals and the supernatants assayed for TCA precipitable  $^{125}\text{I}$ . Each point represents the arithmetic average of three 0.5 ml samples. Vertical bars represent the 95% confidence limits.

TABLE IV

*Effect of Dibutyryl cAMP and Theophylline on the Antigen-Binding Capacity of Primed Spleen Cells*

Cell pretreatment	pg $\beta\text{GZ}$ bound/ $10^6$ cells	Depression %
None	$306 \pm 61$	—
Poly A:U, 100 ng/ml	$134 \pm 46$	56
Dibutyryl cAMP, 1 ng/ml	$116 \pm 39$	62
Theophylline, 1 ng/ml	$219 \pm 44$	28
Poly A:U, 10 ng/ml, + theophylline, 1 ng/ml	$107 \pm 31$	65

Spleen cells from mice primed with  $\beta\text{GZ}$  were incubated for 10 min at  $37^\circ\text{C}$  with the indicated reagent. The suspensions were then centrifuged and resuspended in HBSS + FCS containing, in the case of cAMP or theophylline treatment, 1 ng/ml of the respective reagent. The suspensions were incubated 30 min at  $37^\circ\text{C}$ , washed, and assayed for  $\beta\text{GZ}$ -binding capacity.  $\pm$  values represent the 95% confidence limits.

populations were prepared: thymus cells, spleen cells, nonadherent (glass wool-absorbed) spleen cells, and cortisone-treated spleen cells. Each cell population was treated with poly A:U and then assayed for  $\beta\text{GZ}$  binding capacity. The polynucleotide treatment reduced binding in thymocyte populations by 75%, in intact spleen cell populations by 47%, in nonadherent spleen cell populations by 60%, and in cortisone-treated nonadherent spleen cells by 59% (Table V).



TABLE V  
*Poly A:U-Mediated Depression of Specific Antigen-Binding by Normal Thymus and Primed Spleen Cells*

Cell population	pg $\beta$ GZ bound/ $10^6$ cells	
	Control cells	Poly A:U treated cells
Spleen cells	418 $\pm$ 83	223 $\pm$ 72
Nonadherent spleen cells	367 $\pm$ 69	145 $\pm$ 47
Cortisone-treated nonadherent spleen cells	196 $\pm$ 74	80 $\pm$ 31
Thymus cells	92 $\pm$ 19	21 $\pm$ 6

Spleen cells were obtained from mice primed with  $\beta$ GZ and were prepared as described in Materials and Methods. Thymus cells were obtained from normal (not primed) 6-wk old mice. The cells were incubated 30 min at 37°C after a 10 min exposure to poly A:U, washed, and assayed for  $\beta$ GZ-binding capacity.  $\pm$  values represent the 95% confidence limits.

#### DISCUSSION

The data presented in this paper demonstrate that the antigen-binding capacity of both thymus and spleen lymphocytes can be significantly reduced by incubating the cells with synthetic polynucleotides. This reduction in antigen-binding is not due to a steric interference of antigen-binding by the polynucleotides since: (a) the effect is not seen with concentrations of poly A:U in excess of 1  $\mu$ g/ml; (b) the effect is not seen if the cells are treated with poly A:U at 0°C or in the presence of metabolic poisons; and (c) a similar effect is obtained with dibutyryl cAMP.

Evidence that labeled globulin recovered from whole cells which were iodinated ( $^{125}$ I) by the lactoperoxidase reaction was actually on the cell-surface has been reported by Marchalonis et al. (11); using autoradiography and electron microscopy they found the label associated with the cell-membrane. Vitetta et al. (13), after labeling whole cells, and then disintegrating them, found the activity associated only with the membrane fraction. We have not looked for the  $^{125}$ I in the experiments reported here; but, as noted in the Results,  $\gamma$ -globulin was identified on the surface by immunofluorescence.

It is therefore suggested that the incubation of the cells with polynucleotides results in a loss of the receptors for antigen from the cell membrane. The mechanism by which polynucleotides remove the receptors from the membrane is not entirely understood at this time. However, it has been demonstrated that the immunoglobulin receptors for antigen on both thymocytes (14) and spleen cells (14, 15) are constantly turning over on the cell membrane. Treatment of the cells with the polynucleotides accelerates this turnover within a 30 min period, a time in which the polynucleotide stimulation of RNA and protein synthesis has not yet become apparent (cf. Figs. 3 and 4). The loss of immunoglobulin receptors may therefore be the result of accelerated membrane turnover in the absence of comparable increases in protein synthesis—i.e., the im-

munoglobulin may be released from the cell membrane faster than it can be replaced. The only other evidence for this hypothesis at this time is that respiratory poisons, which have been shown to inhibit the turnover of membrane immunoglobulin (14), prevent the loss of immunoglobulin from polynucleotide-treated cells (Table II).

The physiological significance of receptor depletion is presently under study. Such depletion has been observed after treatment of the cells with anti-Ig reagents at 37°C (16, 17). The time-course of depletion and subsequent recovery of membrane immunoglobulin after treatment with anti-Ig serum (16, 17) is remarkably similar to that reported for the reduction and subsequent recovery of rosette formation by spleen cells treated with polynucleotides (8, 18). The depletion of receptors by polynucleotide treatment has been shown to result in impaired antibody responses after adoptive transfer of the treated cells to irradiated recipients (8). Such impairment is probably due to the decreased antigen-binding capacity of the receptor depleted cells.

The binding of antigen by cell populations from the spleen and lymph nodes of normal mice has been described and reviewed by Sulitzeanu (1), from normal mouse thymus and bone marrow by Sercarz et al. (19), and from normal thymus by Modabber et al. (20). The significance of such cells is not clear, although it is unlikely that it is due to antibody resulting from antigenic stimulation from *Escherichia coli* in the intestine because their numbers are comparable in neonatal, germfree, and adult mice.<sup>2</sup>

#### SUMMARY

The effect of polyadenylic: polyuridylic acid complexes (poly A:U) on the amount of antibody on the surface of various populations of mouse lymphoid cells has been investigated by means of a sensitive measure of such activity—the binding by primed cell populations of  $\beta$ -galactosidase ( $\beta$ GZ) as an antigen. The sensitivity derives from the liberation of fluorescein from an artificial substrate, fluorescein-di- $\beta$ -galactopyranoside (FD $\beta$ G). After incubation with 100 ng/ml of poly A:U, only 40% of the cells previously showing antigen-binding were still active. The optimum range of activity lay between 0.01–1.0  $\mu$ g/ml poly A:U. Such cells showed increased RNA and protein synthesis as indicated by [<sup>3</sup>H]uridine and [<sup>14</sup>C]amino acid incorporation. The polynucleotide effect was abolished by incubation of the cells with sodium azide or iodoacetate, but not by puromycin. When the proteins on the cell surface were labeled by <sup>125</sup>I, poly A:U caused their release into the medium.

Reports by others that the enhancing effect of polynucleotides on the immune response involves the adenylylase system are consistent with the finding reported here that reduction of binding by dibutyl 5'-cyclic monophosphoric

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<sup>2</sup> Swain, S. Personal communication.

acid (cAMP) and poly A:U were parallel in extent, and that theophylline and poly A:U acted synergistically in suboptimal concentrations of each.

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