Antiviral Research, 6 (1986) 285–297
Elsevier

AVR 00207

Prophylaxis of Rift Valley fever with antiviral drugs, immune serum, an interferon inducer, and a macrophage activator

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(Received 26 July 1985; accepted 14 January 1986)

Summary

Rift Valley fever virus (RVFV), a member of the family Bunyaviridae, extended its range from sub-Saharan Africa into Egypt in 1977. Its clinical spectrum is recognized to include severe manifestations such as hemorrhagic fever and encephalitis. For these reasons, as well as the limited knowledge of specific therapy for Bunyaviridae infections, we investigated several prophylactic regimens for RVF in a mouse model. Rimantadine, thiosemicarbazone, and inosiplex were ineffective. Pretreatment with glucan was of some use, but the most encouraging results were obtained with the antiviral drug ribavirin, passive antibody, or an interferon inducer polyriboinosinic-polyribocytidylic acid complexed with poly-L-lysine and carboxymethylcellulose (poly[ICLC]). Ribavirin and poly(ICLC) were also shown to be efficacious in preventing disease in hamsters. Ribavirin (loading dose of 50 mg/kg followed by 10 mg/kg at 8-h intervals for 9 days) suppressed viremia in RVF-infected rhesus monkeys. Ribavirin

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In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals", as promulgated by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council. The facilities are fully accredited by the American Association for accreditation of Laboratory Animal Care.

The views of the authors do not purport to reflect the positions of the Department of the Army or the Department of Defense.

also reduced virus yield in infected cell cultures; sensitivity varied markedly with cell type but not with virus strain. Immune mouse ascitic fluid, with a plaque reduction neutralization titer of 1 : 1024, was effective in a dose of 4 ml/kg, a volume approximately equivalent to administration of a unit of convalescent plasma to a human. Poly(ICLC) may well have functioned through interferon induction, since RVFV was shown to be sensitive to interferon in cell culture, and since another macrophage activator (glucan) was only marginally effective. These studies suggest that ribavirin, poly(ICLC), and convalescent plasma may have a role in prevention or therapy of human RVF.

Rift Valley fever virus; ribavirin; interferon; immune serum; hemorrhagic fever

Introduction

Rift Valley fever virus (RVFV) has been a significant cause of human morbidity in sub-Saharan Africa since its initial isolation in the 1930s (reviewed in ref. 28). In recent years its potential for causing lethal hemorrhagic fever and encephalitis has emerged [28,35]. In 1977, RVFV was recognized in Egypt for the first time. In apparently virgin territory, RVFV caused a devastating epizootic/epidemic with thousands of human cases, at least 600 deaths, and extensive livestock losses [22]. Although the potential for RVF to spread to other receptive areas has been recognized, existing human and livestock vaccines are in limited supply and have shortcomings in controlling RVF outbreaks [21]. We have therefore investigated several drugs suspected of being active against other RNA viruses, a macrophage activator, an interferon inducer, and passive antibody for their efficacy in preventing experimental RVF.

Materials and Methods

Animals

BALB/cAnN or C57BL/6N mice were obtained from the Frederick Cancer Research Center, Frederick, MD. BALB/cMai, C3H/BiMai, or C57BL/6Mai mice were purchased from M.A. Bioproducts, Walkersville, MD. AKR/J mice were purchased from The Jackson Laboratories, Bar Harbor, ME. ICR Swiss mice were from the Walter Reed Army Institute of Research (WRAIR), Washington, D.C. Unless otherwise noted, all mice were 5–12-week-old females. Male Syrian golden hamsters (125–150 g) were obtained from Charles River Lakeview. Rodents were housed in stainless steel or polycarbonate cages, with hardwood chip bedding, in rooms with constant temperature (22°C) and a 12-h light cycle. Commercial rodent diet (Purina Rodent Chow, Ralston Purina, St. Louis, MO) and water were provided at libitum. Adult laboratory-conditioned rhesus monkeys, seronegative for RVF, were housed individually in suspended metal cages. They were fed a commercial monkey diet (Purina High-Protein Monkey Chow, 5045) twice daily, with fresh fruit three times a week.

Viruses

The Zagazig Hospital 501 (ZH-501) strain of RVFV was furnished by Dr. James Meegan, U.S. Naval Medical Research Unit No. 3, Cairo, Egypt. Viremic serum from a fatal human hemorrhagic fever case was passed twice in diploid fetal rhesus lung cells (DBS-103) and stored at -70°C. The ZH-501 strain used in the monkey experiments had, in addition, undergone one spleen and one serum passage in rhesus monkeys. Other RVFV strains used for comparison are described in more detail elsewhere [27], but include a 1951 South African sheep isolate (SA-51), a 1975 South African human isolate (SA-75), and a 1944 Ugandan mosquito isolate (Entebbe). RVFV was assayed as plaques under agar, and serum dilution plaque-reduction neutralization (PRN) tests were performed in Vero cells essentially as described by Earley et al. [9]. All virus dilutions were performed in Hanks' balanced salt solution buffered with Hepes and containing 2% heat-inactivated (56°C for 30 min) fetal calf serum, 50 μg/ml streptomycin, and 50 U/ml penicillin. Animals were inoculated with 0.1 ml, either subcutaneously (s.c.) or intraperitoneally (i.p.). The lethal $dose_{50}$ (LD_{50}) of this virus strain for adult mice was 1–250 PFU s.c. and 1–10 PFU i.p. The s.c. LD_{50} was 0.3 PFU for adult hamsters. Immunity of surviving animals was assessed in some experiments by challenging s.c. with 5×10^3 PFU. The Indiana strain of vesicular stomatitis virus (VSV) was obtained from the American Type Culture Collection (Rockville, MD) and propagated in Vero cells.

Treatments

Glucan was a gift of Dr. N.R. Di Luzio, Tulane University Medical School, New Orleans, LA, and was injected intravenously (i.v.) (1.0 mg in 0.1 ml saline) 8, 5, and 1 day before virus inoculation. Polyriboinosinic-polyribocytidylic acid complexed with poly-L-lysine and carboxymethylcellulose (poly[ICLC]), lot 97A, a gift from Dr. Hilton Levy, was given in a single s.c. dose of 3 mg/kg on day 0. Ribavirin, donated by ICN Pharmaceuticals, Covina, CA, was diluted in saline and given s.c. Inosiplex, inosine-dimethyl-amino-2-propanol-*p*-acetamidobenzoate (Isoprinosine®, Newport Pharmaceuticals, Newport Beach, CA) was supplied as an anhydrous soluble powder, which was dissolved in pyrogen-free saline. Thiosemicarbazone (BH 89974) was donated by the WRAIR and was diluted in saline with 0.2% methylcellulose and 0.4% Tween 80 to aid in suspending the compound. Rimantadine hydrochloride (α-methyl-L-adamantane-methylamino HCl), obtained as a gift from E.I. duPont de Nemours and Co., Wilmington, DE, and was diluted in saline.

Immune mouse ascitic fluid was pooled from BALB/cAnN, AKR/J, C3H/BiMai, C57BL/6Mai and ICR mice receiving multiple injections of formalin inactivated RVFV vaccine NDBR-103 emulsified in complete Freund's adjuvant. Neutralizing antibody titer was 1 : 1024.

All treatments were administered at a separate site from virus inoculation. Controls received injections of diluent in every case.

Determination of ribavirin sensitivity in cell culture

Medium (Eagle's minimal essential medium with supplemental nonessential amino acids, 1 mM sodium pyruvate, 10% heat-inactivated fetal calf serum, 50 μg/ml strepto-

mycin, and 50 U/ml penicillin) was decanted from confluent 75-cm^2 monolayers of the appropriate cell type and replaced with 1000 PFU of RVFV diluted in 3 ml of medium containing graded concentrations of ribavirin (0, 2.5, 5, 10, 25, 50, 100, or 150 g/ml). After 1 h adsorption at 37°C the inoculum was removed, the flask washed once, and 30 ml drug-containing medium added. 2-ml aliquots were removed daily and stored at −70°C before being titrated for virus content. Control flasks and flasks containing virus alone or ribavirin alone were examined daily at 125× for evidence of cytopathic effect (CPE). When different cell types were compared, the ZH-501 strain of RVF virus was used, but three additional RVFV strains were compared in MRC-5 cells. Hepatoma 7777, glial 107, Vero, LLC-MK_2, fetal rhesus lung (DBS-103), and MRC-5 cells were obtained from the American Type Culture Collection, Rockville, MD. BW-J-M and NZW-S2-M have been described [26]. Lewis rat thymus, Wistar-Furth rat thymus, Maxx rat thymus, guinea pig kidney, and guiney pig kidney SV-40 cells are transformed lines initiated in this laboratory.

Determination of sensitivity to interferon

Medium was aspirated from triplicate 24-mm diameter wells of confluent L929 cells (American Type Culture Collection, Rockville, MD) and replaced by 0.6 ml diluted interferon (NIH #G-002-904-5112, Newcastle disease virus-induced L cell interferon). Interferon dilutions were prepared in Eagle's medium, with Earle's salts and Hepes buffer (EBME-H) containing 0.2% heated calf serum plus antibiotics. After 16–20-h incubation at 37°C in 5% CO_2, the interferon solution was aspirated and replaced by 0.1 ml suspension containing 30–100 PFU virus. After a second 1-h incubation, a 1 ml agarose overlay of EBME-H with 4% calf serum at 43°C was applied. In the case of RVFV, it contained 1% agarose and was followed by an identical 1 : 9000 neutral red overlay on day 3; in the case of VSV, it contained 0.3% agarose, which was decanted on day 2 before alcoholic crystal violet staining.

Results

Initial screening studies of antiviral drugs (Table 1)

Thiosemicarbazone, rimantadine, and inosiplex were all inactive in reducing mortality or prolonging survival even though toxic doses were reached with thiosemicarbazone and rimantadine.

Ribavirin, in contrast to the above drugs, proved to be efficacious in protecting mice from otherwise lethal RVFV challenge (Table 2). The interferon inducer poly(ICLC) was employed in the same experiment and was also effective. Interestingly, poly(ICLC), and to some extent ribavirin, presumably even prevented infection, since most of the mice were susceptible to RVF on subsequent challenge. For confirmation, 15 BALB/cAnN mice (female, 6 weeks of age) were infected with 250 PFU virus s.c. and immediately given poly(ICLC) at another site; 14 survived, as compared to 1 of 15 saline control mice. We also extended these observations to hamsters which, like mice, are susceptible to lethal hepatic necrosis after RVFV infection (Table 3). Both ribavirin and poly(ICLC) protected them.

TABLE 1

Treatment of RVFV-infected mice[a] with antiviral drugs

Drug	Dose (mg/kg per day)	Survivors/tested	
		Infected	Uninfected
Thiosemicarbazone[a,b]	0.0	4/10	10/10
	25.0	4/10	5/5
	50.0	2/10	5/5
	100.0	0/10	5/5
Rimantadine[a,b]	0.0	5/10	10/10
	40.0	3/10	5/5
	80.0	2/10	3/4
	160.0	0/10	2/5
Inosiplex [c,d,e]	0.5	0/20	-
	5.0	0/20	-
	100.0	0/20	-
	500.0	0/20	-

[a] Male ICR Swiss mice, 6–8 weeks of age were given 200 PFU RVFV s.c. on day 0.
[b] Given s.c. on days 0, 1, and 2.
[c] Male AKR/J mice weighing 20–25 g received 200 PFU RVFV i.p. at $t = 0$.
[d] Given i.p. for 7 consecutive days.
[e] Identical results obtained when drug administered on days 1–8.

TABLE 2

Treatment of RVFV-infected mice[a] with ribavirin or Poly(ICLC)

Treatment	Survivors/ inoculated	No. immune/ challenged[e]
Ribavirin[b]	18/24	3/10
Poly(ICLC)[c]	25/25	0/10
Saline[d]	0/10	-
None	1/25	-

[a] Male C57BL/6 N mice 4–6 weeks of age were given 200 PFU RVFV s.c. at $t = 0$.
[b] 75 mg/kg s.c. at $t = 8$ h and 25 mg/kg s.c. twice daily for 10 days.
[c] 3 mg/kg s.c. in a single dose 8 h postinfection.
[d] 0.1 ml saline given on same time schedule as ribavirin.
[e] 10 survivors of original infection challenged s.c. with 5×10^3 PFU on day 28. 10 uninfected mice held as controls were all susceptible (data not shown).

Effectiveness of antibody

Passive antibody administration was also protective. When 6–8-week-old ICR mice were infected i.p. with 500 PFU RVFV, all 10 mice given 0.1 ml immune ascitic fluid s.c. 4 h later survived and all 10 controls died. Identical results were obtained when the same experiment was repeated using 4–6-week-old BALB/cAnN mice challenged s.c. with 5×10^3 PFU.

TABLE 3

Protection of RVFV-infected Syrian golden hamsters with ribavirin or poly(ICLC)

Virus dose	Treatment	Survivors
10 PFU	Ribavirin[a]	8/10
	Saline	0/10
5×10^3 PFU	Poly(ICLC)[b]	5/5
	Saline	0/5

[a] 60 mg/kg twice daily s.c. on day 0 and 20 mg/kg twice daily s.c. on days 1–10.
[b] 3 mg/kg s.c. on day 0.

Glucan action

Although glucan has previously been show to protect CD-1 mice from RVF [29], we failed to affect significantly the course of infection when 20 4–6-week old male AKR/J mice pretreated with glucan were challenged s.c. with 250 PFU ZH-501 and compared to 20 saline controls (data not shown). To further analyze the activity of glucan, we treated BALB/cMai mice with glucan or saline and challenged them with graded doses of RVFV (Table 4). Even near the LD_{50}, there was no significant improvement in mortality. However, glucan-treated mice receiving 10 or 100 PFU did have a significant prolongation of survival compared to controls (Fig. 1). Mice dying in the 2nd or 3rd week of infection had obvious neurological signs, including hind-limb paralysis and convulsions. Nine glucan-treated and 4 control moribund mice were subjected to histopathological examination. Mice killed during the first 8 days of infection (4 control and 5 glucan-treated) had evidence of severe diffuse liver necrosis, but glucan-treated mice sacrificed on days 9, 13, and 15 had mild to moderate histological evidence of encephalitis. The disseminated focal necrotic lesions resembled those seen in unmanipulated rodents with RVFV encephalitis [11,29; T.W. Slone, C.J. Peters and A. DePaoli, Pathology and pathogenesis of RVF infection in laboratory rodents, in preparation]. In an attempt to demonstrate the mechanism of glucan protection, we

TABLE 4

Protection of mice[a] from RVF by pretreatment with glucan

Virus dose (PFU)	Survivors/infected (%)		Mean time to death (± S.D.)		P^b
	Glucan	Saline	Glucan	Saline	
1000	1/20 (5)	1/20 (5)	5.2 ± 2.2	4.6 ± 1.5	NS
100	2/20 (10)	1/20 (5)	5.6 ± 1.7	4.3 ± 1.3	0.03
10	3/20 (15)	1/20 (5)	10.3 ± 5.0	5.8 ± 1.6	0.01
1	14/20 (70)	10/20 (50)	8.5 ± 4.4	7.4 ± 1.8	NS

[a] 6–8-week-old male BALB/cMai mice were given 0.1 mg glucan in saline or saline alone i.v. on days −8, −5, −1 before s.c. infection on day 0 with RVFV.
[b] Prolongation of survival in glucan-treated mice assessed by Kolmogorov-Smirnov test.

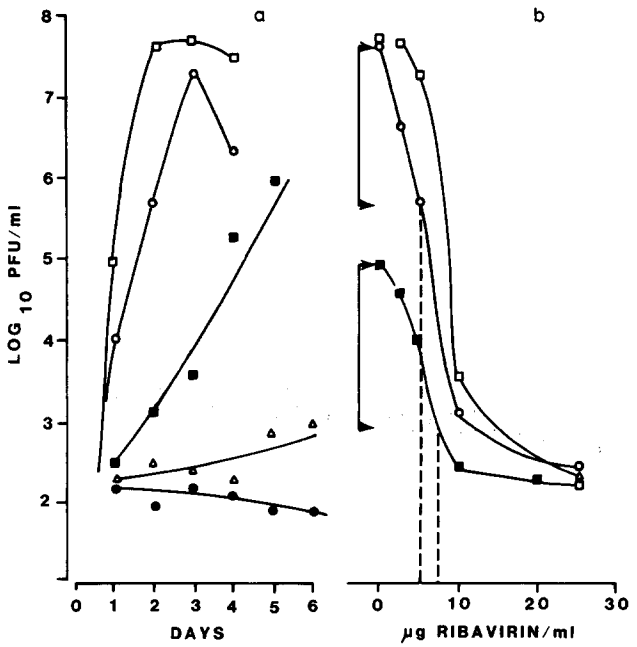

Fig. 1. Ribavirin inhibition of RVFV growth in MRC-5 cells. On day 0, 1000 PFU ZH-501 strain RVFV was inoculated into 75-cm^2 flasks of MRC-5 cells containing 30 ml medium with the indicated concentration of ribavirin. Supernatant virus was measured daily. In (a) virus yield from each flask is plotted as a function of time. In (b) the daily yields are ploted as a function of the concentration of ribavirin in the tissue culture medium. Arrows on vertical axis identify a 2-log reduction in virus yield and dashed lines indicate the extrapolated concentration of ribavirin resulting in this decrease. Symbols: (a) □, no drug, 0.5 μg/ml; ■, 10 μg/ml; △, 25 μg/ml; ●, 50 μg/ml. (b) ■, 24 h; ○, 48 h; □, 72 h.

assessed virus clearance [23] after i.v. injections of 10^7 PFU RVFV. In both saline- and glucan-treated mice, titers were 6.7 $\log_{10}$ PFU/ml 1 min later and fell gradually to 5.8 to 6.0 $\log_{10}$ PFU/ml by 30 min.

Further experiments with ribavirin

Since ribavirin was clearly protective for rodents, we extended these observations to rhesus monkeys (Table 5). Neither treated nor control monkeys were clinically ill. All control monkeys were viremic for 3 days with peak serum virus titers of 4.9 to 5.7 $\log_{10}$ PFU/ml. Only 1 of 4 treated monkeys had detectable viremia with a peak value of only 2.7 $\log_{10}$ PFU/ml. Plaque reducing neutralizing (PRN) antibodies appeared in all animals by day 7, but usually peaked on day 10 or 14 in control as opposed to day 28 in treated monkeys (Table 5).

Since ribavirin was so effective in the intact animal, we assessed its ability to inhibit RVFV replication in cell culture. In a typical test, control virus yields were maximum on day 2 or 3 (Fig. 1a). Increasing drug concentrations diminished virus yield, and in many cases completely suppressed viral replication and CPE. Microscopic evidence of cell damage closely paralleled virus yield measurements. If the concentration of

TABLE 5

Viremia and antibody titers of RVFV-infected[a] rhesus monkeys

	log_{10} PFU/ml on day			PRN_{80} on day			
	1	2	3[c]	7	10	14	28
Control	2.5	5.1	4.6	80	320	320	160
	3.0	4.9	2.4	80	320	1280	320
	5.2	5.7	5.0	80	320	160	640
	4.6	5.3	2.8	320	1280	640	640
Ribavirin[b]	1.7	1.7	1.7	160	640	640	1280
	1.7	1.7	1.7	80	160	160	640
	1.7	1.7	1.7	80	640	640	1280
	1.7	2.7	2.3	80	160	320	640

[a] 4.2 log_{10} PFU injected i.v. on day 0.
[b] 50 mg/kg i.m. 2 h after inoculation and 10 mg/kg i.m. every 8 h for 9 days.
[c] No serum virus was detected on days 4 through 7.
PRN = plaque reduction neutralization; for a calculation of PRN_{80}, see Ref. 9.

supernatant virus is plotted as a function of the ribavirin concentration in the cell culture medium (Fig. 1b), a reproducible and useful extrapolation of the concentration of ribavirin necessary to decrease virus yield 100-fold can be made. When we compared 14 cell types, we found marked differences in their responses. Ten cell types established from kidney, lung, or thymus cultures were relatively resistant to the toxic effects of ribavirin and could be maintained for several days in concentrations up to 50–150 g/ml without morphologic evidence of toxicity (Table 6). Ribavirin clearly decreased virus yield and exerted a protective influence against CPE; the inhibitory concentration varied significantly among the different cells. There was no correlation with the origin of the cells or the maximum virus yield, but there was a tendency for high virus yield on day 1 or 2 to be associated with relative resistance to ribavirin inhibition (not statistically significant by Kendall's tau test). The four cell lines that clearly represented analogues of differentiated cell types (BW-J-M, NZW-S2-M, glial 107, and hepatoma 7777 cells) all responded to concentrations of ribavirin from 2.5 to 10 μg/ml with rounding up and cessation of replication. The two macrophage cell lines then seemed to adapt to ribavirin and regained normal adherence and growth properties, but the other two cell lines continued to decline in cell numbers over time. Antiviral studies were not pursued in detail in these four lines, but they showed marked inhibition of viral growth by concentrations of ribavirin as low as 1–5 μg/ml. ZH-501 and three other RVF isolates were tested with ribavirin in MRC-5 cells. Their inhibition patterns were virtually identical.

In vitro sensitivity to interferon

The Egyptian prototype strain of virus (ZH-501) used in our animal experiments and three additional RVFV isolates were tested in L cell cultures and found to require 2–5 times more mouse interferon to provide 50% inhibition of plaques inoculated as the control prototype VSV-Indiana.

TABLE 6

In vitro inhibition of RVFV replication by ribavirin in different cell types

Cell type	Peak titer	Toxicity[c]	Day 1		Day 2		Day 3	
			Titer	2-log inhibition[d]	Titer	2-log inhibition	Titer	2-log inhibition
Vero	7.5	150	4.2	50[a]	5.2	48	7.5	23[b]
LLC-MK_2	6.7	150	4.0	10	5.9	8	6.6	9
Fetal rhesus lung	7.3	50	3.7	9[a]	7.1	2	7.3	3
MRC-5	7.7	150	4.9	8	7.6	6	7.7	7
Lewis rat thymus	7.8	150	5.7	27	7.8	23	-	-
Wistar-Furth rat thymus	8.1	150	7.2	25	8.1	43[b]	-	-
Maxx rat thymus	7.6	150	5.8	14	7.4	18	-	-
Guinea pig kidney	6.3	150	4.0	5[a]	5.3	2	6.3	2
Guinea pig kidney-SV40	6.6	150	4.0	2	6.2	1	6.3	2
Chicken embryo fibroblast	7.2	150	4.0	1	7.2	3	-	-

[a] No evidence of replication at higher concentrations of drug, but only 1.4–1.8 log decrease compared to control.

[b] Peak titer already reached and therefore may not be accurate.

[c] Minimum concentration of drug causing cytopathic effect.

[d] Concentration of drug resulting in 2-log decrease in viral titer compared to controls. See Fig. 1 and Results for details of calculation.

Discussion

RVF occurs throughout most of the African continent. Its recent extension into Egypt may pose a threat to the Mediterranean basin and further increases the possibility of its introduction into other countries by air travel [28,31]. The ability of many mosquito species to be infected by and/or transmit virus suggests that there may be a real danger of establishing epizootic disease in the United States, or other countries of temperate climates [12]. The virus is highly infectious by aerosol [5] and is present in high concentrations in human serum, a threat both to viral diagnostic and clinical laboratory workers. Although most human infections result in a mild febrile illness, a minority (perhaps 0.1–1.0%) are complicated by retinal lesions, severe encephalitis, or hemorrhagic fever. Thus, effective antiviral treatment modalities could be useful in preventing infection or its sequelae, treating serious infections, or suppressing viremia of amplifying hosts, thereby preventing infection of biting arthropods. Furthermore, RVFV is a member of a large family of human pathogens, Bunyaviridae, for which no

practical therapeutic agents are known [28]. This family includes North American pathogens (such as viruses of the California encephalitis group), virulent exotic agents (such as Congo–Crimean hemorrhagic fever virus), and other members of the phlebotomus fever group [30] to which RVFV belongs (e.g., sandfly fever and Punta Toro viruses).

We performed an initial survey in the RVFV-infected mouse of several avenues of therapy which were reported to be effective in vitro and/or in vivo with other RNA virus infections and emphasized drugs which might find human application. We detected no significant activity for rimantadine [2,18], thiosemicarbazone [2], or inosiplex [13]. When we tested the broad-spectrum antiviral drug ribavirin, however, we found marked activity, confirming our preliminary observations [34]. This drug was also successful in protecting hamsters, a species which resembles the mouse in its susceptibility to lethal RVF infection and in having the liver as a target organ. RVFV-infected monkeys develop viremia, but only mild hepatitis. Ribavirin in modest doses (30 mg/kg per day) was able to suppress monkey viremia to low levels. The antibody response was slightly delayed, possibly a reflection of a smaller antigenic stimulus.

We were also able to add ribavirin to tissue culture systems and inhibit virus production. The amount of drug required for a 100-fold reduction in virus yield varied between 1 and 50 μg/ml ribavirin, depending on the cell type used. Information from radiolabeled drug studies in rhesus monkeys [10] suggests that intracellular concentrations of ribavirin and its metabolites may exceed 30 μg/ml tissue water in the liver 8-h following a single dose of 10 mg/kg. These concentrations in tissue culture medium bathing a cell monolayer were nontoxic and effective in suppressing viral replication in several cell types. Brain concentrations of ribavirin radioactivity indicated drug levels of only 2 g/ml tissue water in the same study. This may explain the late deaths with clinical signs of neurological dysfunction observed in some ribavirin-treated animals.

Poly(ICLC) was employed as a prototype of an effective interferon inducer [20,24,25]. Its administration a few hours after virus infection resulted in virtually complete protection of RVFV-infected mice. Poly(ICLC) also stimulates the reticuloendothelial system and may exert antiviral effects through that mechanism [4]. Although direct data are not yet available, three lines of evidence suggest that interferon mediated the observed protection: first, poly(ICLC) is an established interferon inducer in mice as well as monkeys; second, RVFV was quite sensitive to the effects of murine interferon in vitro; and third, a potent macrophage stimulator (glucan) extended survival of infected mice, but had only marginal effects on the final mortality of murine RVFV. It is also interesting that Bunyamwera virus from the Bunyavirus genus of Bunyaviridae is sensitive to interferon in vitro [19] and in a mouse model [17]. Murine encephalitis following infection with Germiston virus, closely related to Bunyamwera virus, can also be prevented by pretreatment with interferon [1]. However, murine RVFV infection leads to a fatal outcome in spite of high serum interferon levels [16]. Gene-cloned interferon may be as effective as the naturally occurring substance and may now be produced economically, thus increasing the potential usefulness of interferon. Penetration of interferon into the central nervous system is limited [14], but under certain circumstances may have a therapeutic effect [15].

A reticuloendothelial stimulant might be expected to have strong protective effects since macrophages occupy a strategic anatomic position to protect the liver from RVFV invasion [23]. Glucan has protected outbred CD-1 mice from lethal RVFV [29], but in this study it only prolonged the time to eventual death of infected mice. The cause of death in glucan-treated mice dying late was encephalitis rather than liver necrosis. Thus, glucan protected the liver, but presumably viremia occurred and seeded the brain for later development of encephalitis. Whatever the mechanism of glucan action, it did not affect clearance of injected virus [23].

Passive antibody, as reported in other studies [3,8], was effective in protecting susceptible animals from lethal RVFV challenge. The dose of 4 ml/kg given to these mice is the approximate volume per kg administered to a human in a single unit of plasma. Since human RVFV infections usually result in PRN titers of 1 : 1000 to 1 : 10 000 (J.M. Meegan and C.J. Peters, unpublished observations), compared to the 1 : 1024 titer of the experimental ascitic fluid, convalescent plasma might be useful in prophylaxis of a defined human exposure. Although human RVFV is usually a self-limiting disease, retinitis, encephalitis, and hemorrhagic fever are recognized complications which may potentially be prevented by passive antibody administration. More speculatively, gamma globulin prepared with plasma from vaccinees or residents of an endemic area might have considerable value. The use of passive antibody in hemorrhagic fever cases has the potential theoretical disadvantage that acute immune complex disease could precipitate a temporary exacerbation. Since encephalitis and retinal vasculitis both occur when serum antibody levels are high, immune plasma is unlikely to be useful.

Ribavirin provides an additional modality that could be applied to human RVFV in the foreseeable future [33]. Subsequent studies have discovered other candidate compounds which may eventually find application after more extensive testing [6,7].

Ribavirin, however, is a potent antiviral agent with therapeutic efficacy when given intravenously in Lassa fever or by the aerosol route in influenza and respiratory syncytial virus infections of man. Therefore, it could be useful either in prophylaxis of defined RVFV exposure or treatment of hemorrhagic fever. Use in established encephalitis or ocular disease may be limited by its poor penetration into the nervous system. The development of more lipid-soluble analogues may overcome this deficiency [32].

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