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Inhibitory Effect of Synthetic Polyribonucleotides on the Primary *in Vitro* Immune Response (38860)

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Synthetic double-stranded polyribonucleotides are well known as enhancers or adjuvants of the *in vivo* antibody response (1–3). This enhancement has been ascribed to the effect of these polyribonucleotides on the T lymphocytes (4–6). Less recognized and relatively unpursued is the fact that synthetic double-stranded polyribonucleotides can also inhibit the *in vivo* antibody response under certain conditions (3, 7). *In vitro* studies on the immune response also indicate that synthetic double-stranded polyribonucleotides are enhancers of the antibody response, but even here, indications of an inhibitory effect are found (8–10).

Data are presented here that examine the inhibitory aspects of synthetic double-stranded polyribonucleotides on the primary *in vitro* antibody response to both a thymus-dependent and thymus-independent antigen. The inhibitory effect of the polyribonucleotides on the *in vitro* immune response will be shown to be dependent on the presence of functional T lymphocytes in the spleen cell cultures.

Materials and Methods. Mice. C57BL/6 female mice, 8–12 wk old, were obtained from the Laboratory Supply Company, Indianapolis, IN. Congenitally athymic, female, nude mice, 8–10 wk old, homozygous for the nu/nu allele, were supplied by J. G. Michael (11) of the University of Cincinnati. The original breeding nucleus of the nude mice was obtained from I. Lefkovitz, Basel Institute for Immunology, Basel, Switzerland.

Diluent. Spleen cells at the time of harvesting, synthetic polyribonucleotides, and antigens were all suspended or diluted in Eagle's minimal essential medium (MEM) containing spinner salts, but no L-glutamine or sodium bicarbonate (12).

Antigens. High-responding sheep red blood cells (SRBC), the thymus-dependent antigen (13), were obtained from the Colorado serum Company, Denver, CO. The SRBC used throughout the study were obtained from a single sheep (No. 446).

A stock culture of *E. coli* 0127 bacteria, the thymus-independent antigen (14), was grown on BHI agar (15). The organisms were washed four times with phosphate-buffered saline (PBS), pH 7.2, suspended in PBS, and boiled for 1 hr. The organisms were then washed three times with modified MEM and suspended to 1×10^9 organisms/ml.

Cultures. Dissociated mouse spleen cells were cultured for *in vitro* anti-SRBC plaque-forming cell (PFC) response exactly as described by Mishell and Dutton (12). Cultures consisted of 1.0×10^7 to 1.5×10^7 spleen cells/ml and 3×10^6 SRBC. All PFC responses were determined at day 5. Cell viabilities were determined by trypan blue dye exclusion. Direct PFC assays were carried out on microscope slides as previously described (16). A single lot (No. 22301) of fetal calf serum obtained from the Reheis Company, Kankakee, IL, was used throughout the study. All results are expressed as the average of duplicate cultures.

Anti-*E. coli* 0127 PFC responses were carried out as described for SRBC above. A range of *E. coli* organism concentrations, 1×10^4 to 1×10^6/culture, was tested, and 1×10^5 organisms were found to be optimal for specific anti-*E. coli* PFC responses. SRBC were sensitized with 0127 lipopolysaccharide for anti-0127 PFC responses as previously described (17). Appropriate controls, including untreated SRBC, were included in all assays to monitor the so-called polyclonal or nonspecific anti-SRBC response (18). Our results agreed with those of other investigators (11) in that no such non-specific PFC responses to SRBC were observed.

Synthetic polyribonucleotides. The double-

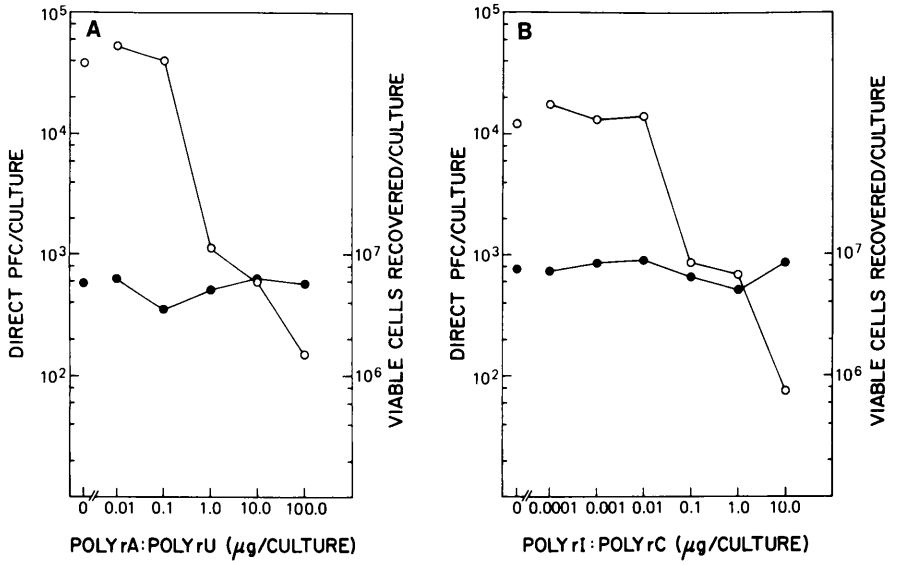

FIG. 1. The effect of poly (rA):poly (rU)(A) and poly (rI):poly (rC)(B) on the primary *in vitro* PFC response to SRBC in C57BL/6 mouse spleen cultures. Polyribonucleotides were added at the time of SRBC addition, and direct anti-SRBC PFC/culture (○——○) and viable cells recovered/culture (●——●) were determined on day 5.

stranded complexes, polyriboadenylate:polyribouridylate [poly (rA):poly (rU)] and polyriboinosinate:polyribocytidylate [poly (rI):poly (rC)], were obtained from Miles Laboratories, Inc., Elkhart, IN. The complexes had minimal molecular weights of 100,000. The potassium salts of the single-stranded polynucleotides, polyriboadenylic acid [poly (rA)], and polyriboinosinic acid [poly (rI)], minimal molecular weights of 100,000, were obtained from Calbiochem San Diego, CA. Stock solutions were stored at −68 C. Various concentrations were added to cultures in 100-μl vol.

Results. Effect of polyribonucleotides on the in vitro PFC response of C57BL/6 spleen cells to SRBC. Representative data on the inhibitory effects of various concentrations of the duplexes poly (rA):poly (rU) and poly (rI):poly (rC) on the PFC response of C57BL/6 spleen cells to SRBC are presented in Fig. 1. Synthetic polyribonucleotides and SRBC were added to cultures at the same time. Poly (rA):poly (rU), 1.0 μg/culture, inhibited the PFC response by >90% (Fig. 1A). Similar inhibitory effects of poly (rA):poly (rU) were obtained in three separate experiments (data not shown). Poly (rI):poly (rC) inhibited the PFC response to SRBC by >90% at 0.1 μg polynucleotide/culture (Fig. 1B). Repeated experiments have shown that the >90% inhibitory concentration of poly (rI):poly (rC) may vary from 0.1 to 10.0 μg polynucleotide/culture (data not shown). A consistent pattern of enhancement was not observed with either polyribonucleotide at any of the concentrations tested. What might be interpreted as slight enhancement (41%) of the PFC response with 0.01 μg of poly (rA):poly (rU) (Fig. 1A) was not responsible in subsequent experiments or at lower concentrations. The same erratic pattern of enhancement was observed with poly (rI):poly (rC) (Fig. 1B). Kinetic data on the effect of the synthetic double-stranded polyribouncleotides (10 μg/culture) on the PFC response at days 3, 4, and 5 also showed a consistent inhibitory effect, but not an enhancement effect (data not shown). Thus, poly (rA):poly (rU) and poly (rI): poly (rC) had profound inhibitory effects on the anti-SRBC PFC response at low concentration ranges (0.1–10.0 μg polynucleotide/culture). The viable cell recovery was not reduced by the PFC inhibitory concentrations of either polyribonucleotide (Fig. 1).

The single-stranded polyribonucleotides,

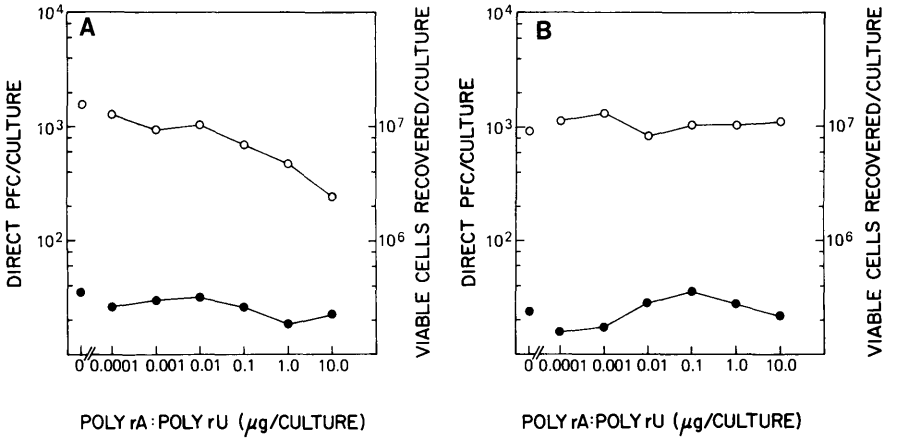

FIG. 2. The effect of poly (rA):poly (rU) on the primary *in vitro* PFC response to *E. coli* 0127 LPS in C57BL/6 (A) and nude (athymic) (B) mouse spleen cultures. The polyribonucleotide was added at the same time as antigen, and direct anti-*E. coli* 0127 LPS PFC/culture (○——○) and viable cells recovered/culture (●——●) were determined on day 5.

poly (rA) and poly (rI), were not inhibitory at the same concentrations as their corresponding duplexes. It required 1000 μg of the single-stranded polynucleotides/culture to inhibit the PFC response by >90%.

The inhibition patterns described above for double- and single-stranded synthetic polyribonucleotides were also observed with synthetic polynucleotides obtained from another source (P. L. Biochemicals, Milwaukee, WI). These results support the validity of the data presented.

Effect of polyribonucleotides on the in vitro PFC response of C57BL/6 spleen cells to E. coli 0127. *E. coli* 0127 lipopolysaccharide (LPS) is a thymus-independent antigen in both *in vivo* (18) and *in vitro* (11) PFC responses. The effect of poly (rA):poly (rU) and poly (rI):poly (rC) on the anti-*E. coli* 0127 PFC response of C57BL/6 spleen cells was examined. Data on the inhibitory effect of poly (rA):poly (rU) are presented in Fig. 2A. The anti-0127 LPS PFC response was inhibited 69 and 84% by 1.0 and 10.0 μg, respectively, of polyribonucleotide/culture without a reduction in the number of viable cells recovered. A similar pattern was obtained with poly (rI):poly (rC), while poly (rA) and poly (rU) were noninhibitory at the same concentrations. Enhancement of the PFC response was not observed at lower concentrations of polyribonucleotides.

Effect of polyribonucleotides on the in vitro PFC response of nude (athymic) spleen cells to E. coli 0127. Spleen cells from the nude (athymic) mice contain functional B lymphocytes but do not contain functional T lymphocytes (11, 19). This fact is in contrast to C57BL/6 spleen cells, which contain both functional B and T lymphocytes (13). Since *E. coli* 0127 LPS is a thymus-independent antigen, the *in vitro* PFC response is not affected by the lack of functional T lymphocytes in nude spleen cells (11). We therefore determined the inhibitory effect of double-stranded polyribonucleotides on the PFC response of nude spleen cells to *E. coli* 0127 (Fig. 2B). Poly (rA):poly (rU), at concentrations (1 and 10 μg/culture) that were inhibitory in C57BL/6 spleen cell cultures (Fig. 2A), was noninhibitory in the PFC response of nude spleen cells to *E. coli* 0127 (Fig. 2B). Poly (rI):poly (rC) was also noninhibitory. This finding is consistent with the interpretation that functional T cells are required for double-stranded synthetic polyribonucleotides to exert an inhibitory effect on the *in vitro* PFC response.

Discussion. Synthetic double-stranded polyribonucleotides are well known as enhancers or adjuvants of the *in vivo* antibody response (1–3). It has also been shown that these same polyribonucleotides are potent inhibitors of the antibody response if given

to the animal 18–48 hr before the antigen (3, 7). *In vitro* studies have indicated that poly (rA):poly (rU) can have a modest enhancing or inhibiting effect on the immune response, depending on the polyribonucleotide concentration and the length of the culture period (8–10).

We have shown here that poly (rA):poly (rU) and poly (rI):poly (rC) are potent inhibitors of the primary *in vitro* antibody response to both a thymus-dependent (SRBC) and a thymus-independent (*E. coli* 0127 LPS) antigen using a mouse spleen culture system (C57BL/6) that contained both functional B and T lymphocytes (13). The PFC response to SRBC and *E. coli* 0127 LPS was inhibited by 84% or more by 0.1–10 μg polynucleotide/culture. Enhancement of the PFC response by lower concentrations of the duplexes was modest and not consistently reproducible. The single-stranded polynucleotides, poly (rA) and poly (rI), were relatively ineffective as inhibitors.

Poly (rA):poly (rU) and poly (rI):poly (rC) had no inhibitory effect on the PFC response to *E. coli* 0127 LPS by a spleen culture system (athymic, nude mice) that contained functional B lymphocytes but lacked functional T lymphocytes (11, 19). This finding raises the possibility that functional T lymphocytes are necessary for double-stranded polyribonucleotides to exert their inhibitory effect on the PFC response.

The T cell has previously been shown to be the target cell for the adjuvant effect of poly (rA):poly (rU) (4–6). The T cell may normally exert its inhibitory effect on the *in vitro* PFC response by production of the lymphokine, interferon (20). Synthetic double-stranded polyribonucleotides are known interferon inducers (21), and it has recently been demonstrated that interferon can inhibit the immune response to SRBC both *in vivo* (22, 23) and *in vitro* (24–26). We have obtained this *in vitro* inhibition with physiological concentrations of interferon (25, 26). Further, interferon inhibited the PFC response to *E. coli* 0127 LPS in both C57BL/6 and nude (athymic) spleen cultures (unpublished data). The above thesis is in agreement with data indicating that functional T lymphocytes are necessary for early induction of interferon in mouse spleen cell cultures by T cell mitogens (20). Studies are in progress to determine the nature of interferon induction by synthetic polyribonucleotides in Mishell-Dutton spleen-cultures (12).

Finally, data have previously indicated that the *in vivo* (27) and *in vitro* (11) PFC response to *E. coli* 0127 LPS was not influenced by T cells. Since double-stranded polyribonucleotides can inhibit the *in vitro* PFC response to this antigen in mouse C57BL/6 spleen cultures, but not in nude (athymic) cultures, we have shown here that functional T lymphocytes can affect the B cell response to *E. coli* 0127 LPS under certain conditions.

Summary. The synthetic double-stranded polyribonucleotides, poly (rA):poly (rU) and poly (rI):poly (rC), were shown to be potent inhibitors of the *in vitro* plaque-forming cell (PFC) response to a thymus-dependent (SRBC) and thymus-independent (*E. coli* 0127 LPS) antigen in mouse C57BL/6 spleen cell cultures. The same polynucleotides had no effect on the PFC response of nude (athymic) mouse spleen cells to *E. coli* 0127 LPS, suggesting that functional T lymphocytes are necessary for the inhibitory effect. Enhancement effects were modest and inconsistent in the cultures. Poly (rA) and poly (rU) were ineffective as inhibitors. The data indirectly suggest that the inhibition may be due to the early production of interferon by functional T lymphocytes.

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