CELLULAR IMMUNOLOGY 39, 345–354 (1978)

Regulation of the Immune System by Synthetic Polynucleotides

VII. Suppression Induced by PREtreatment with Poly A : U [1]

CLARICE K. MORRIS [2] AND ARTHUR G. JOHNSON

Department of Microbiology, The University of Michigan, Ann Arbor, Michigan 48109

Received January 3, 1978

Pretreatment of mouse spleen cells with polyadenylic-polyuridylic acid complexes (poly A : U) either *in vivo* or *in vitro* 24 hr prior to addition of antigen, resulted in a substantial time dependent decrease in anti-SRBC PFC. Enhancement was observed 6 hr after poly A : U, while inhibition did not become evident until 24 hr after pretreatment. Inhibition of the PFC response appeared to result from poly A : U activation of a nylon wool adherent, T suppressor cell, capable of diminishing the response of normal spleen cells exposed to antigen on co-culture.

INTRODUCTION

The homoribopolymer complex of polyadenylic acid-polyuridylic acid (poly A : U)[3] has been shown to be a potent adjuvant to the immune response, acting primarily on the T cell to increase its functional capacity (1). The adjuvant action of poly A : U was greatest when administered at the time of antigenic stimulation; however, if the same adjuvant dose of poly A : U were administered 24 hr prior to antigenic stimulation, the serum antibody response was drastically inhibited (2). The inhibition has been confirmed with both poly A : U and poly I : C in other laboratories (3, 4). In addition, Colmerauer *et al.* (5) and Cunnington and Naysmith (6) have broadened this phenomenon by documenting that double stranded RNA also inhibits cell mediated immunity and humoral immunity if given 1 day before antigen. With the conceptual clarification by Gershon (7) and others, that the negative aspect of immunoregulation might be due to suppressor cells, we have studied the possible generation of suppressor cells by poly A : U when given prior to antigen. The data described herein support this concept as the mechanism of immunosuppression by poly A : U.

[1] This work was supported by USPHS Grant AM 14273 and the American Cancer Society Institutional Research Grant IN-40-0.

[2] F. G. Novy Research Fellow. This work was submitted as partial fulfillment of the requirements for the degree of Doctor of Philosophy at The University of Michigan, 1977.

[3] Abbreviations used in this paper: poly A : U, equimolar complex of polyadenylic and polyuridylic acid; NSC, normal spleen cells; AUSC, spleen cells from mice pretreated with poly A : U; FCS, fetal calf serum; SRBC, sheep red blood cells; F_1 mice, (C57Bl/6 × C3H)F1 mice; PFC, plaque forming cells.

0008-8749/78/0392-0345$02.00/0

MATERIALS AND METHODS

Animals. Hybrid (C57Bl/6 × C3H)F_1 mice, herein termed F1 mice, inbred in our laboratory from parental strains obtained from Microbiological Associates (Baltimore, MD), or Jackson Laboratories (Bar Harbor, Maine) were used.

Antigen. Sheep red blood cells (SRBC) were obtained from the Colorado Serum Co., Denver, CO, from a single sheep, No. 466 (4, 8). The cells were prepared for use in the *in vitro* culture system by the method described previously (9).

Homopolyribonucleotides. Polyadenylic acid (poly A), potassium salt (Lot 81) and polyuridylic acid (poly U), ammonium salt (Lot 92) were purchased from Miles Laboratories, Kankakee, IL. Polymers were complexed *in vitro* to form poly A : U by mixing equal amounts of the polynucleotides in 0.15 *M* NaCl before use.

Cell preparations. Single cell suspensions from spleens of F_1 mice were prepared as described previously (9). Spleen cells depleted of B cells were obtained by treatment with anti-immunoglobulin serum and/or by passage of the spleen cells over a nylon wool column. Rabbit anti-mouse immunoglobulin serum was prepared by Dr. J. Latham Claflin, University of Michigan, and spleen cells treated with a 1 : 6 dilution in the manner described previously (10). Approximately 70% of the presumed B cells were killed by this procedure. Spleen cells, 5×10^7, were passed over nylon wool columns according to the method of Julius (11). The nylon wool was packed in all but the top one-fourth inch of the 10 ml, glass pak syringe. The viability of the 20 to 30% cells recovered in the eluate was greater than 90%.

Spleen cells depleted of T cells were obtained by treatment with anti-theta serum and/or by passage of the cells over nylon wool columns. The method of Reif and Allen (12) was used for the preparation of antisera specific for the mouse C3H-θ antigen (Thy 1.2). Briefly 10^7 spleen cells/ml were treated with an equal volume of anti-theta serum diluted 1 : 16 (a dilution at which 98% of the thymocytes were killed) for 20 min at 37°C. The cells were washed once, resuspended to the original volume in culture medium, as defined below, treated with an equal volume of guinea pig complement for 45 min at 37°C, and washed twice. Thirty to 40% of the cells were recovered. To elute the B cells that had adhered to the column, 4 ml of culture medium were put on the column and forced through with a 10 ml syringe plunger, and the process repeated. The viability was greater than 90%, and 20 to 30% of the cells were recovered.

Spleen cells depleted of macrophages were obtained by treatment of 2×10^8 cells in 5 ml medium with 100 mg sterilized carbonyl iron (Atomergic Chemical Co., New York N.Y.), and separation via a Ficoll-Hypaque gradient after incubation for 2.5 hr in a shaking water bath at 37°C. This treatment resulted in a population containing about 99% lymphocytes as determined by morphology, and a recovery rate of 50 to 58%.

The populations derived from the above procedures were evaluated for purity by the ability to mount an antibody response and to respond to T and B cell mitogens. The mitogens used were LPS, (SMTCA-AA4), and CON A (Lot C-20 101 Sigma Chemical Co.).

Cultures. Culture conditions were essentially those of Mishell and Dutton (13) with some modifications. Culture media consisted of: distilled, deionized, pyrogen-free water—85 ml; 10× Hank's minimal essential medium without $NaHCO_3$–10 ml; 100× vitamins—1 ml; 100× nonessential amino acids mixture–1 ml; 50× essential

amino acids mixture—2 ml; 200 m*M* glutamine—1 ml; 100 m*M* sodium pyruvate—1 ml; 7.5% $NaHCO_3$—3.9 ml; 1 *M* Hepes buffer—1.17 ml; and 10^4 units/ml penicillin—10^4 mcg/ml; streptomycin—250 mcg; fungizone—1 ml, all from Microbiological Associates, Bethesda, MD. Other reagents included: heat-inactivated fetal calf serum (FCS) (Lot 23012, Reheis Chem. Co., Chicago, IL)—10 ml and 2-mercaptoethanol, 5×10^{-5} *M* final concentration. Cultures consisted of 1 to 1.2×10^7 spleen cell/ml and 8 to 9×10^6 SRBC. Generally PFC responses were determined on Day 5. Cell viabilities were determined by trypan blue dye exclusion. Direct PFC assays were carried out as described previously (9). The responses of the experimental groups were converted to percentages of the control group with the latter equated to 100%.

Poly A: U activation of spleen cells. In vivo activation was achieved by injecting 5 to 7 month old hybrid mice iv with 300 μg poly A: U 24 hr prior to removal of their spleens. *In vitro* activation was achieved by treatment of 10^7 spleen cells in serum-free H3 medium with 1 to 0.1 μg poly A: U for 24 hr under Mishell–Dutton conditions. The cells recovered from this activation were washed twice; the recovery was 70%.

Statistical methods. The *t* test for the difference between two means was employed when animals were pretreated *in vivo* with poly A: U, as in these cases assays were performed on individual mice. In studies involving poly A: U induced suppressor cells (*in vitro*), the *t* test for paired observations was used since the assay system for both the suppessor and control cells came from the same pooled populations.

RESULTS

Inhibition induced by poly A: U in vivo. F_1 mice, 5 to 7 months of age, were injected with 300 μg poly A: U 24 hr prior to removal of their spleen cells and stimu-

TABLE 1

Inhibition of the *in Vitro* PFC Response Following Injection of Poly A:U 1 Day Prior to Culture[a]

Experiment No.	% of control PFC/culture on Day							
	3		4		5		6	
	NSC	AUSC	NSC	AUSC	NSC	AUSC	NSC	AUSC
1	100[b] (135)[c]	11	100 (2,275)	8	100 (3,900)	8	100 (2,500)	20
2	100 (1,634)	15	100 (8,600)	7	100 (23,000)	9	100 (10,000)	42
3	100 (1,900)	4	100 (16,000)	4	100 (17,500)	13	100 (7,750)	25
Mean ± 95% confidence	100 ± 10[d]	10 ± 14	100 ± 10	6 ± 5	100 ± 10	10 ± 6	100 ± 10	29 ± 28

[a] (C57B1/6 × C3H)F_1 mice were injected iv with 300 μg poly A:U or saline 24 hr prior to sacrifice. 10^7 spleen cells from poly A:U treated (AUSC) or saline treated (NSC) mice were stimulated with 9×10^6 SRBC, and assayed 3–6 days later.

[b] The data were normalized such that the response of the saline treated animals was equated to 100% on each day of assay, and the experimental response converted to the appropriate percentage of the control response.

[c] Values in parenthesis are the actual numbers of PFC/culture.

[d] *P* values for all days were 0.01.

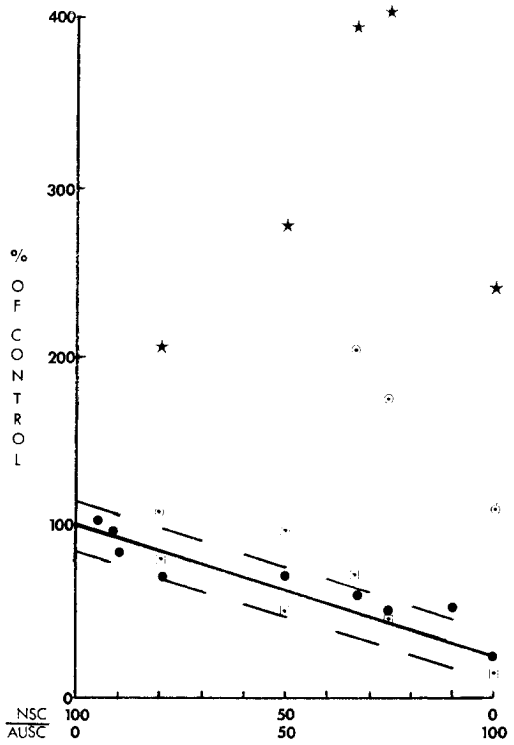

FIG. 1. Spleen cells were taken from normal mice or mice that had been injected intravenously with 300 μg poly A:U 6 (*), 12 (⊙), 24 (●), or 48 (⊡) hr prior to culture separately or in varying ratios of normal to A:U treated cells. The cell density was kept constant at 10^7 cells. The antigen-stimulated cultures were assayed for direct PFC's on Day 5. The data were normalized such that the normal PFC response was equated to 100%. The solid line represents the expected response if no interaction occurred between the two cell populations and is based on the normalized control value of 100% (NSC:AUSC = 100:0) and the mean percentage of 26% for the poly A:U treated group (NSC:AUSC = 0:100). The standard error of the population was 7% and the dashed lines represents the 95% confidence limits for the expected response.

lation with SRBC *in vitro*. When direct PFC were measured 3 to 6 days later, drastic inhibition of the response of mice pretreated with poly A:U as compared to the normal was evident regardless of the day of assay (Table 1). In further dose response experiments (data not shown) in which mice were injected with either 30, 100, 300, or 500 μg poly A:U 24 hr prior to spleen cell culture, like inhibition was seen at the 30 and 100 μg levels (10 to 15% of normal), as well as the 300 μg dose (30% of normal); no inhibition was induced by the high dose of 500 μg.

To determine the minimum time interval after poly A:U injection for inhibition to become measureable, spleens were removed 6, 12, 24, or 48 hr following injection of the polynucleotides, and subsequently cultured with SRBC for 5 days. When just the AUSC populations were examined, it appeared that an induction period of greater than 12 hr following *in vivo* activation with poly A:U was required to generate the inhibitory effect. Interestingly, enhancement was observed after 6 hr of pretreatment (239%), while treatment for 12 hr did not alter the response as compared to the control (111%). However, 24 and 48 hr after poly A:U in-

TABLE 2

Inhibition of the PFC Response Following Pretreatment of Spleen Cells with Poly A:U *in Vitro* 24 hr Prior to Antigenic Stimulation[a]

Pretreatment of spleen cells with:	% Control PFC/culture in Expt.	
	1	2
Medium	100 (1100)	100 (4000)
1 μg poly A:U	11	14
0.5 μg poly A:U	35	17
0.1 μg ploy A:U	71	47

[a] 10^7 spleen cells from (C57B1/6 × C3H)F_1 mice were put in culture on Day −1 and treated with 0.1 ml medium or poly A:U[1]. On Day 0 ,SRBC were added and the PFC response determined on Day 5. The experimental groups (poly A:U) were compared to the control group (medium) which was standardized to 100%. The actual number of plaques for the control is presented in parentheses below the normalized value.

jection, the response was only 26 and 11% respectively of the normal (Fig. 1, NSC: AUSC = 0: 100).

To determine whether the inhibition observed was transferrable and therefore indicative of suppressor cell activity, various proportions of normal spleen cells and spleen cells from mice pretreated with poly A: U for the various lengths of time were mixed and co-cultured with antigen. As may be seen in Fig. 1, under these conditions the inhibition evident when the AUSC were cultured alone (NSC: AUSC = 0: 100) could not be transferred to a normal population at any of the ratios tested, regardless of the length of pretreatment. In fact, an enhanced state was transferred in several of the mixtures.

However, transfer of the inhibition could be achieved when we utilized a completely *in vivo* system. For example, mice were injected with 300 μg poly A: U or saline 24 hr prior to spleen removal, and either AUSC or NSC transferred in graded doses iv into normal mice. Five days following antigenic stimulation *in vivo* with 8×10^7 SRBC ip, a 77% inhibition of the normal response following transfer of 10^8 AUSC as compared to 10^8 NSC was seen. The extent of suppression appeared to be dependent on the number of poly A: U treated cells transferred, in that 10^7 cells gave only a 33% inhibition and 10^6 cells resulted in an actual enhancement of the response (290%). A repeat of this experiment gave essentially similar values.

In vitro activation of suppressor cells with poly A: U. Analysis of this suppressive phenomenon was facilitated by development of a system wherein suppression was both induced and tested *in vitro*. Thus, normal spleen cells were incubated *in vitro* with graded doses of poly A: U for 24 hr prior to the addition of antigen to the cultures. Five days later, it was found (Table 2) that strong dose dependent inhibition, similar to that resulting from *in vivo* petreatment could be achieved by an *in vitro* exposure to poly A: U.

To determine whether this inhibition observed *in vitro* was transferrable, graded doses of poly A: U were incubated with normal spleen cells in serum-containing H3 medium for 24 hr. AUSC cells, 10^6, then were added to normal spleen cells plus antigen in Mishell–Dutton cultures on Day 0. The results varied from experiment

TABLE 3

Inhibition of Normal PFC Response Following Co-culture with Poly A:U Activated Spleen Cells

10^7 NSC + 10^6 cells treated with:	Mean % control PFC/culture
Medium	100 (560)
10 μg Poly A:U	83 ± 43
1 μg Poly A:U	58 ± 17
0.1 μg Poly A:U	75 ± 30

10^7 spleen cells from (C57B1/6 × C3H)F_1 mice were activated in serum containing medium with or without poly A:U for 24 hr. The cells were scraped from the culture dish, washed three times and counted. 10^6 of these cells were added to 10^7 NSC plus 8 to 9 × 10^6 SRBC's and cultured for 5 days. IgM PFC's were determined and the poly A:U treated groups were compared to the control group, which was standardized to 100%. The actual number of plaques for the control group is presented in parentheses. Values represent the means and standard deviations from five to seven experiments. Using paired *t* statistics, the difference between the response of control cultures and cultures containing cells treated with 1 μg poly A:U were significantly different at the 0.01 level.

to experiment. Although all three doses of poly A:U induced suppression > 50% at one time or another in five to seven experiments, routine statistically significant transfer of suppression under these conditions occurred via spleen cells incubated for 24 hr with 1 μg poly A:U/10^7 spleen cells (Table 3).

Since fetal calf serum has been shown to be a B cell mitogen (14), and is capable of generating suppressor cells (15), it was eliminated from the activating medium

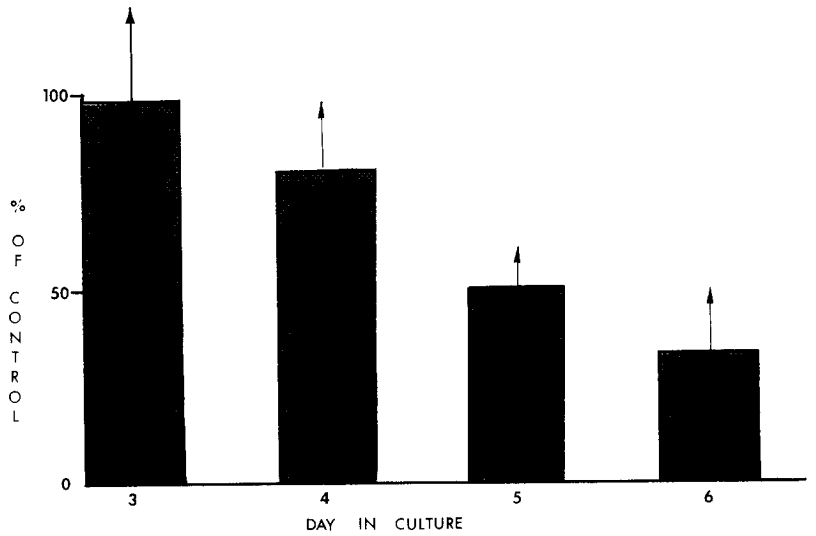

FIG. 2. 2 × 10^6 poly A:U induced suppressor cells were added to 10^7 antigen stimulated normal spleen cells on Day 0. PFC's were determined on Days 3 through 6 of the culture period. The control PFC response for each day was equated to 100% and the experimental value given as percent of the control. The mean of seven experiments is presented with the arrows defining the 95% confidence limits. The responses on Days 5 and 6 differed from the control at greater than the 0.01 level.

TABLE 4

Retention of Suppressor Cell Activity Following Treatment with Carbonyl Iron

10^7 NSC +	% of control PFC/culture in Expt.			Mean ± 95% confidence limits
	1	2	3	
Untreated				
Control cells	100 (9,300)	100 (11,900)	100 (11,900)	100 ± 10
Suppressor cells	67	67	49	61 ± 25
Carbonyl iron treated				
Control cells	100 (8,800)	100 (13,500)	100 (13,500)	100 ± 10
Suppressor cells	62	61	52	58 ± 17

Spleen cells were activated with 0.1 μg poly A:U or medium, washed, treated with carbonyl iron for 2.5 hr and separated on ficoll-hypaque. 2×10^6 experimental or control cells were added to 10^7 antigen-stimulated spleen cells and the PFC response determined on Day 5. Experimental values are given as a percent of each control value and the actual number of plaques for the controls is presented in parentheses. The means and 95% confidence limits were calculated by the Student's *t* test. Using paired *t* statistics for the difference in the responses of the control and suppressed cultures, the *P* value before treatment was less than 0.05 and after treatment was less than 0.01.

in all subsequent experiments. However, to obtain maximal suppression in the absence of serum, 2×10^6 spleen cells induced by poly A:U were required (data not shown).

To ascertain whether poly A:U might be carried over on the transferred cells and thus induce suppression, the polynucleotide complex was added just prior to harvest to control or medium treated spleen cells under identical conditions. Carry over of poly A:U appeared not to be responsible for the suppression, inasmuch as the addition at 0 time of 1, 0.5, and 0.1 μg poly A:U slightly stimulated rather than inhibited PFC formation to 136, 112, and 135 percent respectively of the control.

To examine the kinetics of the suppression, 2×10^6 spleen cells exposed to 1 μg poly A:U were added to 10^7 normal spleen cells along with 8 to 9×10^6 SRBC, and the direct PFC response measured on Days 3 to 6. The initial response of the suppressed cultures was essentially the same as the control (Fig. 2). However, their response to antigen began to abort on Day 4, and the differences became more apparent as the culture time was increased.

Three preliminary experiments were carried out to determine whether the induction of suppressor cells by poly A:U resulted in a soluble suppressor factor in the supernatant fluids. The supernatant fluids from the poly A:U treated spleens resulted in an enhanced response, rather than the inhibition observed in cultures containing the suppressor cell population, suggesting that inhibitory factors were not released, or that they were masked by the poly A:U induced helper factor (AUSF) which we have described previously (16).

Characteristics of the suppressor cell induced in vitro. To determine whether macrophages were responsible for the suppression, carbonyl iron was added to the suppressor control cell populations after they had been activated and washed. Cells, 2×10^6 of the untreated or carbonyl iron treated cells then were added to the

TABLE 5

Attempts to Identify the Inhibitory Cell Evoked *in Vivo* by Poly A:U

Group	Spleen cells	% PFC/culture after deletion with			
		Antiserum		Nylon Wool + Antiserum	
		Expt. 1	Expt. 2	Expt. 3	Expt. 4
1	NSC	100 (16,300)	100 (4,300)	100 (14,500)	100 (17,000)
2	AUSC	64	15	14	63
3	T(NSC) + B (NSC)	100 (8,350)	100 (7,900)	100 (5,000)	100 (8,850)
4	T (AUSC) + B (AUSC)	61	54	301	188
5	T (AUSC) + B (NSC)	46	36	120	118
6	T (NSC) + B (AUSC)	167	94	128	119

Spleen cells from animals that had been injected with 300 μg poly A:U 24 hr previously and the normal counterparts were separated into T and B cell populations as described in the Materials and Methods Section. 10^7 cells of either unseparated or 5×10^6 each of the separated T and B cell populations were placed in culture with 8 to 9×10^6 SRBC's for 5 days and the direct PFC response measured. The pretreated, unseparated population (AUSC) was compared to the normal, unseparated population (NSC). After separation, all experimental groups were compared to the reconstituted, normal spleen cells (Group 3) as normalized to 100%. The actual number of plaques for the control groups is presented in parentheses.

antigen stimulated cultures. As may be seen in Table 4, the poly A : U stimulated populations were equally effective in suppressing the PFC response before or after treatment with carbonyl iron. Thus, depletion of macrophages from the suppressing cell population had no discernible effect on the capacity to suppress.

Depletion experiments designed to deplete the suppressing cell population selectively of T cells, utilized (a) spleen cells which had adhered to nylon wool and subsequently eluted and treated with anti-theta serum and complement, or (b) the spleen cells were treated twice with cytotoxic anti-θ serum plus complement without passage through nylon wool. To deplete B cells, spleen cells were either (a) treated twice with anti-immunoglobulin serum and complement or (b) first passed over a nylon wool column and the eluate treated once with cytotoxic anti-immunoglobulin serum + C′.

The results are shown in Table 5. Groups 1 and 2 represent the unseparated reference groups documenting again the expected inhibition of the response after preinjection with poly A : U. Group 3 represents the normalized control value (assigned 100%) resulting from reconstitution of a T cell depleted with a B cell depleted population from normal mice. In experiments 1 and 2, where the T and B cells from either normal or poly A : U treated spleen populations were recombined after depletion with the respective antiserum alone, without passage over nylon wool, the inhibition was observed in Group 5 rather than Group 6 suggesting a T-cell population was necessary for inhibition. The necessary control test (Group 4) resulted in retention of the inhibitory capacity when both T- and B-cell populations were taken from poly A : U treated animals. On the other hand, in experiments 3 and 4 in which the poly A : U pretreated cells were separated first by nylon wool followed by antiserum treatment and reconstituted, the inhibition was lost in

the positive reference control (Group 4) and enhancement was observed. Inhibitory activity in these latter two experiments was not found in either the T- or B-cell (AUSC) population. Thus, a nylon wool adherent subpopulation appeared to have been lost during this purification procedure. The enhanced response in the control (Group 4) revealed that pretreatment with poly A: U was activating helper cells as well as suppressor cells.

When five similar experiments were done, purifying the cell populations activated by poly A: U *in vitro,* essentially similar conclusions could be drawn (data not shown). Thus, after separation by nylon wool and testing the suppressor activity of the separate or combined T- and B-cell populations, the suppressive effect was lost, indicating the nylon wool had removed the responsible cell. Two-fold enhancement was observed again after nylon wool passage, suggesting poly A: U was activating both helper and suppressor cells. Removal of the latter by nylon wool may have allowed the helper cells to exert their activity.

DISCUSSION

These experiments confirm and extend our previous finding (2) of the immunosuppressive capabilities of poly A: U, in that exposure of murine spleen cells to this adjuvant either *in vivo* or *in vitro,* 24 hr prior to antigen, stimulates the host to suppress antibody synthesis. Consequently, the regulatory capacity of these synthetic polynucleotides on the immune response is not restricted to an enhancing action. It is now becoming increasingly evident that many adjuvants have a time dependent regulatory characteristic of enhancing antibody synthesis when given with antigen, and inhibiting this response when given prior to antigen (1, 17–20). The events which transpire over this 24 to 72 hr period to reverse the enhancing effect of the adjuvant are as yet unknown.

Of relevance in this respect we believe, are the experiments wherein the suppression was lost after passage over nylon wool columns, and the remaining population proved to be capable of *enhancing* antibody synthesis (Table 5). This suggests that during the 24 hr period after injection of poly A: U (or addition *in vitro*), both T helper and T suppressor cells are activated non-specifically and coexist. The helper cells, and/or events leading to enhancement of antibody, apparently develop very quickly after poly A: U stimulus, whereas suppressor cells require greater than 12 hr to become functional. Perhaps the latter period represents the time necessary for helper cells to provide a stimulus which is a prerequisite for development of suppressor cell activities. We hypothesize that if antigen is given near the time of poly A: U, it drives the activated helper cells into a state of dominance, which results in enhancement of PFC synthesis. On the other hand, should antigen be delayed until 1 day after poly A: U when the T suppressor cells have become dominant, it serves to inhibit the subsequent chain of events leading to PFC formation. Removal of the suppressor cells by nylon wool permits the coexisting helper cells to once again express their function. Although the suppression induced was profound and dramatic, transfer of this suppressive property via poly A: U induced spleen cells to normal spleen cells making antibody, was variable and generally resulted in only a 50% suppression. Thus, the degree of suppression actually measured probably reflects an equilibrium between a helper and suppressor cell population, both induced by poly A: U. Consequently total suppression may be difficult to

transfer in "adjuvant" induced models. Experiments designed to test the above hypothesis are in progress.

The failure to demonstrate transferrable suppressor cells induced *in vivo* and transferred *in vitro* has been attributed by Gershon (7) to the rapid loss of suppressor characteristics by the removal of T cells from the environment in which feedback induction occurred. The suppression produced by transfer of suppressor cells often became help when the transferred cells were sufficiently diluted. This was evident also in our experiments when spleen cells were pretreated *in vivo* and transferred to normal mice. Thus, transfer of 10^6 spleen cells resulted in 290% enhancement while transfer of 10^8 cells resulted in 77% inhibition. It appears that dilution of the postulated suppressor T cells with normal T cells may tip the balance in favor of helper activity.

The non-specific inhibitory cell induced following injection of poly A: U appears to be a nylon wool adherent T cell similar to the antigen specific suppressor cell reported by Basten *et al.* (20). In the case of the suppressor cell induced *in vivo* by poly A: U, no definitive statement can be made other than it appeared to be a non-phagocytic nylon wool adherent cell. A higher degree of purity of the subpopulations was attained in these experiments by putting only 5×10^7 spleen cells on nylon wool columns rather than the higher concentrations suggested by Julius *et al.* (11). This was in keeping with the finding that suppressor T cells adhere to the columns when the wool was not saturated with B cells and macrophages (Stout, R. D., personal communication).

These results documenting suppression of the immune response by pretreatment with poly A: U raise questions as to the eventual controlled use of synthetic polynucleotides as adjuvants in human beings. It is of interest in this respect, that the suppression induced by pretreatment with polyA: U can be overcome by simultaneous injection of poly A: U with antigen (2).

REFERENCES

1. Johnson A. G., *In:* "Immune RNA" (E. P. Cohen Ed.), pp. 17–34. The Chemical Rubber Co., Cleveland, Ohio, 1976.
2. Schmidtke, J. R., and Johnson, A. G. *J. Immunol.* **106**, 1191, 1971.
3. Collavo, D., Finco, B., and Chieco-Bianchi L., *Nature New Biol.* **237**, 154, 1972.
4. Johnson, H. M., Bukovic, J. A., and Smith, B. G., *Proc. Soc. Exp. Biol. Med.* **149**, 599, 1975.
5. Colmerauer, M., Rumi, L., Saal, F., Pasqualini, C. D., and Rabasa, S. L., *J. Immunol.* **111**, 743, 1973.
6. Cunnington, P. G., and Naysmith, J. D., *Immunology* **28**, 451, 1975.
7. Gershon, R. K., *Transplant. Rev.* **26**, 170, 1975.
8. McCarthy, M. M., and Dutton, R. W., *J. Immunol.* **115**, 1316, 1975.
9. Han, I. H., and Johnson, A. G., *J. Immunol.* **117**, 423, 1976.
10. Weiner, H. L., Moorehead, J. W., and Claman, H. N., *J. Immunol.* **116**, 1656, 1976.
11. Julius, M. H., Simpson, E., and Herzenberg, L. A., *Eur. J. Immunol.* **3**, 645, 1973.
12. Reif, A. E., and Allen, J. M., *Nature* **209**, 521, 1966.
13. Mishell, R. J., and Dutton, R. W., *J. Exp. Med.* **126**, 423, 1967.
14. Coutinho, A., and Moller, G., *Adv. Immunol.* **21**, 113, 1975.
15. Burns, F. D., Marrack, P. C., Kappler, J. W., and Janeway, Jr., C. A., *J. Immunol.* **114**, 1345, 1975.
16. Bick, P. H., and Johnson, A. G., *Scand. J. Immunol.* **6**, 1133, 1977.
17. Schwab, J. H., *Bact. Rev.* **39**, 121, 1975.
18. Kind, P., and Johnson, A. G., *J. Immunol.* **82**, 415, 1959.
19. Persson, U., *J. Immunol.* **118**, 789, 1977.
20. Basten, A., Miller, J. F. A. P., and Johnson, P., *Transplant. Rev.* **26**, 130, 1975.