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Interferon Induction in Cynomolgus and Rhesus Monkeys After Repeated Doses of a Modified Polyriboinosinic-Polyribocytidylic Acid Complex

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Serum interferon activity was determined in 12 cynomolgus and 12 rhesus monkeys injected intravenously once daily for 10 days with from 0.1 to 6.0 mg of a stabilized polyriboinosinic acid·polyribocytidylic acid complex per kg, composed of polyriboinosinic acid·polyribocytidylic acid, poly-1-lysine, and carboxymethylcellulose [poly(ICLC)]. Interferon activity was detected 2 h after the first injection, with maximum activity occurring 8 h after the second injection. A period of hyporesponsiveness occurred after the third injection of poly(ICLC) in all monkeys and lasted until the sixth injection in the rhesus monkeys, when interferon activity again became more elevated. The delayed rebound was not as apparent in cynomolgus monkeys. Rhesus monkeys injected with 6 mg/kg did not exhibit serious side effects.

Polyriboinosinic acid·polyribocytidylic acid [poly(I)·poly(C)] is effective as an interferon inducer in rodents (3-6, 14), but not in cattle, humans, or subhuman primates (7, 13). Failure of poly(I)·poly(C) to induce interferon in these latter species has been ascribed to the action of a serum nucleolytic enzyme, which inactivates the compound (10, 11). By complexing the poly(I)·poly(C) with poly-1-lysine and carboxymethylcellulose, it was found that interferon could be induced in rhesus monkeys and chimpanzees by intravenous injection of the resulting stabilized compound [poly(ICLC)] (11).

The effectiveness of this stabilized compound for therapy against viral infection has been demonstrated in rhesus monkeys infected with simian hemorrhagic fever virus (11), yellow fever virus (our unpublished observations), or street rabies virus (G. Baer, S. Baron, and H. B. Levy, unpublished data). A possible barrier to antiviral therapy with poly(ICLC) is the period of hyporesponsiveness, manifested by diminished levels of serum interferon activity during the administration of repeated doses of interferon inducers or viral agents which has been reported by others in rodents and cattle (2-5, 8, 13, 14). This study describes the early interferon responses and a period of hyporesponsiveness that occurs after repeated injections with multiple doses of poly(ICLC) in cynomolgus (*Macaca fasicularis*) and rhesus (*Macaca mulatta*) monkeys.

MATERIALS AND METHODS

Drugs. Poly(ICLC), lot 66, prepared at the National Institutes of Health as previously described (11), was diluted with equal volumes of pyrogen-free 0.85% NaCl prior to injections into monkeys. Ketaset (ketamine hydrochloride, Bristol Laboratories, Syracuse, N.Y.), 10 mg/kg, was injected intramuscularly for restraint of the monkeys to facilitate injection of poly(ICLC).

Monkeys. Twelve healthy, young adult, male cynomolgus monkeys, weighing 1.8 to 2.2 kg, and 12 healthy, young adult, male rhesus monkeys, weighing 3 to 4 kg, were housed in individual cages and maintained throughout the experimental period at a constant room temperature (25°C) and a 12-h light cycle. Monkeys were fed four to six commercial monkey chow biscuits per day (Wayne Monkey Diet, Allied Mills, Inc., Chicago, Ill.), and water was available ad libitum. Apples were given each day 3 h after injection of poly(ICLC).

Injection and bleeding schedules. Four groups of three cynomolgus monkeys each were injected intravenously with 0.1, 0.3, 1.0, or 3.0 mg of poly(ICLC)/kg of body weight per day for 10 days. In addition, four groups of three rhesus monkeys each were injected intravenously with 0.3, 1.0, 3.0, or 6.0 mg of poly(ICLC)/kg per day for 10 days. A 3- to 4-ml amount of venous blood was collected twice daily, prior to the time of each injection of poly(ICLC) and again 8 h later. Two serum samples were collected from each monkey before treatment was initiated to determine base line values of interferon activity. A concurrent control group consisted of four monkeys injected daily with saline and bled according to the same schedule as the monkeys that received

poly(ICLC). These dose levels were generally well tolerated, except for some anorexia and fever produced by the higher doses. Details of the clinical responses to the daily injections of poly(ICLC) will be published separately.

Interferon assays. One-milliliter aliquots of serum from each sample were stored at −70°C until assayed for interferon activity. Interferon activity was measured by determining the dilution of the sample that would cause a 50% reduction of the cytopathic effect induced by vesicular stomatitis virus in HFS-1 tissue culture cells, as previously described (1). Activity was expressed as interferon units per milliliter of serum normalized to reference units per milliliter of human serum interferon standard (National Institute of Allergy and Infectious Diseases reference reagent catalog no. G023901-527).

Statistics. Geometric mean interferon titers were calculated for each group of monkeys. Samples that showed no detectable interferon activity were arbitrarily assigned a value of 10 interferon units/ml, one-half of the lower limit of the test. Interferon activity at various times was plotted, and the area under the curves, representing total serum interferon activity, was determined with a planimeter for each monkey. Two-way analysis of variance was used for intergroup comparisons of the area.

RESULTS

Interferon activities over the 10-day experimental period are shown in Fig. 1 for cynomolgus monkeys and in Fig. 2 for rhesus monkeys. Serum interferon activity was not detected in samples collected prior to the first injection of poly(ICLC) or in samples from control monkeys. Interferon activity was detected in rhesus monkeys 2 h after the first injection of poly(ICLC). To minimize blood loss in the smaller animals, cynomolgus monkeys were not bled at 2 h. Twenty of the 24 monkeys had serum interferon activity ranging from 25 to 800 interferon units/ml 8 h after the first injection of poly(ICLC). Interferon activity 24 h after the first injection of poly(ICLC) remained above base line in 19 of the 20 monkeys that responded to the first injection. Maximum interferon activity was found in 23 of 24 monkeys 8 h after the second injection of poly(ICLC), with individual monkeys having interferon activity up to 16,000 interferon units/ml.

The area under the interferon curves, representing total interferon activity (Fig. 3 and 4) for the cynomolgus and rhesus monkeys, re-

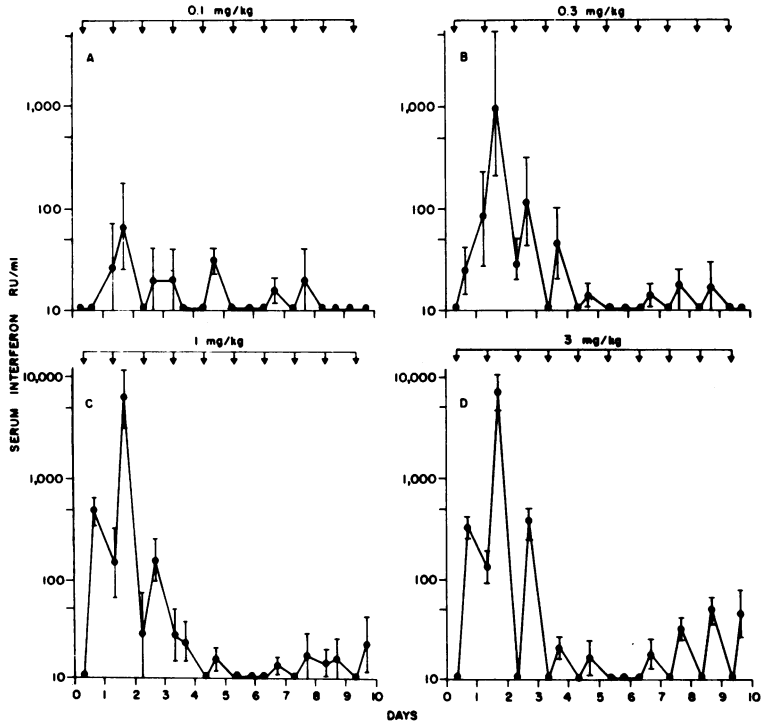

FIG. 1. *Serum interferon response of cynomolgus monkeys to multiple intravenous injections of poly(ICLC) in varying amounts. Monkeys were injected daily (arrows), and serum interferon activity was determined at times indicated by (●). Standard errors of the means are shown.*

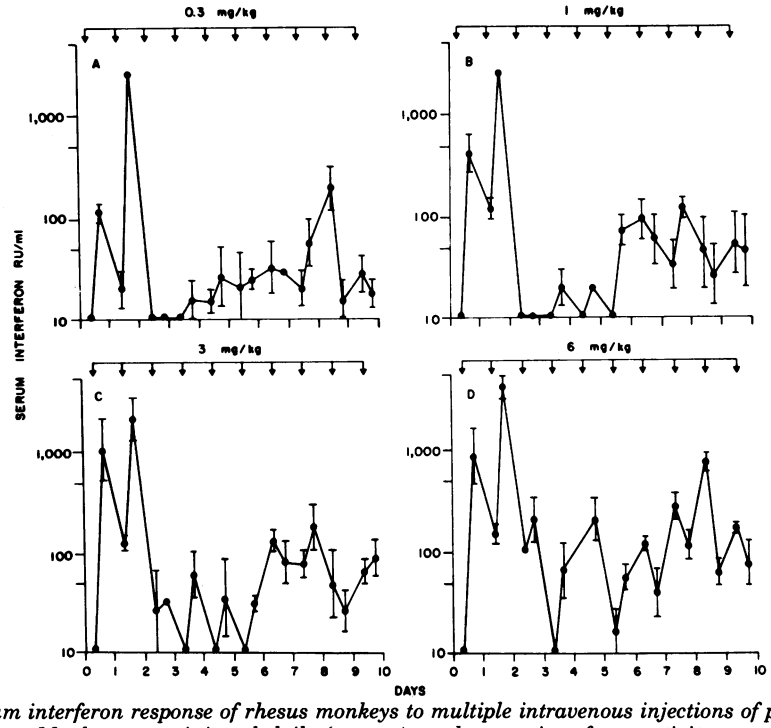

FIG. 2. *Serum interferon response of rhesus monkeys to multiple intravenous injections of poly(ICLC) in varying amounts. Monkeys were injected daily (arrows), and serum interferon activity was determined at times indicated by (●). Standard errors of the means are shown.*

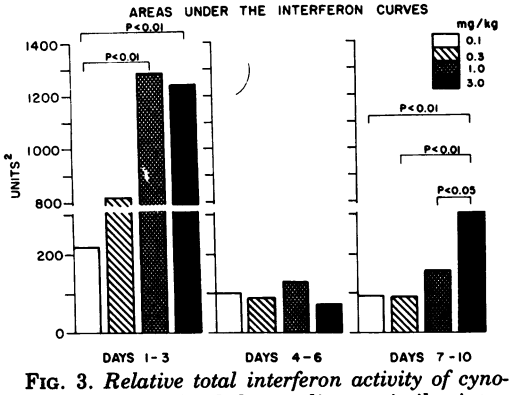

FIG. 3. *Relative total interferon activity of cynomolgus monkeys divided according to similar interferon responses.*

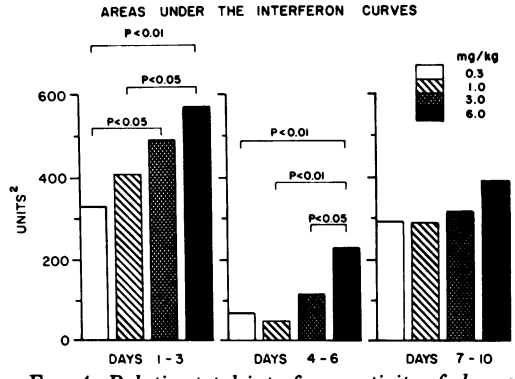

FIG. 4. *Relative total interferon activity of rhesus monkeys divided according to similar interferon responses.*

spectively, showed dose-related increase of interferon response during the first 3 days. Significantly lower interferon activity was noted throughout the remainder of the experiment. This period was considered to represent the hyporesponsive period. Serum interferon activity in the rhesus monkeys was higher from days 7 to 10 than on days 4 to 6. The recovery phase was not as evident in cynomolgus monkeys. The 0.1-mg/kg dose of poly(ICLC) was not an effective interferon inducer in cynomolgus monkeys. The rhesus monkeys injected with 6 mg of poly(ICLC) per kg were anorectic and had a fever after each injection, but no severe clinical signs were observed and deaths did not occur.

DISCUSSION

We found that poly(ICLC) effectively induced serum interferon activity in cynomolgus and rhesus monkeys. Further, the first injection of

poly(ICLC) primed the monkey for a potentiated response to the second injection of poly(ICLC). A similar potentiation has been demonstrated in vitro using mouse L cells with moderate concentrations of interferon (12).

The hyporesponsiveness in interferon activity after the third injection of poly(ICLC) is consistent with similar observations in rodents and cattle (2-5, 13, 14) and may be a limiting factor in the potential clinical usefulness of poly(ICLC) as an antiviral compound. However, serum interferon may not be a complete measure of the degree of resistance to virus in animals. The monkeys given 3.0 or 6.0 mg of poly(ICLC) per kg had more interferon activity than the other groups during the hyporesponsive period. One possible explanation for the difference in dose response during the hyporesponsive phase and subsequent increase of interferon activity in the rhesus monkeys is that some population of new cells normally being produced each day in the monkey could be induced to produce interferon by the higher doses of poly(ICLC). Other possible interferon control mechanisms include circulating, refractory repressor products, which, if depleted by repeated injections of interferon inducers, could also result in interferon activity in the higher-dosage groups and in the recovery phase reported in rhesus monkeys (14).

Homan et al. (9) reported that poly (I)·poly(C) was toxic and lethal at 5 mg/kg after four doses in rhesus monkeys. Poly(ICLC) was not lethal when 6.0 mg/kg was given daily for 10 days to these macaques. Our studies indicate that poly(ICLC) is safe for use in monkeys as an interferon inducer. This is supported by the observation that poly(ICLC) induces interferon in rhesus monkeys and chimpanzees and is an effective antiviral compound against simian hemorrhagic fever (11) and yellow fever virus infections in rhesus monkeys.

In view of changing interferon responses after sequential injections of poly(ICLC), the time of initial antiviral therapy and frequency and length of treatment in relation to infection with interferon-sensitive viruses are important potential factors to consider when using poly(ICLC). As might have been predicted from experiments in rodents, the hyporesponsive period cannot be avoided by increasing the dosage of inducer. Higher dosages (3.0 or 6.0 mg/kg) do, however, induce the release of significant amounts of serum interferon during the hyporesponsive period in monkeys. Since the anorexia and fever are more pronounced with higher dosages of poly(ICLC) and 0.3 mg of the complex per kg stimulated the release of significant amounts of serum interferon, a regimen of therapy consisting of two injections at 0.3 mg/kg followed by three injections at 3.0 or 6.0 mg/kg might be expected to induce maximum interferon responses and concomitant antiviral activity.

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