# Depression of the Interferon Response to Polyinosinic·Polycytidylic Acid by Specific Antibody (35620)

A. D. STEINBERG, S. BARON, C. UHLENDORF, AND N. TALAL

*National Institute of Arthritis and Metabolic Diseases and National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20014*

NZB/NZW (B/W) mice, like human patients with systemic lupus erythematosus, spontaneously develop antibodies to nucleic acids. Sera from such mice will bind the double-stranded RNA, polyinosinic·polycytidylic acid (poly I·poly C). Treatment of B/W mice with multiple intraperitoneal injections of poly I·poly C stimulates interferon production but also induces higher titers of anti-RNA antibodies (1). Mice can be made immunologically tolerant to poly I·poly C if cyclophosphamide is given after the synthetic RNA (2). As part of these experiments we have studied the effect of immunization and of specific tolerance to poly I·poly C on interferon induction in B/W mice.

*Methods. Animals.* NZB/NZW $F_1$ and A/LN mice were obtained from colonies maintained at the National Institutes of Health, Bethesda, Maryland. All NZB/NZW $F_1$ hybrids were obtained by NZB female $\times$ NZW male matings.

*Polynucleotides.* The double-stranded RNAs, poly I·poly C and poly A·poly U, were formed by annealing equimolar amounts of the single-stranded homopolymers, polyinosinic and polycytidylic acid or polyadenylic and polyuridylic acid, in 0.15 *M* NaCl (3).

*Interferon assay.* Interferon titers in sera and interferon-like resistance were determined as the reciprocal of the highest dilution of the sample which inhibited the yield of vesicular stomatitis virus in primary mouse embryo culture by 0.5 $\log_{10}$ (4).

*Antibody effects on interferon induction.* In vivo *method.* One-month-old B/W females were injected intraperitoneally (ip) with poly I·poly C (150 $\mu$g) emulsified with an equal volume of complete Freund's adjuvant. Two weeks later they were bled for antibody assay. Immediately after bleeding they were injected ip with poly I·poly C either 10 or 100 $\mu$g and bled again 4, 12, and 24 hr later for determination of interferon titers. Additional control B/W mice were injected with Freund's adjuvant alone or left uninjected. They also were bled for assay of interferon titers 4 hr after receiving 10 or 100 $\mu$g poly I·poly C. These control mice did not have significant antibody titers to poly I·poly C.

In vitro *and* in vivo *method.* One-month-old B/W females were immunized with poly I·poly C in complete Freund's adjuvant as described above. Two weeks later they were bled for assay of antibodies to poly I·poly C. High-titered antisera (binding capacity 32–100 $\mu$g/ml (2) ) and serum from immunized animals with binding capacity of $<3$ $\mu$g/ml were pooled separately. Three milliliters of each pool were mixed with 30 $\mu$g poly I·poly C *in vitro* and incubated at 37° for 15 min and then at 4° for 120 min. One fifth of the mixture was injected intraperitoneally or intravenously into each of five untreated young B/W females. Four hours later the recipient mice were bled for assay of interferon titers. Control young B/W females were injected with 6 $\mu$g poly I·poly C and bled 4 hr later.

In vitro *method.* Immunization of A/LN mice with poly A·poly U produces large quantities of antibody that cross reacts with poly I·poly C (5). One-month-old A/LN mice were immunized with one or two ip injections of poly A·poly U in complete Freund's adjuvant. Two weeks later they were bled and six very high-titered antisera were obtained (antigen-binding capacity $>200$ $\mu$g/ml). Each antiserum (0.2 ml) and control A/LN sera were individually mixed

TABLE I. Reduced Interferon Response by B/W Mice Containing Antibodies to Poly I • Poly C.

| Antibody to poly I • poly C | Poly I • poly C injection ($\mu$g) | Interferon titer ($\log_{10}$) at 4 hr | Range |
|---|---|---|---|
| No[a] | 100 | 3.6 | 3.3–3.9 |
| Yes | 100 | 2.2 | 2.0–2.4 |
| No | 10 | 3.2 | 3.0–3.3 |
| Yes | 10 | 1.6 | 1.5–1.8 |

[a] Unimmunized controls.

with 1 $\mu$g poly I·poly C in Eagle's medium and incubated at 37° for 30 min and 4° for 60 min. The mixtures were then assayed for ability to induce interferon-like resistance in the presence and in the absence of neomycin (6). In a second experiment antisera were diluted serially prior to addition of 1 $\mu$g of poly I·poly C.

*Tolerance effects on interferon induction.* One-month-old B/W female mice were made tolerant to poly I·poly C by treating them with two courses of poly I·poly C (100 $\mu$g) ip followed 24 hr later by cyclophosphamide (75 $\mu$g/g) ip. The two courses were separated by 5 days. Control B/W females were treated with either poly I·poly C alone or cyclophosphamide alone. Twelve days after the last injection all mice were injected with 100 $\mu$g of poly I·poly C and bled 4 and 12 hr later for interferon assay. They were then challenged with poly I·poly C in complete Freund's adjuvant. Two weeks later they were bled. The absence of anti-RNA antibodies confirmed the presence of immunological tolerance (2).

*Results.* Mice with circulating antibody to poly I·poly C injected with either 10 or 100 $\mu$g of poly I·poly C showed a reduced interferon response in each of three experiments. Table I shows the reduced interferon titers at 4 hr in a representative experiment. This reduction was also present at 12 and 24 hr.

In an attempt to define the role of antibody in this reduction, sera from mice with high titers of anti-poly I·poly C antibody were mixed *in vitro* with poly I·poly C and then injected into untreated mice. Such antisera strongly depressed the interferon responses to poly I·poly C (Table II) at an antibody excess of 3- to 10-fold. Antisera with poly I·poly C-binding capacities of $<3$ $\mu$g/ml did not significantly reduce the interferon response.

*In vitro* mixture of poly I·poly C and antibody to poly I·poly C also led to a reduction in interferon-like resistance when the mixture was added to the *in vitro* assay. This took place at high antibody concentrations, but fell off rapidly with dilution of antibody. When the poly I·poly C-binding capacity of the antiserum was 80–160 times the quantity of poly I·poly C added, the resistance-inducing capacity of the poly I·poly C was eliminated (Table III). Twenty-five- to fifty-fold excess of antibody showed some reduction in the amount of interferon-like resistance; however, lesser amounts of antibody did not inhibit interferon induction. Dilution of the antigen–antibody mixture also reduced the suppressive effects of the antibody. The requirement for antibody excess in the *in vitro* method was greater than that of the *in vitro* plus *in vivo* method.

To determine the relationship between immunological tolerance to poly I·poly C and interferon induction by poly I·poly C experiments were performed using tolerant and nontolerant mice. Mice made tolerant to poly I·poly C had normal interferon responses at 4 and 12 hr after induction.

*Discussion.* The finding that antibodies to poly I·poly C reduce the interferon response *in vivo* and *in vitro* implies that poly I·poly C antibody complexes are not capable of inducing interferon as well as poly I·poly C

TABLE II. Reduced Interferon Response After Mixing Poly I • Poly C with Antibodies to Poly I • Poly C and Injecting the Mixture into Untreated Mice.

| Mixture received by each mouse | | |
|---|---|---|
| Serum[b] with binding capacity of: | Poly I • poly C ($\mu$g) | Interferon titer ($\log_{10}$)[a] |
| 32–100 $\mu$g/ml | 6 | 1.8 |
| $<3$ $\mu$g/ml | 6 | 2.7 |
| Control | 6 | 2.9 |

[a] Mean of five mice assayed individually.

[b] Volume of serum 0.6 ml.

TABLE III. Suppression of Poly I • Poly C Induction of Interferon-Like Resistance by Excess Antibody.

| Expt. no. | Serum | Antibody/antigen ratio[a] | Inhibition of yield of VSV ($log_{10}$)[b] |
|---|---|---|---|
| 1 | a | 80 | 0.0 |
| | b | 80 | 0.0 |
| | c | 160 | 0.0 |
| | d | 160 | 0.0 |
| | Four control sera | 0 | >1.7 |
| | Medium control | — | >1.7 |
| 2 | e | 50 | 0.3 |
| | | 12 | >1.0 |
| | | 3 | >1.0 |
| | | 1 | >1.0 |
| | f | 50 | 0.7 |
| | | 12 | >1.0 |
| | | 3 | >1.0 |
| | | 1 | >1.0 |
| | g | 50 | 0.0 |
| | | 25 | 0.5 |
| | h | 50 | 0.3 |
| | Four control sera | 0 | >1.0 |
| | Medium control | — | >1.0 |

[a] [Poly I • poly C-binding capacity of antiserum ($\mu$g/ml) × antiserum volume (ml)]/($\mu$g Poly I • poly C).

[b] Vesicular stomatitis virus.

alone. Antisera to poly I·poly C demonstrated this suppressive effect *in vitro* only when present in marked excess. The effect was observed when the poly I·poly C binding capacity of the serum was greater than 25 times the quantity of poly I·poly C in the mixture. When the serum was diluted to the point of 12-fold antibody excess the suppressive effect was not apparent. This finding suggests that the poly I·poly C molecules had to be largely completely coated with antibody to be ineffective as interferon inducers *in vitro*. Dissociation of some antigen–antibody complexes could contribute to the requirement for antibody excess. Antibodies to poly I·poly C probably are responsible also for the reduced interferon response to poly I·poly C in immunized rabbits (7).

High-titered antibody to poly I·poly C, such as that used in the present study, may be a useful tool in studying the mechanism of interferon induction by poly I·poly C. In addition, the suppression of interferon induction by antibody to poly I·poly C may have practical importance in man since production of antibodies to poly I·poly C would decrease its effectiveness. A lower molecular weight poly I·poly C might be less antigenic and yet retain interferon-inducing properties.

The immune response requires the participation of lymphoid cells. These cells also make interferon when stimulated nonspecifically by mitogens (8, 9) or specifically by viral (10) or nonviral (11) antigens to which they are sensitive. These results suggest an association between immunity and interferon induction. However, many cell types other than lymphocytes produce interferon in response to a variety of stimuli (12). This observation indicates that immune stimulation is probably not required for all types of interferon induction. The relative contribution of immune lymphoid cells to *in vivo* interferon production has not been previously studied. The present study demonstrates a dissociation between the immune response and the interferon response to poly I·poly C since mice made tolerant to poly I·poly C could produce interferon in response to this synthetic RNA. Apparently nonimmune cells which were not killed by cyclophosphamide remained to produce normal quantities of interferon.

*Summary.* The relationship in mice between immune status to polyinosinic·polycytidylic acid (poly I·poly C) and interferon induction by poly I·poly C was studied *in vivo* and *in vitro*. Circulating antibody to poly I·poly C reduced the *in vivo* interferon response to poly I·poly C. A similar but less profound inhibitory effect of specific antibody upon interferon induction was demonstrated *in vitro*. The findings imply that antibody-poly I·poly C immune complexes are less potent interferon inducers than poly I·poly C without antibody. Immunological tolerance to poly I·poly C did not alter interferon induction by poly I·poly C indicating dissociation between immunological status and interferon induction with this synthetic RNA *in vivo*.

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