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NOTES

Effect of Administered Human Interferon on Experimental Rabies in Monkeys

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Cynomolgus monkeys infected with rabies virus were protected by repeated intramuscular administration of human leukocyte interferon beginning 24 h after infection.

It is known that mice, hamsters, or rabbits infected with rabies virus can be protected from the disease by administering interferon or inducing interferon by polyriboinosinic polyribocytidylic acid in the animals around the time of infection (3, 7, 10). We examined whether human leukocyte (HIF), administered 24 h after infection, prevented rabies in primates.

Cynomolgus monkeys (*Macaca fascicularis*) were used because a high antiviral activity of HIF has been reported in these animals (11), and because we knew the infectious dose of rabies virus and the progress of the disease from immunological investigations on these monkeys (R. Barth, O. Jaeger, and E. Weinmann, Symp. Ser. Immunobiol. Stand., in press). Procedures for the preparation of HIF and the interferon assay have been published elsewhere (4). The antiviral activity is given in units of the British research human interferon standard A 69/19 (9). The HIF preparation used in these experiments had an antiviral activity of 1.5×10^5 U/ml and $\geqq 1 \times 10^5$ U/mg of protein.

We first estimated the antiviral activity of HIF in human U cells (established, amniotic cells) and cynomolgus monkey primary kidney (MCK) cells against Semliki Forest virus (SFV) as challenge, and the sensitivity of HIF-treated MCK to SFV and rabies virus propagation. Rabies virus, strain Flury LEP, was used instead of rabies street virus in these in vitro experiments because the latter was not adapted to MCK. These experiments were undertaken as yield reduction assays. Dense cell cultures (1×10^7 to 2×10^7 cells) with 10 ml of the indicated HIF concentrations (Table 1) were incubated overnight in complete medium and infected with SFV (10^3 plaque-forming units per culture) or rabies virus (10^4 mean lethal doses [LD_{50}] [mouse]/culture). After approximately 50% of the cells in the culture which were not treated with interferon had been damaged by virus propagation (2 to 3 days after infection), the supernatants of all cultures of one series were harvested, and the virus yield was determined. Infectivity of SFV was determined by plaque titration in U cells. Infectivity of rabies virus, strain Flury LEP, and rabies street virus was determined by the mortality rate of NMRI mice. The results listed in Table 1 indicate (i) that the antiviral activity of HIF is lower in MCK than in U cells, and (ii) the inhibition of virus propagation in HIF-treated MCK is lower in the case of rabies virus than in SFV. Therefore it was decided to administer high amounts of HIF, totaling 1×10^6 to 2×10^6 U/cynomolgus monkey.

The conditions of the in vivo experiment are schematically summarized in Table 2. The monkeys, weighing 2.2 to 3.0 kg, were distrib-

TABLE 1. *Inhibition of Semliki Forest virus and rabiesvirus replication by human leukocyte interferon in primate cell cultures*

Interferon (U/ml)	Yield of virus propagation		
	Semliki Forest virus (plaque-forming units/ml)		Rabiesvirus (LD_{50}/ml)
	U cells	MCK	MCK
0	4.8×10^7	7.2×10^5	5.0×10^6
25	1.0×10^5	ND[a]	ND
100	4.1×10^2	1.3×10^4	1.6×10^6
400	<10	2.2×10^3	1.0×10^6
1,600	ND	ND	1.6×10^5
6,400	ND	1.6×10^1	3.2×10^2

[a] ND, Not done.

TABLE 2. *Human leukocyte interferon administration to cynomolgus monkeys infected with rabiesvirus*

Group	No. of animals	Infection per animal (LD_{50})	Amount of administered HIF (U × 10^5/animal) on post-infection day:						
			−4 h	0	1	3	5	7	9
A	10	$10^{5.7}$							
B	5	$10^{5.7}$	10	10					
C	10	$10^{5.7}$			5	2	1	1	1
D	3	$10^{5.7}$	10 given per day and animal after appearance of clinical symptoms						
E	3	None			5	2	1	1	1

uted at random among the five groups. They were infected with $10^{5.7}$ LD_{50} (mouse)/monkey rabies street virus, strain NYC, by the intramuscular (i.m.) route into the nape of the neck. HIF dissolved in 0.15 M NaCl was injected i.m. into both thighs simultaneously.

The results in groups A, B, and C are demonstrated in Fig. 1. One animal in group C died without either rabies symptoms or rabies virus antigen, proven by direct immunofluorescence in cell smears of the medulla oblongata, and was no longer considered. In the medullae oblongatae of all investigated animals which died of rabies, rabies virus antigen was demonstrable by this method, whereas all survivors on postinfection day 44 were negative. A protective efficacy of HIF in group C can be concluded from the length of survival time and the increase in the survival rate compared to group A. The effect of HIF in group B, administered shortly before infection, was to our surprise minimal.

The application of HIF to rabies-infected monkeys (group D) at a time when clinical symptoms could be observed did not influence the progress of the disease when compared with rabid animals in group A. This result coincides with the suggestion that there is a blood-brain barrier for the distribution of interferon (5), so that HIF administered i.m. could not inhibit virus propagation in the brain. The animals with HIF analogous to group C, but not infected (group E), behaved normally. Neutralizing antibodies were determined in the sera of all survivors (1). Little if any antibody could be detected in protected animals (Table 3), whereas Fenje and Postic (3) observed distinctly higher antibody concentrations in the sera of rabbits infected with rabies but protected by interferon.

HIF was injected i.m., because Skreko et al. (8) have observed that the concentration of interferon in the blood decreased more rapidly after intravenous than after i.m. administration. The different efficacy of HIF in groups B and C caused us to investigate whether these results corresponded to different clearance of HIF in both groups. Three monkeys were each treated with HIF as groups B and C but were not infected. The interferon levels in the sera of

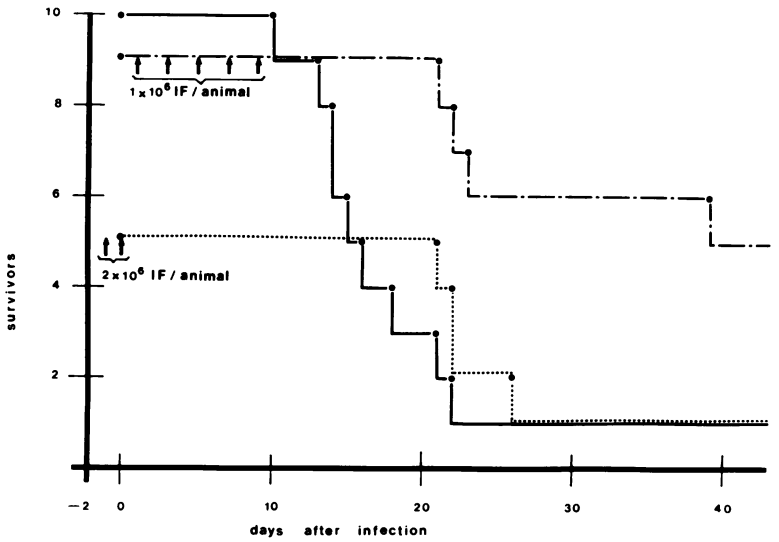

FIG. 1. *Effect of i.m.-applied human leukocyte interferon on the survival time of rabies virus-infected cynomolgus monkeys. Arrows indicate the time of interferon administration. At zero time the animals were infected with $10^{5.7}LD_{50}$ (mouse)/animal i.m. in the nape of the neck. Symbols: ●——●, group A; ●- - - -●, group B; ●—·—●, group C.*

TABLE 3. *Neutralizing antibodies against rabiesvirus in the sera of surviving animals on postinfection day 44*

Group	No. of animals	Antibody level[a]
A	1	<2
B	1	<2
C	5	<2
		<2
		2.5
		4.0
		5.5
E	3	<2
		<2
		<2

[a] 50% end points for sera tested against 100 LD_{50} of challenge virus strain of rabies virus.

TABLE 4. *Interferon concentrations determined in the sera of cynomolgus monkeys after human leukocyte interferon administration corresponding to groups B and C*

Time (h) after first interferon administration	Interferon (U/ml) in the sera of interferon-treated animals corresponding to:	
	Group B	Group C
0	<12	<12
2	25	12
4	25	25
8	50	25
12	25	25
24	<12	<12
30	<12	<12

the animals during the first 30 h after starting HIF treatment showed no differences between the groups (Table 4), nor could we determine any antiviral activity in the sera of group C monkeys 24 h after delayed administration of HIF. If a longer lasting interferon level exists in the blood of animals of group C, our interferon assay is not sensitive enough to detect it. Sera of untreated monkeys, diluted 1:5 (this dilution, giving 50% reduction of plaque yield in our assay, corresponds to 12 U of HIF/ml), neither showed any antiviral activity nor inhibited the activity of added interferon (3 and 6 U of HIF/ml).

The results discussed here suggest that primates can be protected from rabies by administration of HIF, starting 24 h after infection. Further investigations will be undertaken concerning the influence of the dosage of HIF and the number of repeated HIF doses, as well as the combination of intralumbal and i.m. application of interferon as discussed by Ho et al. (6).

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