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Intramuscular and/or Intralumbar Postexposure Treatment of Rabies Virus-Infected Cynomolgus Monkeys with Human Interferon

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From 9 to 10 of 10 cynomolgus monkeys infected with rabies street virus died of rabies about 20 days postinfection (pI). Symptoms of illness appeared 1 to 4 days before death. In an attempt to protect infected animals from the disease, human leukocyte interferon (HIF) was administered intramuscularly (i.m.) near the site of infection or into the cerebrospinal fluid between the first and second lumbar vertebrae (i.e., intralumbarly [i.l.]). Multiple HIF doses given over a period of several days proved more effective than a single HIF dose. In every experiment, i.m. HIF treatment was started 1 day pI. The best result obtained was a survival rate of 7 of 10 monkeys. The i.l. HIF administration schedules, consisting of multiple doses given over a period of at least 8 days, were started on day 3, 7, or 11 pI. Here the best result noted was the protection of 5 of 10 treated monkeys. The latest successful postexposure i.l. HIF treatment began on day 11 pI. The highest protection rate, 8 survivors of 10 treated monkeys, was achieved by a combined i.m. and i.l. HIF treatment. From these results we conclude that human patients severely bitten by rabid animals should in addition to an active immunization be i.m. and i.l. treated with HIF. Particularly, i.l. HIF administration could be effective, even when given several days pI. Whether an HIF administration starting after the appearance of clinical symptoms of rabies can help cannot be decided upon from the studies made in this monkey model. The most obvious difference between rabies in humans and cynomolgus monkeys is the duration of illness between the outbreak of the disease and death (1 to 4 days only in this animal model). It might have been due to this short period of illness that i.l. and i.m. HIF treatment at the appearance of clinical symptoms failed to help any of the monkeys treated.

The efficacy of human interferon (HIF) has been studied in patients with herpes keratitis (19, 30), herpes zoster (22), and chronic hepatitis (5, 8), as well as in cancer patients (28). Since HIF, to date not commercially available, will be an expensive substance, it obviously will not be used for the treatment of normally harmless diseases (e.g., common cold), with the exception of those which can be cured with low HIF doses locally. An example for such a case could be herpes keratitis, where a few drops of a highly concentrated HIF solution put into the eye seems to be sufficient to prevent the patient from contracting the disease (19, 30).

Rabies rates among the most severe viral infectious diseases and is thus a candidate for HIF treatment in the near future (7; T. C. Merigan, personal communication). From various in vitro and in vivo studies in nonprimate animal models it has been established that the rabies virus is sensitive to interferon (6, 17, 24, 33). Furthermore, Baer et al. reported that monkeys infected with rabies virus can be protected from rabies by a combined postexposure treatment of interferon inducers or HIF in addition to vaccination (2). In these experiments the inducer or HIF was injected intramuscularly (i.m.).

Since the rabies virus spreads from the site of infection via the nerves to the central nervous system, HIF administration should not only be effective by the i.m. route but also by the intralumbar (i.l.) route (23). We therefore studied the efficacy of HIF administered postexposure i.m., i.l., or i.m. plus i.l. on rabies virus-infected monkeys. The aim of this study was to investigate the efficacy of HIF in connection with the route of HIF administration, the dose of HIF used, and the start and length of postexposure treatment. It was not our aim to protect the animals from rabies by all means, and therefore HIF treatment was combined with neither active nor passive immunization. Some of the preliminary

data of our first experiments, which are included in this report, have been published previously (14, 15).

MATERIALS AND METHODS

Virus and mode of infection. Cynomolgus monkeys (*Macaca fascicularis*), originally caught in Malaysia, were quarantined after purchase over a period of at least 6 weeks. Then the animals, weighing 2.2 to 3.0 kg, were randomly distributed into groups of 10 and i.m. infected with 1.0 ml of rabies virus suspension into the nape of the neck. Rabies street virus strain New York City, purchased from the American Type Culture Collection (Rockville, Md.), was intracerebrally propagated in our laboratory for at least one up to six further passages in NMRI mice. For details refer to Table 1. Brains of rabid mice were homogenized, the homogenate was diluted 10-fold with phosphate-buffered saline, and after clarification by low-speed centrifugation the virus preparations were stored frozen at −80°C. Infectivity of virus preparations was determined by the mortality rate of NMRI mice infected intracerebrally (21), and consequently infective doses of challenge virus were expressed in mouse 50% lethal doses.

Preparation and administration of HIF. HIF was obtained from peripheral leukocytes of healthy donors according to the procedure described by Strander and Cantell (29) and concentrated and partially purified to an antiviral activity of 10^5 U/ml and 10^5 U/mg of protein as published elsewhere (13). Antiviral activities were expressed in units of the British research HIF standard preparation A 69/19 (31).

In the case of i.m. administration, HIF was injected into both thighs of each monkey in experiment no. 1 only; in the other experiments it was injected at four adjacent points into the nape of the neck around the site of infection. In the case of the i.l. route HIF was administered by lumbar spinal injection between the first and the second lumbar vertebrae. Control monkeys were only infected, and not i.m. or i.l. mock-HIF treated. All groups of monkeys having the same first figure in their number were members of the same experiment, i.e., they were infected with a portion of the same thawed challenge virus sample on the same day.

TABLE 1. *Results of rabies street virus infection on cynomolgus monkeys*

Group	Rabies virus		Survivors/ challenged	Survival time	
	Prepn. no.[a]	Dose (mouse 50% lethal dose)[b]		Mean ± SD[c]	Range
1.1	1	5.7	1/10	16 ± 4	10–22
2.1	1	5.7	1/10	20 ± 2	16–23
3.1	2	6.3	1/10	17 ± 3	12–20
4.1	3	6.6	0/10	17 ± 3	11–23

[a] Preparation no. 1 had been propagated in mice by at least six passages after delivery of the virus sample from the American Type Culture Collection. Preparations no. 2 and 3 had been propagated once in mice after isolation of the virus from the brains of rabid cynomolgus monkeys of group 2.1.

[b] Logarithmic values are given.

[c] Means and standard deviations (SD) are rounded to whole numbers.

Interferon and antibody titers from body fluids. Cerebrospinal fluids (CSF) from non-interferon-treated monkeys were taken between the first and second vertebrae, and those from HIF-treated animals were taken between the head and the first cervical vertebra. HIF clearance studies after i.l. HIF administration were made on different monkeys which were not used for the protection experiments. Blood was taken from the femoral vein. Interferon titers of these body fluids were determined by the reduction of plaque number of Semliki Forest virus-infected, pretreated U cell cultures as described elsewhere (13). To exclude any unspecific effects simulating interferon activity at high serum or CSF concentrations, some virus control cultures were preincubated with the lowest serum or CSF dilution taken from non-interferon-treated monkeys. On the other hand, to make sure that there was no HIF-inhibiting activity in these body fluids, the antiviral activity of HIF solutions containing 5 U of HIF per ml and serum or CSF from untreated monkeys was tested. Neither interferon-simulating nor interferon-inhibiting activities were observed under these conditions, although in some assays U cell cultures pretreated with medium containing 20% monkey control serum yielded up to twofold higher plaque counts. In these cases the respective HIF test culture (containing the same monkey serum concentration) was related to this virus control.

Titers of neutralizing serum antibodies against rabies virus were measured in NMRI mice according to the procedure described by Atanasiu (1). Antibody levels were expressed as reciprocals of 50% end points tested against 100 mouse 50% lethal doses of the challenge virus strain of rabies virus.

RESULTS

Effects of rabies virus infection on cynomolgus monkeys. In our experiments fairly high mouse infective doses of rabies virus were necessary to kill at least 9 to 10 of 10 infected monkeys (Table 1). This concurs with previous results obtained by Sikes et al. in rhesus monkeys (25).

The animals died within a short period of about 2 weeks, starting with day 10 postinfection (pI). Those animals that survived for 4 weeks pI never died. Usually the monkeys were observed for 6 weeks after infection, with the exception of one experiment (no. 2), where the observation period was extended to 4 months without yielding a higher mortality rate.

First symptoms of illness were refusal of food and reduced mobility. On the following day the monkeys no longer sat on their perches as usual but at the bottom of their cages. They had difficulty in climbing up the cage bars, and a day later they were unable to do so at all. During

the last stage of the disease the animals lay at the bottom of their cages with their legs hanging through the grates. This was also the position in which the animals died. The entire period of illness lasted between 24 h and 4 days.

The clinical picture of rabies in cynomolgus monkeys can easily be diagnosed by a veterinarian. Nevertheless, at the onset of this study, this diagnosis was proven by direct immunofluorescence in cell smears of the medulla oblongata. In the first experiment (groups 1.1, 1.2, and 1.3), rabies virus antigen was demonstrated in all animals that died of rabies, whereas all survivors on pI day 44 were negative (14). In the other experiments this immunofluorescence method was only used when the rabies symptoms did not seem to be completely clear, e.g. when the first symptoms and death occurred within 24 h.

The fact that virus preparations no. 2 and 3 had been obtained from the brains of rabid monkeys followed by only one passage in NMRI mice, whereas preparation no. 1 had been passaged at least six times in mice, had no obvious influence on the efficacy in these monkeys.

Clearance of HIF administered i.l. or i.m. One hour after i.l. administration of 10^5 U of HIF per monkey, the antiviral activity titer in the CSF was 2,000 U/ml, decreasing to 60 U/ml 7 h later, while no activity was found in the serum (Table 2). The i.m. administration of a fivefold-higher amount of HIF led to titers of 25 U/ml or less in the serum. At 24 h after i.l. or i.m. treatment, no antiviral activity was demonstrable in either the CSF or the serum. After i.m. administration of HIF doses lower than 5×10^5 U per monkey, no antiviral activity could be detected at all. This might be due primarily to the sensitivity of our interferon assay, which was limited to 12 U/ml in the serum. Our results shown are comparable with those obtained in a more detailed study by Habif et al. (9), who also reported that only after very high i.l. HIF doses (10^7 U per monkey) could any antiviral activity be found in the serum at all.

i.m. postexposure HIF treatment. The efficacy of i.m.-administered HIF is shown in Table 3. In none of the groups were all animals protected. The highest rate of protection was achieved in group 3.3, but with respect to the 10-fold-higher amount of HIF administered in this group, as compared with that of group 3.2, the increase of protection was rather low. In some of the groups one animal died significantly later ($P < 0.05$) compared with the control groups, but this did not occur in each group of i.m. HIF-treated monkeys, e.g., none of the group 2.3 monkeys that died survived longer than animals of the respective control group. Furthermore,

TABLE 2. *Interferon titers in sera and CSF of i.l. or i.m. interferon-treated cynomolgus monkeys*

Time (h)	Antiviral activity (U/ml) after interferon administration		
	HIF (10^5 U per animal) i.l.[a]		HIF (5×10^5 U per animal) i.m.[b]
	CSF	Serum	Serum
0	<25	<12	<12
1	2,000	<12	ND[c]
2	ND	ND	12
4	125	<12	25
8	60	<12	25
12	ND	ND	25
24	<60	<12	<12

[a] Mean values of six monkeys treated simultaneously.

[b] Mean values of three monkeys treated simultaneously.

[c] ND, Not done.

TABLE 3. *Efficacy of postexposure i.m. administration of human interferon in rabies-infected cynomolgus monkeys*

Group[a]	Virus dose (mouse 50% lethal dose)[b]	Amt. of HIF ($U \times 10^5$ per animal) administered on day pI:								Total HIF dose ($U \times 10^5$ per animal)	Survivors/challenged		Survival time[c]	
		0	1	3	5	7	9	11	13		Test group	Control group	Mean ± SD	Range
1.2	5.7	20[d]								20	1/5	1/10	23 ± 2	21–25
2.2	5.7		10							10	4/10	1/10	19 ± 5	14–27
1.3	5.7		5	2	1	1	1			10	6/9	1/10	26 ± 8	21–38
2.3	5.7		5	2	1	1	1	1	1	12	6/10	1/10	18 ± 3	15–20
3.2	6.3		5		3		3			11	6/10	1/10	19 ± 7	12–28
3.3	6.3		50		30		30			110	7/10	1/10	21 ± 10	12–31
4.2	6.6		5	3	3	3	3	3		20	2/10	0/10	13 ± 2	11–16

[a] In experiment no. 1 (groups 1.2 and 1.3), HIF was i.m. administered into the thighs of the monkeys, whereas in the other experiments it was injected into the nape of the neck adjacent to the site of infection.

[b] Logrithmic values are given.

[c] Only animals of the test groups are considered here; for survival times of the corresponding control group refer to Table 1. Means and standard deviations (SD) shown are rounded to whole numbers.

[d] This amount of HIF was administered in two equal doses 4 h prior to infection and at the time of infection.

there was no considerable prolongation of illness in any of the HIF-treated monkeys, and none of these monkeys, after becoming ill, survived. This proved true not only for i.m. but also for i.l. or i.m.-plus-i.l. HIF-treated animals.

Repeated HIF administration over a longer period starting 24 h after infection was more effective than one dose administered at the time of infection or 24 h later (compare groups 1.2 and 2.2 with groups 1.3 and 2.3). Whether a single dose injected 24 h pI (group 2.2) is more advantageous than one given at the time of infection (group 1.2) cannot be concluded from these data, because in group 1.2 HIF was administered into the thighs whereas in group 2.2 it was administered into the nape of the neck. In the case of a single HIF dose the site of i.m. HIF injection might play an important role, though no different effect was observed in the case of repeated HIF administration (compare groups 1.3 and 2.3). The low protection rate of group 4.2 could be due to the high challenge virus dose, though a similar HIF dose resulted in a considerably higher rate of protection in monkeys infected with an only twofold-lower challenge dose (group 3.2).

i.l. postexposure HIF treatment. The results of postexposure administration of HIF into the CSF are summarized in Table 4. In contrast to the i.m. HIF treatment, these animals received the first HIF dose not earlier than on day 3 pI. In the first experiment (group 2.4), HIF administered at 2-day intervals between days 3 and 13 pI protected 5 of 10 animals. To find out which period after infection was the most suitable for i.l. HIF administration, three groups of monkeys were treated between pI days 3 and 11, 7 and 15, and 11 and 19, respectively. In these groups increased HIF doses at 4-day intervals were injected per monkey, in comparison with the HIF doses in group 2.4 at 2-day intervals. The purpose of this alteration of the HIF schedule was to reduce the number of treatments and thus the stress for the rabies-infected monkeys. In contrast to our expectation there was no difference in the survival rates of the groups. However, it must be stated that only some of the animals of these groups received the total amount of HIF before the outbreak of illness or even before death (Fig. 1). Whereas all monkeys of group 3.4 (Fig. 1A) could actually be treated with HIF according to the protocol, in group 3.6 (Fig. 1C) only the four survivors of this group had the chance of receiving the entire amount of HIF planned. Furthermore, the most extreme situation was observed in one case of this group on which HIF treatment was started after the appearance of the first rabies symptoms. Since i.l. HIF administration in this animal model is not effective after the outbreak of the disease, as will be shown later, in retrospect HIF treatment of this monkey might have been senseless. Based on the results shown in Table 4, we therefore assume that i.l. HIF treatment beginning on day 7 pI and continuing up to day 17, giving 5×10^5 U per monkey at intervals of 2 instead of 4 days, might lead to better results.

Previously we assumed that i.l. HIF-treated animals would show a higher specific immune response than i.m. HIF-treated animals provoked by increasing amounts of viral antigen due to virus propagation before HIF-dependent virus inhibition occurred in the central nervous system (15). In contrast to results obtained with experiment no. 2, the antibody titers obtained in experiment no. 3 on day 42 pI do not support this assumption. First of all, low antibody titers (up to 6) could be detected in the sera of monkeys treated with β-propiolactone-inactivated challenge virus. Furthermore, a few of the animals protected by interferon showed some higher antibody titers (up to 20), but these animals were found in i.m.- as well as i.l.-treated groups and presented only solitary cases, whereas all the other i.l.-treated monkeys showed titers no higher than 6. Sera taken at random from 40 non-rabies-treated monkeys were negative (titers of <3).

TABLE 4. *Efficacy of postexposure i.l. administration of human interferon in rabies-infected cynomolgus monkeys*

Group	Virus dose (mouse 50% lethal dose)[a]	Amt. of HIF ($U \times 10^5$ per animal) administered on day pI:									Total HIF dose ($U \times 10^5$ per animal)	Survivors/challenged		Survival time[b]	
		3	5	7	9	11	13	15	17	19		Test group	Control group	Mean ± SD	Range
2.4	5.7	5	3	2	1	1	1				13	5/10	1/10	19 ± 3	16–23
3.4	6.3	10		10		10					30	3/9	1/10	18 ± 4	14–24
3.5	6.3			10		10		10			30	4/10	1/10	18 ± 4	13–23
3.6	6.3					10		10		10	30	4/10	1/10	15 ± 3	12–19

[a] Logarithmic values are given.

[b] Only animals of the test groups are considered here; for survival times of the corresponding control groups refer to Table 1. Means and standard deviations (SD) shown are rounded to whole numbers.

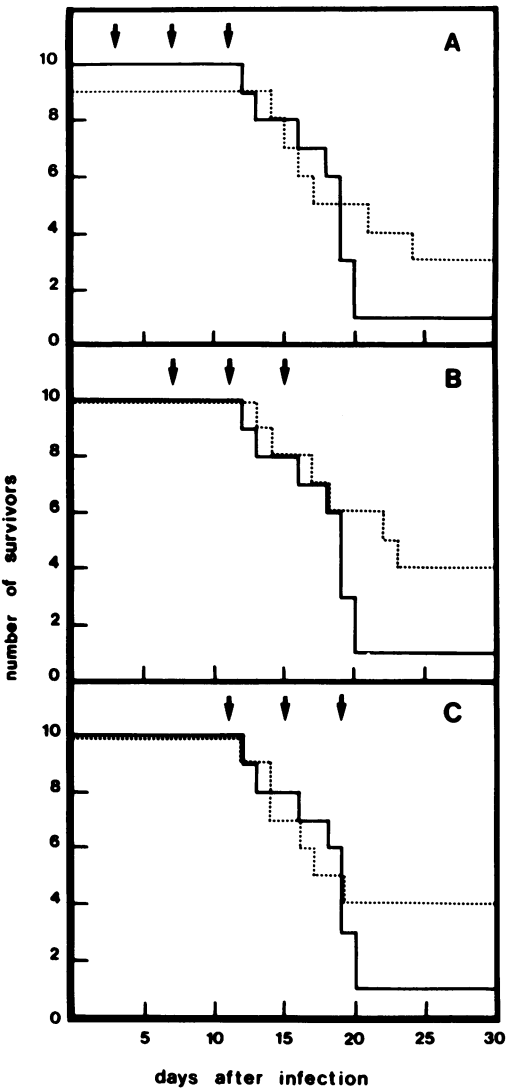

FIG. 1. *Effect of HIF administered i.l. to cynomolgus monkeys at various times after rabies virus infection. HIF treatment was started on pI day 3 (group 3.4) (A), 7 (group 3.5) (B), or 11 (group 3.6) (C). In each graph the HIF-treated group* (-----) *is compared with the control group (group 3.1)* (———). *Arrows indicate the times when 10^6 U of HIF per monkey were injected. The surviving animals were observed over 42 days pI.*

Combined i.l. and i.m. postexposure HIF treatment. In two experiments groups of 10 monkeys were HIF treated, i.m. beginning on day 1 pI and i.l. beginning on day 3 or 5 pI (Table 5). This combined treatment led to higher protection rates than i.m. or i.l. treatment alone. These data also suggest that a combined i.m./i.l. HIF treatment should be preferred to i.l. or i.m. administration of the same total amount of HIF.

HIF treatment at the beginning of illness. From a previous experiment we knew that i.m. HIF administration at the time of outbreak of the disease had no protective effect (Hilfenhaus, Majer, and Weinmann, unpublished data). We therefore studied whether combined daily i.l. and i.m. HIF treatment from the appearance of the first clinical symptoms showed any better effect. The HIF dose on the first day was 10^6 U per route per monkey, which was reduced to 3×10^5 U per route per monkey on the following days. Four of five infected monkeys that became ill were treated this way, but none of them could be protected from death (Fig. 2). Furthermore, there was also no extension of the duration of illness, since even the longer period of illness of monkey no. 2040 did not considerably deviate from the times of all the other monkeys that died from rabies.

DISCUSSION

The protective effect of exogenous or endogenous interferon against rabies has been proved in various nonprimate mammals (6, 17, 24, 33), and more recently the efficacy of postexposure treatment of rabies-infected monkeys with rabies vaccine and interferon inducers has been reported (2). In this paper we showed that postexposure treatment of rabies-infected cynomolgus monkeys with HIF only, beginning with HIF administration no earlier than 24 h pI, is also effective. Since rabies virus is believed to replicate initially in striated muscle cells at the site of infection and then invades via the peripheral nerves into the central nervous system (23), protection of the muscle cells around the site of infection or protection of the cells of the central nervous system from virus replication should preserve the host from the disease. Our results, which show that monkeys i.m. or i.l. treated with HIF can be protected, support this assumption in spite of the fact that in none of the interferon-treated groups did all animals survive.

Although no group of i.l. HIF-treated monkeys produced a higher rate of protection than 50%, this route of interferon administration was effective to some extent in our model, whereas previously, in a similar investigation done by Ho et al. in rabbits (16), no protective effect was obtained. The absence of complete protection could have two reasons. (i) The HIF doses were too low and/or the animals should be treated daily rather than at 3- or 4-day intervals. If this were the case, then the results could be improved by a corresponding alteration of our HIF treatment schedule. (ii) It could, however, be

TABLE 5. *Efficacy of combined i.m. and i.l. human interferon administration in rabies-infected monkeys in comparison to HIF administered i.m. or i.l. alone*

Group	Virus dose (mouse 50% lethal dose)[a]	Route of HIF administration	Amt. of HIF (U × 10^5 per animal) administered on day pI: 1	3	5	7	9	11	13	15	17	Total HIF dose (U × 10^5 per animal)	Survivors/challenged: Test group	Control group	Survival time[b]: Mean ± SD	Range
2.5	5.7	i.m. + i.l.	5	2	1	1	1	1	1			25	8/10	1/10	20	20, 20
				5	3	2	1	1	1							
2.3	5.7	i.m.	5	2	1	1	1	1	1			12	6/10	1/10	18 ± 3	15–20
2.4	5.7	i.l.		5	3	2	1	1	1			13	5/10	1/10	19 ± 3	16–23
4.3	6.6	i.m. + i.l.	5	3	3	3	3	3				43	6/10	0/10	15 ± 1	14–17
					5	3	3	3	3	3	3					
4.2	6.6	i.m.	5	3	3	3	3	3				20	2/10	0/10	13 ± 2	11–16

[a] Logarithmic values are given.

[b] Only animals of the test groups are considered here; for survival times of the corresponding control groups refer to Table 1. Means and standard deviations (SD) are rounded to whole numbers.

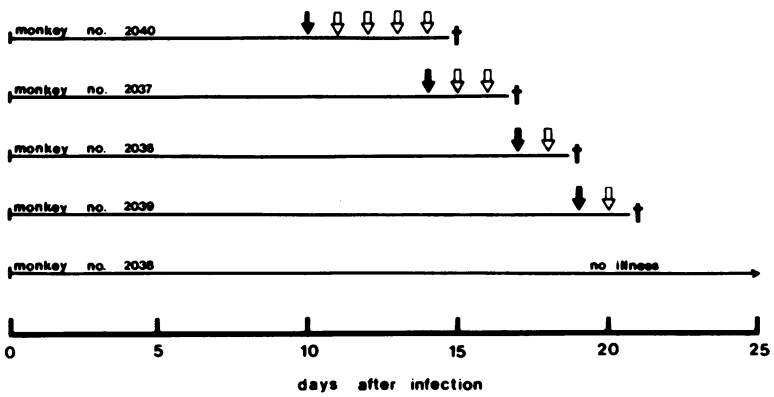

FIG. 2. *Combined i.l. and i.m. HIF administration at the appearance of first symptoms of rabies. The first HIF dose was 10 × 10^5 U per monkey (⇩); this was reduced to daily doses per route of 3 × 10^5 U per monkey for the following days (⇩).*

possible that the complete inhibition of rabies virus propagation in the brain by interferon is principally impossible if interferon in the CSF cannot get to all the brain cells needing protection or if at least the transfer of viral resistance (3) to all the respective cells is not possible. The higher efficiency of the combined i.l. and i.m. HIF treatment, though again not yielding 100% protection, could be explained by the suppression of intramuscular amplification of infectious virus, followed by the incomplete inhibition of virus spread in the brain.

Based on our results, we suggest postexposure i.l. plus i.m. HIF treatment of patients infected by a rabid animal. Particularly, those patients who have been severely bitten in any part of the head or of the body near the head should receive i.l. HIF treatment. Such treatment could possibly protect the patient even when started considerably later than 24 h pI, since some monkeys were protected when HIF had been injected into the subarachnoid space as late as 11 days pI. Of course, any HIF treatment of rabies-infected patients should be supplemented by an active vaccination with killed rabies vaccine. In contrast to the currently used combined postexposure prophylaxis of anti-rabies immunoglobulins with vaccination, which raises the problem of suppression of the specific immune response to the vaccine (10), the HIF treatment in combination with vaccination could have the advantage of showing no essential effect on the immune response.

Although postexposure prophylaxis with HIF

seems to be of some value, HIF administered either i.m. or i.l. plus i.m. after outbreak of the disease had no effect. This could be due to the short period of illness. A possible explanation could be that the total amount of infectious rabies virus in the brain is too high for death to be prevented by HIF treatment at this time or that the damage of the respective cells of the central nervous system has reached a stage at which complete inhibition of virus replication could not protect the host any more.

Besides the special problem of prevention of rabies, we are also interested in this rabies virus monkey model in respect to HIF generally. (i) Studies on the efficacy of interferon injected into the CSF should answer the question of whether virus infections of the brain can be stopped by this means. (ii) Comparison of i.m.- or i.l.-administered HIF obtained from leukocytes or fibroblasts should show whether both are equally effective. Since it has been shown that both interferons can be distinguished by their antiviral activities on different human cell types in vitro (11, 32), it seems to be necessary to compare their efficacy on infection of various organs with the same virus in vivo, e.g., on rabies virus-infected muscle or brain. (iii) Finally, this model offers the possibility of testing in vivo other properties than the antiviral activity of various HIF preparations administered by various routes, e.g., anticellular activity (12, 20, 27), suppression or stimulation of the humoral or cellular immune response (4, 18, 26), compatibility, etc. This could in particular be related to a schedule of HIF treatment that has shown itself to be effective against a serious, life-threatening virus infection.

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