Decreased Interferon Response to Polyinosinic-Polycytidylic Acid in Rabbits Immunized Against the Inducer (36085)

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Previous preliminary observations (1) showed that rabbits immunized against the interferon (IF) inducer polyinosinic-polycytidylic acid (In·Cn) failed to produce IF when injected intravenously with In·Cn. However, it could not be determined whether the lack of IF production by immunized rabbits was due to neutralization of the inducer by specific antibodies or due to degradation by increased levels of depolymerizing enzymes (2).

The present study was undertaken to explore this problem further *in vivo* and *in vitro*. Specifically studies were carried out to determine: (i) IF production in immunized rabbits treated with In·Cn or with a form of In·Cn obtained by complexing it with DEAE-dextran—the resulting complex was highly resistant to serum and pancreatic RNase (2, 3); and (ii) the effect of immune sera and purified immunoglobulins on the IF-inducing activity of In·Cn in tissue cultures.

Materials and Methods. Young New Zealand male rabbits (3 kg av wt) were immunized as previously described (1, 4) using In·Cn (P-L Biochemicals) complexed with methylated bovine serum albumin in Freund's complete adjuvant. Other rabbits were treated with methylated serum albumin alone in Freund's adjuvant as control. Antibody levels and specificity were tested by simple and radial immunodiffusion and by complement fixation (CF) tests as previously described (2, 5).

IF production in immune and control rabbits was determined by injecting 10 μg of In·Cn or In·Cr, DEAE-dextran complex, intravenously. Immediately before injection and 4 hr later, blood samples were collected for IF titration. The IF titer was determined in primary rabbit kidney cell cultures (RK) as the reciprocal of the highest dilution which inhibited the yield of vesicular stomatitis virus (VSV) by 68.4% (6).

The effect of the immune and control sera on the IF-inducing activity of In·Cn was studied by mixing a 1:10 dilution of serum with the same volume of Eagle's medium containing 2 μg/ml of In·Cn or In·Cn, DEAE-dextran complex. Each mixture was incubated 30 min at 4° and 15 min at 37° and then it was inoculated into 3 tubes of RK cells. Neomycin (200 μg/ml) was added to each tube to potentiate the antiviral action of the inducers (7). After 5 hr of incubation at 37°, the cultures were carefully washed and challenged with VSV as previously described (8). A VSV yield reduction of 0.5 $\log_{10}$ (68.4%) was considered significant for antiviral activity of the inducers.

The same procedure was used to test the effect of serial dilutions of immunoglobulins (IG) obtained from pools of immune and control sera on the antiviral activity of In·Cn. IG were purified by ammonium sulfate precipitation followed by DEAE-Sephadex (Pharmacia, Uppsala) chromatography. RNase activity was determined before and after purification as previously described (2).

Results. Interferon production in immune and control rabbits treated with In·Cn or with In·Cn, DEAE-dextran complex. All the immunized rabbits showed precipitating and CF antibodies and no detectable antibody activity was found in controls. The IF response after induction with In·Cn or with In·Cn, DEAE-dextran complex is reported in Table I.

As shown, both inducers stimulated IF production in control animals but not in those with circulating antibodies to In·Cn.

Effect of immune and control sera on antiviral resistance induced by In·Cn or In·Cn,

TABLE I. Interferon Production by Immune and Control Rabbits Injected Intravenously with 10 μg of In • Cn or with In • Cn, DEAE-dextran Complex.

Rabbits	Log_{10} IF titer at 4 hr[a]	
	Treated with In • Cn	Treated with In • Cn, DEAE-dextran complex
Control	2.0	2.5
	2.5	2.0
	2.5	2.5
	2.5	
Immune	<1.0	<1.0
	<1.0	<1.0
	<1.0	<1.0
	<1.0	

[a] Preinjection sera contained no detectable IF.

DEAE-dextran complex in RK cell cultures. In order to test whether the inhibition of the IF-inducing activity could be related to a serum factor present in immunized animals, sera of immune and control rabbits were mixed with the inducers *in vitro* as described above and the residual antiviral activity was tested in RK cell cultures. The results are reported in Table II.

As shown, the antiviral activity by In·Cn and the ribonuclease-resistant In·Cn, DEAE-dextran complex was inhibited by immune and not by control sera. Control cultures treated with each of the sera without the inducer and challenged with VSV produced full yield of virus ($10^{-5.5}$ log_{10}/ml).

To test whether the effect of immune sera could be related to neutralizing antibodies, an experiment was carried out in which the antiviral activity of In·Cn was assayed in RK cells after treatment of the inducer with IG obtained from pools of immune and control sera. With respect to whole immune sera, IG preparations showed an increase of antibody activity of about 10-fold; whereas they showed a decrease of RNase activity of about 36-fold (0.13 units versus 4.7 units). The results are reported in Table III.

Discussion. Our preliminary observations (1) showed that rabbits immunized against In·Cn failed to produce IF when injected with this inducer. The present report indicates that the suppressive effect is mainly due to the presence of specific circulating antibodies rather than to increased levels of depolymerizing enzymes which have been demonstrated in immunized animals (2). This conclusion is substantiated by the finding that (i) suppression of IF production takes place also when an RNase-resistant form of In·Cn was used as inducer; and (ii) the inhibitory activity of immune sera *in vitro* is separated with the IG fraction; whereas RNase activity is strongly reduced in this fraction. In addition, it also was shown recently that antibodies to In·Cn are responsible for reduced IF response in mice (8). Natural and synthetic polynucleotides like In·Cn sometimes can evoke antibodies also in the absence of immunological adjuvants, such as in the New Zealand strain of mice or in patients with lupus erythematodes (8). Any application would be impaired in such situation and also potentially dangerous, even though our immunized rabbits did not show any detectable pathologic effect.

Summary. Rabbits immunized with the double-stranded polynucleotide complex formed by polyinosinic and polycytidylic acid coupled with methylated bovine albumin and emulsified in Freund's complete adjuvant failed to produce interferon after intravenous injection of this inducer. Suppression of interferon production also occurred

TABLE II. Effect of 5% Immune and Control Sera on the Ability of 1 μg of In • Cn or In • Cn, DEAE-dextran Complex to Induce Antiviral Resistance in RK Cell Cultures.

Serum	Presence of In • Cn antibodies	% of control VSV yield	
		In • Cn[a]	In • Cn, DEAE-dextran complex[a]
1	No	15.8	7.9
2	No	12.8	10.0
3	No	10.0	5.0
4	Yes	79.4	63.1
5	Yes	100.0	79.4
6	Yes	100.0	79.4
None (inducer control)		12.6	7.9

[a] 31.6% of control virus yield is statistically significantly different.

TABLE III. Effect of Immunoglobulins Obtained from Immune and Control Sera on Antiviral Activity Induced by In • Cn in RK Cell Cultures.

Immunoglobulins obtained from	% of control VSV yields at IG dilution: 1:40	1:160	1:640
Immune serum	79.4	100.0	63.1
Control sera	12.6	12.6	15.8

when immune rabbits were injected with an RNase-resistant form of the inducer obtained by complexing it with DEAE-dextran. This result indicates that increased serum RNase was not responsible for the inhibitory activity. In rabbit kidney cell cultures, sera obtained from immunized rabbits suppressed the antiviral activity of the inducers. This effect was associated with the immunoglobulin serum fraction despite a strong decrease of RNase activity in this fraction.

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