Stimulatory Effect of Polynucleotides on Short Term Leukocyte Cultures[1] (34336)

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Several investigators have documented the adjuvant action on antibody synthesis of preparations rich in DNA, RNA, or their breakdown products (1). Recently, a synthetic copolymer of polyadenylic and polyuridylic acids (poly A–poly U) has been shown to act in a similar manner in several different experimental systems (2–4) and in addition these copolymers were found to stimulate interferon production (5). This report describes an extension of the effects of poly A–poly U complexes on immune systems to include the cellular hypersensitivities as manifested by tuberculin, phytohemagglutinin, or mixed leukocyte stimulation of short-term human leukocyte cultures (6).

Materials and Methods. Leukocyte cultures were performed initially according to a modification of the method of Bach and Hirschorn (6). Blood, anticoagulated with preservative-free heparin, was allowed to settle at 37°. The leukocyte-rich supernate was mixed with minimal essential Eagle's medium (MEM) and allowed to settle for a second time in glass, flat walled bottles, permitting most of the polymorphonuclear leukocytes and monocytes to adhere to the glass. The decanted cell mixtures contained 70–80% lymphocytes. Cultures of 2.5×10^6 cells in 12-ml screw cap, glass centrifuge tubes were incubated at 37° in 4 ml of culture medium containing MEM, 20% fetal calf serum, glutamine, penicillin, and streptomycin. To various cultures were added purified protein derivative of tuberculin (PPD, Parke Davis), phytohemagglutinin (PHA-M Difco), or poly A–poly U (Sigma). Most PHA cultures were harvested after 6 days. The degree of lymphocyte transformation was determined either by visual counting of cells stained with acetic orcein or by measurement of the radioactivity of the dissolved cell button in a Packard Tri Carb scintillation counter following a 2-hr pulse label with 1.5 μCi of tritiated thymidine.

In a second series of experiments, the culture method of Moorhead *et al.* was used (7). This differed from the first technique in three respects: No attempt was made to eliminate phagocytic cells, only disposable sterile plastic syringes, pipettes and culture tubes (Falcon 6) were allowed to contact cells, and 20% autologous plasma was substituted for fetal calf serum.

Results. Stimulation by PPD alone of lymphocytes from tuberculin-positive donors was obtained consistently in cultures from which most of the monocytes were removed prior to the addition of PPD. In this system, no enhancement of lymphocyte transformation by poly A–poly U complexes was detectable, even with the addition of 200 μg/culture tube. However, when whole leukocyte suspensions were used, poly A–poly U, 100 μg/tube, increased stimulation as much as 12-fold (Fig. 1). Ten μg poly A–poly U was ineffectual. Enhancement of PPD stimulation was most apparent when adding suboptimal amounts of this antigen, but was still evident with the optimal concentration of 5 μg of PPD/culture tube.

Mixed leukocyte cultures behaved in a similar manner (Fig. 2). Thus, mixture of 4.75×10^6 human leukocytes from one individual with 0.25×10^6 human leukocytes obtained from a second individual resulted in

[1] Supported by USPHS research grant AI-1524 and Training Grant AI-00044-10.

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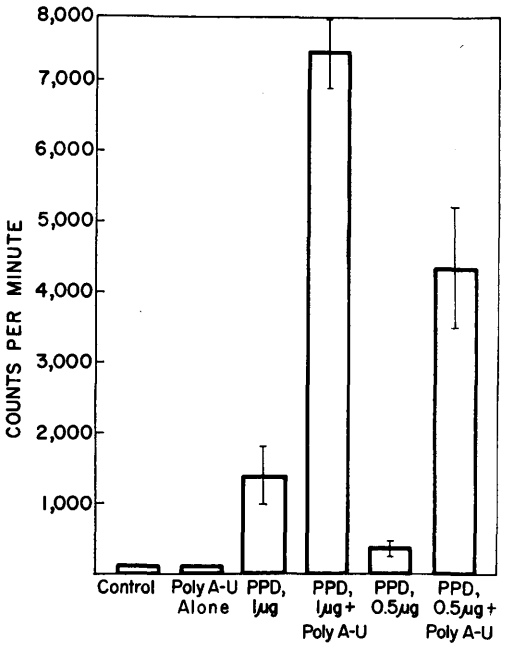

FIG. 1. Enhancement of tritiated thymidine uptake of PPD-stimulated leukocytes by 100 μg of Poly A-U per culture.

a 30-fold increase in uptake of tritiated thymidine as compared to unmixed cells. Addition of 100 μg of poly A–poly U more than doubled this value. In addition, it was found that the leukocytes of a patient who had an allergic reaction to streptomycin did not respond significantly to this antibiotic alone, but when poly A–poly U was added to the cultures, the rate of labeling by tritiated thymidine was 5 times the control values.

Figure 3 illustrates the fact that the poly-

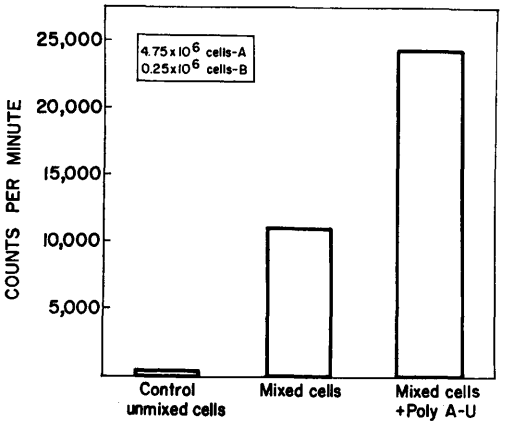

FIG. 2. Enhancement of tritiated thymidine uptake of mixed leukocyte-stimulated cultures by Poly A-U.

nucleotide complex could be added to cultures 4 hr after PPD without loss of activity. Addition of the homoribopolymers prior to antigen resulted in a slight diminution in enhancing action. Microscopic examination of the cultured cells revealed an increased number of mitotic figures as well as higher numbers of blast forms in those tubes incubated with poly A–poly U and PPD as compared with PPD alone.

Conversely, the addition of 50–100 μg of poly A–poly U to cultures of PHA-stimulated leukocytes harvested after 24, 48, or 72 hr in culture resulted in a definite *depression* of DNA synthesis (Fig. 4). Decreasing the PHA/culture tube to 0.1 the maximum stimulatory amount also (*i.e.*, 0.01 ml) did not

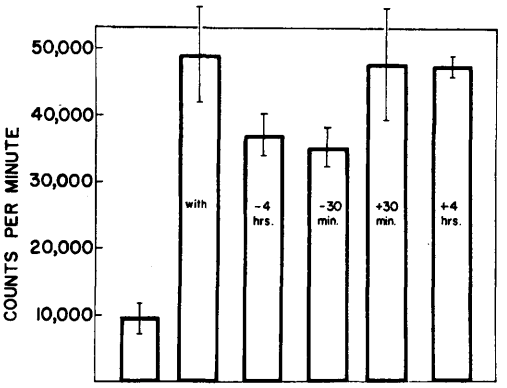

FIG. 3. Effect of time of addition of Poly A-U to culture before and after PPD; in columns 2–6 is given the time of addition of 100 μg of Poly A-U in relation to PPD; all cultures received 1 μg of PPD.

reveal any adjuvant action of poly A–poly U on this response.

Discussion. The mechanism of the adjuvant effect of polynucleotides on antibody synthesis is not known. There is evidence that adjuvants increase the rate of division of antibody producing clones (1, 8), but conceivably this could be secondary to alterations induced in the function of monocytes or macrophages (4, 9). Freedman and Braun reported an increased clearance of carbon particles from the circulation of mice treated with deoxyribonucleotides implicating an effect on macrophages (10). More direct evidence was obtained when 98% pure mouse peritoneal macrophages were incubated with bovine gamma globulin as antigen with poly

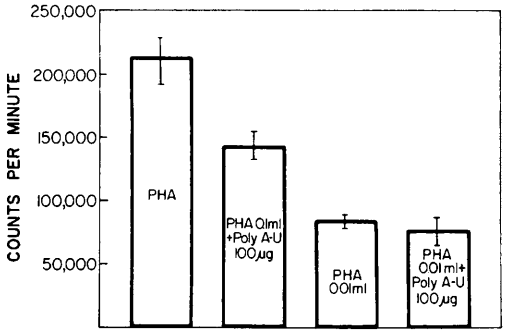

FIG. 4. Depressant effect of Poly A-U on PHA stimulated leukocytes.

A–poly U. These cells showed a significant increase in tritiated-uridine uptake, and caused a 70-fold increase in antibody titer as compared to macrophages incubated with BGG alone when they were reinjected into the peritoneal cavity of mice (4). The lack of response to poly A–poly U in enhancing PHA-stimulated transformation would also favor a primary effect on monocytes or macrophages. PHA, unlike antigen, does not require the presence of both monocytes and lymphocytes to induce transformation (11).

Increasing the rate of pinocytosis, a property of other macromolecules, may be an important function of the polynucleotides. Cohn and Parks (12) reported an extensive list of polyanions and cations capable of increasing pinocytosis of macrophages. Oppenheim *et al.* (13) found that diethylaminodextran and heparin can enhance proliferation of PHA, antigen, or mixed leukocyte-stimulated lymphocytes. The difference between their results and ours regarding the contrasting effects on PHA stimulation remains unexplained, as yet. It would seem likely, however, that a more complex series of events is initiated by the polynucleotides than just enhancement of antigen processing. It takes only a few seconds for PPD to activate sensitized lymphocytes in culture (14), while the poly A–poly U still exerted an effect when added 4 hr after the antigen. At any rate, it is clear that such adjuvants may increase the sensitivity and usefulness of leukocyte cultures for clinical studies.

Conclusions and Summary. Polyadenylic acid when complexed with polyuridylic acid, exerted an adjuvant effect in short-term human leukocyte cultures activated by purified protein derivative of tuberculin or in mixed leukocyte interactions. In contrast, stimulation of DNA synthesis in leukocytes by phytohemagglutinin was depressed by these homoribopolymers.

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Received July 2, 1969. P.S.E.B.M., 1969, Vol. 132.