Yonsei Medical Journal
Vol. 31, No. 2, 1990

Effect of Polyadenylic.Polyuridylic Acid on the Proliferative Responsiveness of Mouse Thymus and Spleen Cells

Bong Ki Lee[1], Youn Jung Yu[2] and Jung Koo Youn[1]

The effects of polyadenylic.polyuridylic acid [poly(A).poly(U)] on in vitro proliferations of thymus and spleen cells from C57BL/6 mice were investigated. Mice were injected intravenously with 30μg of poly(A).poly(U) or placebo. Two days later, thymus, spleen and peritoneal cells from these mice were prepared and cultured in pooled or non-pooled conditions. Cell proliferations were assessed by the technique of incorporation of tritiated thymidine. It has been revealed that the in vitro proliferations of thymus and spleen cells as well as the productions of interleukin-1 by peritoneal adhering cells and interleukin-2 by spleen cells were significantly enhanced in the cultures of cells from poly(A).poly(U)-treated mice. These enhancing effects were observed only in the cultures of pooled cells from mice whose genetic homogeneity is suspected. Furthermore, thymus cells from poly(A).poly(U)-treated mice acted as strong responder cells but not as stimulators in one way mixed cultures. Thus, the enhanced cellular responsiveness may be mediated by the increased production of cytokines and antigen recognitions of thymus-derived cells following activations via the adjuvant effect of poly(A).poly(U).

Key Words: Poly(A).poly(U), thymus, spleen, interleukin-1 & 2

Polyadenylic.polyuridylic acid [poly(A).poly(U)] is a synthetic double-stranded complex of polyribonucleotides capable of stimulating the immune system and its immunostimulatory effect was initially reported by Braun and Nakano (1967).

Later, it was proved that poly(A).poly(U) not only enhanced humoral and cell-mediated immune responses in nomral adult hosts but also provoked high levels of antibody production in newborn mice (Winchurch and Braun 1969), restored immune responsiveness in aging mice that normally displayed impaired antibody responses (Braun *et al.* 1971), and improved the capacity of neonatally thymectomized mice to respond to skin homografts (Cone and Johnson 1971). Recently, anti-tumor effects of poly(A).poly(U) have been reported in experimental tumors (Youn *et al.* 1983) as well as in human malignancies (Lacour *et al.* 1984; Youn *et al.* 1990).

The cellular targets of poly(A).poly(U) have been shown to be a variety of cell types including immunocompetent (Johnson 1979) as well as nonimmunocompetent cells (Kim and Han 1975). Concerning the effect on the immunocompetent cells, poly(A).poly(U) appeared to accelerate immunological maturation of macrophages in newborn mice (Winchurch and Braun 1969), to restore deficient thymic activity through a return to normal levels of T cells and to increase the expression of T cell functions (Cone and Johnson 1972). However, the mechanisms implicated in this biological responsiveness are not fully understood.

In this study, we investigated the effects of poly(A).poly(U) on the biological responsiveness of mouse immunocompetent cells and found that in vitro proliferations of thymus and spleen cells from mice inoculated with the agent were significantly enhanced and this might be partly due to its adjuvant effects on the production of cytokines as well as on the recognition of antigens.

Received February 27, 1990
Accepted April 18, 1990

Department of Microbiology[1], Yonsei University College of Medicine, Seoul, Korea
Department of Oral Biology[2], Yonsei University College of Dentistry, Seoul, Korea

Supported by the grant of The Korea Science and Engineering Foundation, 1987. (No. 871-0407-023-2)

Address reprint requests to Dr. B K Lee, Department of Microbiology, Yonsei University College of Miedicine, C.P.O. Box 8044, Seoul, Korea

MATERIALS AND METHODS

Mice

C57BL/6 mice of both sexes and 6 to 8 weeks of age were used in most of the experiments. These were obtained from two different breeding centers: one was from Yonsei (YS) University, Seoul and the other from Hanrhim (HR) University, Choonchun, Korea. C3H/HeJ mice, used for interleukin-1 (IL-1) assays, were obtained from Osaka Univeresity, Japan.

Poly(A).poly(U)

Poly(A).poly(U), kindly supplied by Dr. A.M. Michelson, (Institut de Biologie et Physico-Chimie, Paris 5e), was dissolved in 0.15M NaCl solution and injected into the mice, 30μg, intravenously (i.v.) through tail veins (Youn *et al.* 1983). Unless otherwise stated, poly(A).poly(U) was administered by a single injection 2 days before sacrificing mice for experiments.

In vitro Cultures of Thymus and Spleen Cells

Mice were injected i.v. with 30μg of poly(A).poly(U) or placebo (0.15M NaCl) and sacrificed by cervical dislocation 2 days later. Thymuses and spleens were aseptically removed, minced separately with scissors, passed through stainless steel mesh (No. 50) and prepared as single cell suspensions in RPMI medium (Hazleton Biologics Inc. Denver, PA, USA). Pooled cell suspensions were prepared from 3 to 5 mice of each group. Red blood cells in spleen cell suspensions were lysed by hypotonic shock using sterilized distilled water. They were washed three times with the same medium, resuspended in RPMI 1640 medium supplemented with 5% heat-inactivated fetal calf serum (J.R. Scientific, Wooland. CA, USA), 2mM glutamine, 10mM HEPES, 100 units/ml pencillin and 100 μg/ml streptomycin (referred to as complete culture medium hereafter), and the number of viable cells were counted by trypan blue dye exclusion. They were seeded in 96-well round-bottom microtiter plates (Costar, Cambridge, MA, USA) at a concentration of 1×10^6 thymus cells/well or 2×10^5 spleen cells/well. The plates were incubtaed for 4 days at 37°C in a humidified 5% CO_2 incubator. After incubation, the plates were pulsed with 10ul (1.0 uCi)/well of labeled tritiated-thymidine (^{3}H-TdR, New England Nuclear Boston, MA, USA) for 8 hrs. The cells were harvested onto glass fibers using a cell harvester (Skatron Inc., Stering, VA, USA) and ^{3}H-TdR incorporations were measured by a beta counter (Beckman, LS 5000TA, CA, USA).

In some experiments, the cultures were made similarly with non-polled thymus cells prepared from an individual mouse. In one-way mixed cultures, equal numbers of thymus cells (10^5/well) prepared from 2 individual mice were mixed and similarly cultured. In this case, the cells from one mouse were irradiated with 2000 rads before seeding and served as stimulator cells.

In vitro Production of Interleukin-1 (IL-1) and Interleukin-2 (IL-2) and Their Assays

Peritoneal cells were collected from mice injected intraperitoneally with 10ml of RPMI1640 medium using syringes with no.18-gauge needles. Pooled cells from 3 to 5 mice were suspended in complete culture medium, seeded at a concentration of 2×10^7 cells/5ml in each of 25cm^2 plastic tissue culture flasks (Costar, Cambridge, MA, USA) and incubated for 3 hrs at 37°C in a humidifed 5% CO_2 incubator. Nonadherent cells were removed by repeated washings with RPMI1640 medium. Adherent peritoneal cells were harvested using a rubber policeman after leaving the flasks in ice for 30 min, and adjusted to 1×10^5 cells/ml in complete culture medium. One ml of the cell suspensions was delivered into each well of 24-well tissue culture plates (Costar, Cambridge, MA, USA) and incubated at 37°C in a humidifed 5% CO_2 incubator. Twenty-four hrs later, the culture supernatants were harvested and assayed for IL-1 as follows.

The IL-1 activity in the culture supernatants was measured by the technique of thymocyte comitogenic response described by Mizel *et al.* (1978). Fresh thymus cells from C3H/HeJ mice were suspended to a density of 1×10^7 cells/ml in complete culture medium and 1×10^6 cells/well were seeded in 96-well round bottom microtiter plates. These were cultured for 72 hrs at 37°C in a humidified 5% CO_2 incubator in the presence or absence of 1 μg/ml concanavalin A and 100μl (4x final dilution) of the culture supernatants to be tested. Tritiated (^{3}H)-TdR incorporations of the cultured cells were measured as described above.

For in vitro production of IL-2, pooled spleen cells were suspended in complete culture medium and 10^6 cells in 1ml were plated in each well of 24-well tissue culture plates. They were cultured at 37°C in a humidified 5% CO_2 incubator for 3 days and the culture supernatants were harvested and assayed for IL2. The IL2 activity was determined using IL2-dependent mouse T lymphocytes (CTLL2) as described by Gillis *et al.* (1978). Five million cells per

well were seeded in 96-well round bottom microtiter plates and the culture supernatants (100μl) to be tested were added. The plates were incubated at 37°C in a humidifed 5% CO_2 incuabtor. After 24 hrs, cells in each well were pulsed with 10ul (1.0 uCi) of ^{3}H-TdR for 4 hr and measured for ^{3}H-TdR incorporation.

Statistical Analyses

Proriferations of thymus cells in Table 1 and 2 and IL-1 production in Figure 3 were analysed by paired Student's t test.

RESULTS

In vitro Proliferations of Thymus and Spleen Cells

In order to study the in vivo and in vitro effects of poly(A).poly(U) on the proliferative responsiveness of lymphocytes, C57BL/6 mice (YS origin) were injected i.v., with 30μg of poly(A).poly(U) or 0.15M NaCl solution as placebo. Two days later, the mice were sacrificed and thymus and spleen cells from 3 to 5 mice of each group were respectively pooled. They were cultured in the presence (30 μg/ml) or absence of poly(A).poly(U) in culture media and cell proliferations were estimated by the technique of incorporation of ^{3}H-TdR as described in Material and Methods.

Figure 1 shows the representative result obtained from thymus cell cultures. When thymus cells were cultured in culture medium without poly(A).poly(U), the mean ^{3}H-TdR incorporation of cultured cells from poly(A).poly(U)-treated mice was approximately

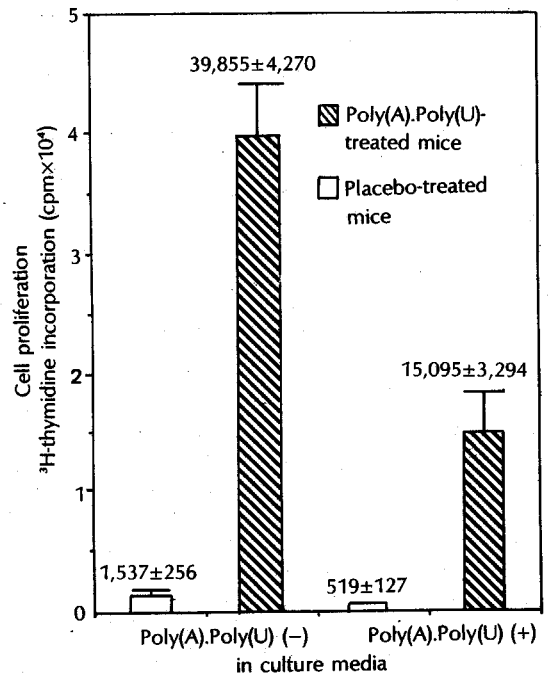

Fig. 1. *Proliferations of thymus cells from poly(A).poly(U)- or placebo-treated C57BL/6 mice (YS origin) following in vitro culture for 4 days in the presence or absence of poly(A).poly(U) in culture media.*
Thymuses were harvested from 3 mice of each group 2 days after poly(A).poly(U) (30μg, i.v.) or placebo injections. Pooled thymus cells were seeded in 96-well microplates (10^6 cells/well) and cultured in medium with (30 μg/ml) or without poly(A).poly(U) for 4 days. Cell proliferations were determined by the technique of ^{3}H-TdR incorporation.

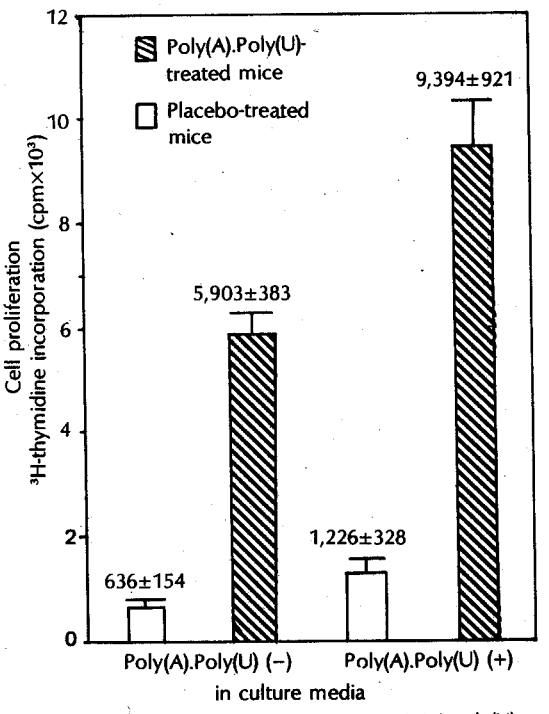

Fig. 2. *Proliferations of spleen cells from poly(A).poly(U)-or lacebo-treated C57BL/6 mice (YS origin) following in vitro culture for 4 days in the presence or absence of poly(A).poly(U) in culture media.*
Spleens were harvested from 3 mice of each group 2 days after poly(A).poly(U) (30μg, I.V.) or placebo injections. Pooled spleen cells were seeded in 96-well microplates (2×10^5 cells/well) and cultured in medium with (30 μg/ml) or without poly(A).poly(U) for 4 days. Cell proliferations were determined by the technique of ^{3}H-TdR incorporation.

26-fold higher (39,855±4,270 cpm) than that of thymus cells from plaebo-treated control mice (1,537±256 cpm). Thus, in vivo treatment of mice with poly(A).poly(U) highly significantly enhanced ($p<0.001$) in vitro proliferation of their thymus cells. However, when the same batch of the thymus cells were cultured in parallel in culture medium containing 30 μg/ml of poly(A).poly(U), their proliferations were significantly decreased in both groups as compared to those cultured in the absence of poly(A).poly(U): 15,095±3,294 cpm for poly(A).poly(U)-treated mice and 519±127 cpm for control mice.

In spleen cell cultures, as shown in Figure 2, the mean ^{3}H-TdR incorporation of cultured cells in the absence of poly(A).poly(U) was 5,903±383 cpm for poly(A).poly(U)-treated mice and 636±154 cpm for control mice, showing approximately 9-fold higher in the former than the latter. Nevertheless, when the spleen cells were similarly cultured in the presence of 30 μg/ml poly(A).poly(U) in culture medium, their proliferations were, contrary to the thymus cell cultures, rather accelerated: 9,394±921 cpm for poly (A).poly(U)-treated mice and 1,226±328 cpm for control mice.

Thus, the injections of mice with poly(A).poly(U) significantly enhanced in vitro proliferations of their thymus and spleen cells and this effect was much more pronounced in thymus cells than spleen cells. Furthermore, the additions of poly(A).poly(U) in culture media have resulted in an adverse effect on their proliferations, showing a suppression for thymus cells and an acceleration for spleen cells.

In vitro Productions of IL-1 by Pertioneal Cells and IL-2 by Spleen Cells

Adherent peritoneal cells and spleen cells were obtained from poly(A).poly(U)- or placebo-treated C57BL/6 mice of YS origin and cultured for 24 hrs without any additive in culture media. The presence of IL-1 or IL-2 in culture supernatants was estimated by the assay techniques of ^{3}H-TdR incorporation into fresh thymus cells from C3H/HeJ mice for IL-1 and CTLL2 cells for IL-2.

The proliferations, expressed by the mean ^{3}H-TdR incorporation, of the thymus cells in the medium added to the culture supernatants of adherent peritoneal cells from poly(A).poly(U)- or placebo-treated mice were 36,969±8,680 and 24,517±2,320 cpm respectively and this difference was statistically ($p<0.02$) significant (Fig. 3).

For IL-2 assay, when CTLL2 cells were cultured in the medium containing the culture supernatants of spleen cells, their proliferations were very significantly ($p<0.001$) increased in the cultures containing the culture supernatants of spleen cells from poly(A).poly(U)-treated mice (29,404±4,157 cpm) as compared to those of spleen cells from placebo-treated mice (7,560±990 cpm) (Fig. 4).

Thus, the injections of mice with poly(A).poly(U) also significantly enhanced in vitro productions of both IL-1 and IL-2 by their peritoneal and spleen cells respectively.

Pooling Effects on the Proliferations of Thymus Cells

In the above experiments, the in vitro cultures of thymus or spleen cells were performed with pooled

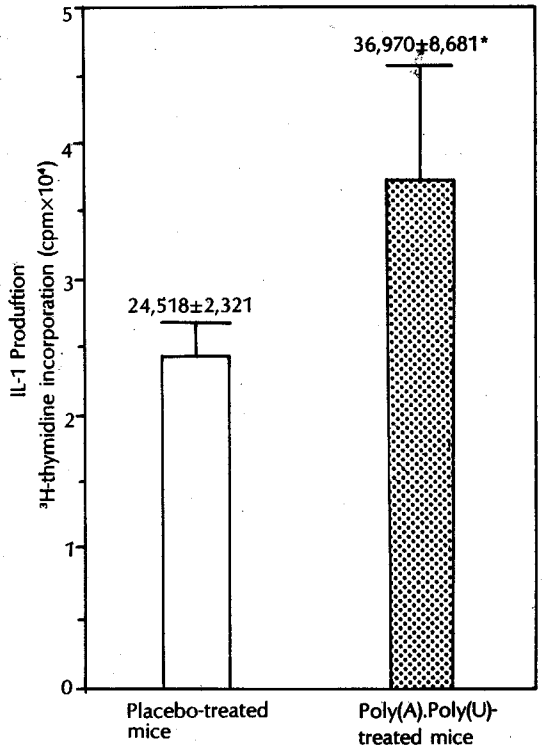

Fig. 3. IL-1 production of adhering peritoneal cells from poly(A).poly(U)-or placebo-treated C57BL/6 mice (YS origin) following in vitro culture for 24 hrs. Pooled adhering peritoneal cells from 3 mice were seeded in 24-well Costar plates (10^6 cells/well) and cultured for 24 hr. IL-1 activities of the culture supernatants were assayed by ^{3}H-TdR incorporation of fresh thymus cells from C3H/HeJ mice.
** $p<0.02$ vs Placebo-treated mice.*

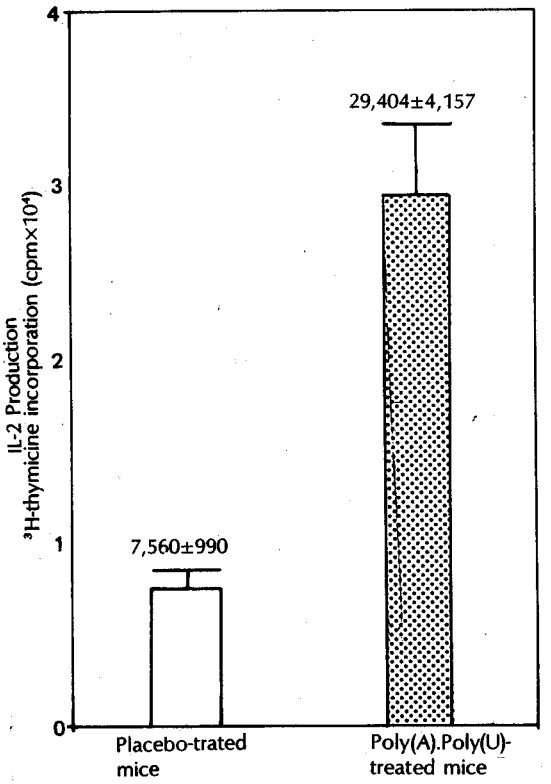

Fig. 4. *IL-2 production of spleen cells from poly(A).poly(U)- or placebo-treated C57BL/6 mice (YS origin) following in vitro culture for 3 days.*
Pooled spleen cells from 3 mice were seeded in 24-well Costar plates (2×10⁵ cells/well) and cultured for 24 hrs. IL-2 activities of the culture supernatants were assayed by ³H-TdR incorporation of CTLL2 cells.

cell suspensions of each organ prepared from 3 to 5 C57BL/6 mice obtained from the YS breeding center. When the same experiments were conducted with a group of the same strain of mice but originated from another source, the HR breeding center, surprisingly little growths of pooled thymus cells (758±98 cpm) were observed, whereas the parallel cultures of those from mice of YS origin showed, as expected, highly enhanced proliferations (28,502±4,852 cpm) (Table 1). Thus, the enhancing effect observed in the above experiments might be due to a pooling effect of thymus cells prepared from a group of mice of YS origin.

To verify this eventuality, non-pooled thymus cells from each individual mouse of both origins were cultured in the same condition and their proliferations were compared. No significant growths were present in the cultures regardless of whether the thymus cells were from mice of YS or HR origin.

In order to gain more insight into the effect of poly(A).poly(U), regarding notably the recognition and/or expression of putative poor antigen(s) which could not be revealed by the mixed culture technique of cells from placebo-treated normal mice, thymus cells prepared from each individual mouse of YS origin pretreated with poly(A).poly(U) or with placebo were divided into two parts, responder and stimulator cells, and they were cross-mixed and similarly cutlured. The stimulator cells were irradiated with 2000 rads before culture. As shown in Table 2, when stimulator cells from a placebo-treated mouse were cultured with responder cells from a poly(A).poly(U)-treated mouse, a strong proliferative response could be observed (11,968±2,948 cpm), whereas reversely, when stimulator cells from a poly(A).poly(U)-treated mouse and responders from a placebo treated-one were mixed, a very low level of proliferation could be detected (964±141 cpm). Thus, it appears that the enhancing effect of poly(A).poly(U) on the proliferative responsiveness of thymus cells resides primarily on responder

Table 1. Pooling effects on the proliferations of thymus cells from poly(A).poly(U)-treated C57BL/6 mice

Source of C57BL/6 mice	³H-TdR incorporation (cpm)	
	Pooled cell culture	Non-pooled cell culture
YS origin	28,502±4,852	719±132*
HR origin	758±98	535±76**

C57BL/6 mice of both YS and HR origin were injected i.v. with 30μg of poly(A).poly(U); Two days later, pooled thymus cells from 3 mice and non-pooled thymus cells from each individual mouse were seeded in 96-well microplates (10^6/well) and cultured for 4 days in medium without poly(A).poly(U). Cell proliferations were determined by ³H-TdR incorporations.

* p: Not significant vs Non-pooled cell culture of HR origin

** p: Not significant vs Pooled cell culture of HR origin

Table 2. Proliferations of thymus cells from C57BL/6 mice of YS origin following one-way mixed culture

Responder cells	Stimulator cells (irradiated)	^{3}H-TdR incorporation (cpm) in mixed thymus cell culture
Thymus cells from placebo-treated mouse	Thymus cells from poly(A).poly(U)-treated mouse	964±141*
Thymus cells from poly(A).poly(U)-treated mouse	Thymus cells from placebo-treated mouse	11,968±2,948

Thymus cells from each individual mouse (C57BL/6, YS origin) treated with poly(A).poly(U) or placebo were divided into two groups: responder cells and stimulator cells. The stimulator cells were irradiated with 2000 rads. An equal number (10^6) of responder and stimulator cells were mixed and cultured in 96-well microplates for 4 days and their proliferations were measured by ^{3}H-TdR incorporations.

* $p<0.001$

cells, enabling them to recognize more effectively antigen(s) which is expressed on stimulator cells.

DISCUSSION

It has been revealed from our experiments that injections of mice with poly(A).poly(U) significantly enhanced in vitro proliferations of thymus and spleen cells and the proliferative responsiveness was much higher in cultures of thymus cells than those of spleen cells.

These results suggest that such enhanced responsiveness is probably due to the types of cell populations existing in thymuses and spleens, implying that the major target of poly(A).poly(U) may be the thymus-derived cells. This can be partly supported by the evidence that poly(A).poly(U) restored deficient thymic activities (Cone and Johnson 1972), induced polyclonal activations of cytotoxic T cells (Bick and Moller 1977), and modulated both precursor T (Bick and Johnson 1977) and activated helper T cells (Eardley *et al.* 1978).

The presence of poly(A).poly(U) in culture media, in our experiments, significantly inhibited the proliferations of thymus cells but accelerated those of spleen cells from poly(A).poly(U)-treated mice. The reason for such differential effects of the agent is not clear. The concentration of poly(A).poly(U) used in these experiments was 30 μg/ml, a dose largely exceeding the effective dose range of 0.001-10 μg/ml that Johnson (1976) used in his experimens for the stimulation of in vitro cell proliferations. The question of whether or not such in vitro phenomenon observed in our experiments is related to a dose effect of poly(A).poly(U) remains to be answered.

Bick and Johnson (1977) reported that poly(A).poly(U) enhanced T cell function by secreting helper lymphokines. It is well known what IL-2 exerts various effects on immune functions including proliferations and activations of lymphoid cells and secretions of several other lymphokines from the latter (Robb 1984). In our experiments, effectively increased productions of IL-2 were found in culture supernatants of spleen cells from mice treated with poly(A).poly(U).

IL-2 is produced by T cells in response to two signals provided by antigen-pulsed accessory cells (Oppenheim and Gery 1982). The first signal is stimulations by antigens, and the second is IL-1 that is produced chiefly by marcophages or monocytes and that activates and differentiates several types of cells, particularly thymus derived cells (Oppenheim *et al.* 1986). If this is so, direct and/or indirect effects of IL-1 might be implicated in the mechanisms for the enhanced proliferations of thymus and spleen cells. In concordance with this, the in vitro productions of IL-1 by peritoneal macrophages in our studies were significantly higher in poly(A).poly(U)-treated mice than placebo-treated controls.

Thus, our results indicate that in vivo treatment of mice with poly(A).poly(U) stimualted their macrophages and lymphocytes to more efficiently produce IL-1 and IL-2 which may be involved at least in part in the increased proliferations of thymus and spleen cells.

Friedman *et al.* (1969) demonstrated that poly(A).poly(U) remarkably enhanced the uptake of ^{3}H-TdR by human lymphocytes which had been sensitized and reexposed to the diphtheria toxodis and emphasized that for the enhancing effect of poly(A).poly(U), some stimulation of antigen(s), even of threshold doses, is required. Wagner and Cone (1974) also showed that the enhanced cytotoxicity of T cells by poly(A).poly(U) is dependent on the antigenic stimulation. In contrast, Bick and Möller (1977)

reported that poly(A).poly(U) caused a polyclonal activation of cytotoxic T cells even in the absence of any antigen. In our experiments, all the enhanced biological responsiveness of lymphocytes and macrophages was obtained from mice treated with poly(A).poly(U) alone without the addition of any exogenous antigen.

The mice used in the first part of our experiments were of C57BL/6 strain originated from the YS breeding center. When the experiments were similarly conducted with the same strain of mice but with those originated from another breeding source (HR center), no more such enhanced proliferations of thymus cells were observed. These unexpected findings raised the question about whether this enhanced responsiveness was induced by the mitogenic effect of poly(A).poly (U) alone or by a pooling effect of the cells from the mice of YS origin which may not be genetically homogenous.

To clarify this question, we have undertaken experiments in which single cultures with non-pooled thymus cells obtained from each individual mouse of both origins were similarly attempted. From these experiments, it was found that thymus cells from poly (A).poly(U)-treated mice of HR origin did not show any proliferative responsiveness in both single and pooled mixed cultures, whereas those from the mice of YS origin treated similarly witht he agent did not proliferate in single cultures but did in mixed cultures, as expected. These results indicate that the mice of HR origin seem to be genetically homogenous while those of YS origin are not. Thus, the enhanced proliferations of thymus cells observed in our experiments might be due to an allogenic effect resulting from the pooling rather than the mitogenic effect of poly(A).poly(U). Moreover, the fact that thymus cells from the placebo-treated mice of YS origin did not proliferate in mixed cultures, but those from the poly(A).poly(U)-treated mice of the same origin did, strongly suggests that poly(A).poly(U) augments the ability of thymus cells to respond to antigen(s) which may be difficult for thymus cells of normal, untreated mice to recognize.

The effect of poly(A).poly(U) on such enhanced proliferations was further investigated by one- way mixed cultures of thymus cells from each mouse of YS origin, and it was found that thymus cells from the poly(A).poly(U)-treated mouse acted as strong responder cells but not as stimulator cells, suggesting that the mode of action of poly(A).poly(U) may be in the augmentation of antigen recognition rather than antigen expression on these cells. This concept is further supported by the evidence that poly(A).poly(U) modulates T cells to increase their antigen specific surface receptors and has the capacity to evoke the expression of certain T cell antigens (Scheid *et al.* 1973).

From our results, it can be concluded that the injections of mice with poly(A).poly(U) significantly enhanced the in vitro proliferations of thymus and spleen cells and the productions of IL-1 by macrophages and IL-2 by spleen cells. This enhanced cellular responsiveness may be due to the potentiation not only by these cytokines produced but also by the increased antigen recognition of thymus-derived cells activated through the adjuvant effect of poly(A). poly(U).

REFERENCES

Bick PH, Johnson AG: PolyA.U-induced secretion of T lymphocyte helper factors. *Scand J Immunol 6:1133-1139, 1977*

Bick PH, Möller G: Cytotoxic T-cell activation by polyribonucleotides: DNA synthesis is not required. *J Exp Med 146:844-856, 1977*

Braun W, Nakano M: Antibody formation: Stimulation by polyadenylic and polycytidylic Acids. *Science 157: 819-821, 1967*

Brawn W, Ishizuka M, Yajima Y, Webb D, Winchurch R: *Spectrum and mode of action of polyA : PolyU in the stimulation of immune responses.* In: Beers RF, Braun W, eds. *Biological Effects of Polynucleotides.* New York, Springer Verlag, *1971, 139-156*

Cone RE, Johnson AG: Regulation of the immune system by synthetic polynucleotides: III. Action on antigen-reactive cells of thymic origin. *J Exp Med 133:665-675, 1971*

Cone RE, Johnson AG: Regulation of the immune system by synthetic polynucleotides. IV. Amplification of proliferation of thymus influenced lymphocytes. *Cell Immunol 3:283-294, 1972*

Eardley DD, Hugenberger J, McVay-Bouddreau L, Shen FW, Gershon RK, Cantor H: Immunoregulatory circuits among T cell scts. I. T-helper cells induce other T cells to exert feedback inhibition. *J Exp Med 147:1106-1115, 1978*

Friedman HM, Johnson AG, Pan P: Stimulatory effect of polynucleotides on short–term leukocyte cultures. *Proc Soc Exp Biol Med 132:916-919, 1969*

Gillis S, Ferm MM, Ou W, Smith KA: T cell growth factor: Parameter of production and a quantitative microassay for activity. *J Immunol 120:2027-2032, 1978*

Johnson AG: *Modulation of the immune system by synthetic polynucleotides.* In: Springer Seminars in Immunopathology. New York, Springer-Verlag, *1979 (Vol. 2), 149-168*

Johnson AG: *The adjuvant action of synthetic polynucleotides on the immune response.* In: Cohen EP, ed. *Immune RNA.*

Cleveland, CRC Press, Inc. *1976, 17*

Johnson HG, Johnson AG: Regulation of the immune system by synthetic polynucleotides. II. Action on peritoneal exudate cells. *J Exp Med 133:649-663, 1971*

Kim YG, Han SS: Stuides on hypoxia. X. Effect of synthetic polynucletides on pancreas from hypoxic and control rats, an electron microscopic and biochemical study. *Exp Mol Pathol 23:43-58, 1975*

Lacour J, Lacour F, Spira A, Michelson M, Petit J, Delage G, Sarrazin D, Contesso G and Viguier J: Adjuvant treatment with polyadenylic-polyuridylic acid in operable breast cancer: Updated result of a randomized trial. *Br J Med 288:589-592, 1980*

Mizel SB, Oppenheim JJ, Rosenstreich DL: Characterization of lymphocyte-activating factor (LAF) produced by the macrophage cell line, P388D, I. Enhancement of LAF production by activated T lymphocytes. *J Immunol 120:1497-1503, 1978*

Oppenheim JJ, Gery I: Interleukin 1 is more than an interleukin. *Immunol Today 3:113-119, 1982*

Oppenheim JJ, Kovacs EJ, Matsushima K, Durum SK: There is more than one interleukin 1. *Immunol Today 7:45-56, 1986*

Robb RJ: Interleukin 2: the molecule and its function. *Immunol Today 5:203-209, 1984*

Scheid MP, Hoffmann NK, Komuro K, Hammerling U, Abbott J, Boyse EA, Cohen GH, Hooper JA, Schulof RS, Goldstein AL: Differentiation of T cells induced by preparations from thymus and by non-thymic agents. *J Exp Med 138:1027-1033, 1973*

Wagner H, Cone RE: Adjuvant effect of poly(A).poly(U) upon T cell-mediated in vitro cytotoxic allograft responses. *Cell Immunol 10:394-403, 1974*

Winchurch R, Braun W: Antibody formation: Premature initiation, by endotoxin or synthetic polynucleotide in newborn mice. *Nature 223:843-844, 1969*

Youn JK, Lacour F, Hue G: Inhibition of C3H/He mouse mammary tumor growth by combined treatment with cyclophosphamide and polyadenylic.polyuridylic acid. *Cancer Res 42:4706-4711, 1982*

Youn JK, Kim BS, Min JS, Lee KS, Choi HJ, Lee YB, Lee DW, Park IS, Roh JK, Chung JB, Koh EH, Park YJ, Kim HI and Lee KB: Adjuvant treatment of operable stomach cancer with polyadenylic.polyuridylic acid in addition to chemotherapeutic agents: A preliminary report. *Int J Immunopharmacol 12:289-295, 1990*