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EMC virus infection in baboons as a model for studies on antiviral substances

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Summary

EMC virus causes a lethal infection in baboon monkeys within 4–8 days following subcutaneous injection with 10^4–10^8 pfu of virus. The infection is accompanied by viremia, invasion of heart muscle and of brain. Monkeys infected with 10^6 pfu of EMC virus were treated with human leukocyte interferon. The interferon was injected intramuscularly first 0, 0.5, 6 and 24 h post-infection, then twice daily with a dose of 3 $\times$ 10^6 units for 5 consecutive days. All the monkeys treated with interferon remained alive and healthy. Animals infected with EMC virus, but not treated with interferon died within 6 days with evidence of myocarditis.

The EMC virus–interferon interaction in baboon monkeys seems to provide a useful primate model system for testing the prophylactic and therapeutic antiviral activity of interferons or other antiviral substances.

EMC virus; antiviral substances; interferon; baboons

Introduction

The cardioviruses genus of the Picornaviridae family comprises several antigenically related strains of viruses, such as encephalomyocarditis (EMC), Mengo, Columbia-SK and M.M. virus [4,10]. There is no antigenic relationship between the EMC group of viruses and the poliomyelitis viruses [22]. Members of the EMC group of viruses have been isolated from humans, from a variety of mammals and from birds' arthropods in widely dispersed geographic areas [18]. Initially EMC viruses had been isolated from latent virus infections in laboratory rodents [21]. In 1945 a virus was recovered from a chimpanzee which had died suddenly. The most significant pathological finding in the autopsied chimpanzee was myocarditis. The virus isolate produced encephalomyocarditis and myocarditis in various laboratory animals [11]. Naturally occurring

outbreaks of clinical and often fatal illness in swine and non-human primates were described [12]. Later other members of the EMC group of viruses were isolated from human patients with encephalitis and meningitis [6]. Owl monkeys, night monkeys and marmosets are highly susceptible to EMC virus infection and develop a fulminating fatal disease [11].

In rabbits, rhesus monkeys and *Cynomolgus irus* monkeys EMC virus causes only an inapparent infection accompanied by viremia. It has been assumed that rodents are the natural reservoir of EMC virus [13]. EMC virus infection, however, has not yet been studied in baboon monkeys which, because of their relatively low price and availability are now the primate of choice for experiments with viruses.

Interferons have been tested in vitro and in vivo as antiviral and antitumor agents in humans [19]. The production cycle of the virus in the infected cell is inhibited at the transcriptional or translational level, depending on the virus–cell system studied [5]. The antiviral effect of interferon in vivo is also dependent on the host. Interferon may activate several defence systems of the host, for example the cytotoxicity of the natural killer cells and macrophages [15].

The greater availability of different types of human interferons makes it desirable to find a model system which would be as close as possible to the human organism for evaluating efficacy of the different preparations of interferons against viral infections. Human interferons were already tested in Rhesus monkeys infected intradermally with vaccinia virus [16,17], or in squirrel monkeys infected with EMC virus [7]. Another model system for evaluating the activity of human interferons against viral infection involves baboon monkeys. We show here that baboon monkeys are highly susceptible to EMC virus infection, which is lethal in this species. This lethal infection can be prevented or suppressed by suitable treatment with human leukocyte interferon.

Experimental

Encephalomyocarditis virus (EMC) was propagated in L-929 cells and titered by plaque assay on BHK monolayers. Baboon monkeys (*Papio cynocephalus*, 4–6 kg) were imported from Kenya. The monkeys were kept in quarantine 2 weeks before the start of the experiments and for 4 weeks after their completion. All animals were screened for the presence of circulating interferon or neutralization antibodies against EMC virus. Only baboons lacking interferon and antibodies were used. Human leukocyte interferon was prepared by the method of Cantell [3]. Its specific activity was 3×10^6 units/mg protein. Interferon activity was determined by its inhibitory effect in a cytopathic assay of VSV (vesicular stomatitis virus) in MDBK cells [14].

The course of EMC virus infection in baboons

Baboon monkeys were infected subcutaneously (s.c.) with EMC virus, at doses ranging from 10^4 to 10^8 pfu. The monkeys were observed twice daily at 12-h intervals for clinical symptoms. The infected monkeys showed clinical symptoms about 1 day before death. The time of death depended on the dose of virus (Fig. 1) and ranged from 3.5 to 9 days. Viremia was monitored for up to 10 days following the inoculation with

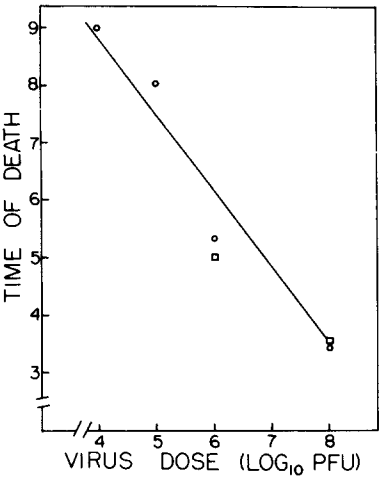

Fig. 1. Time of death of EMC virus-infected baboon monkeys as a function of virus dose. Six monkeys were infected subcutaneously with EMC virus at doses ranging from 10^4 to 10^8 pfu and were observed till death.

the virus (Fig. 2). It usually appeared 2–4 days before death. Thus, after a dose of 10^8 pfu, the viremia began on day 2, and after a dose of 10^4 pfu, on day 6. Viremia followed a similar course in all infected animals (Fig. 2A). After a dose of 10^5 pfu, blood levels reached about 1.8×10^5 pfu/ml, and following injection of 10^6 pfu the virus concentration in blood was 3.8×10^6 pfu/ml.

Monkeys were autopsied within 1–2 h after death. Specimens for histopathology were taken. Extracts of hearts, brains, kidneys, spleen and lungs were titered for the

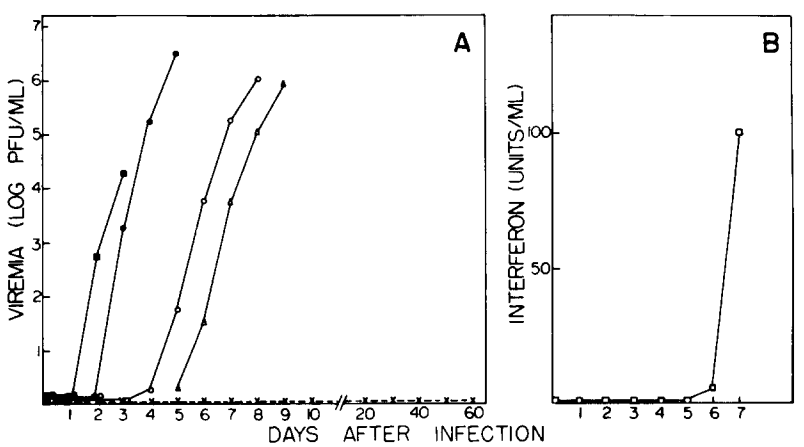

Fig. 2. Viremia in EMC virus-infected monkeys with and without interferon treatment. (A) Viremia. EMC virus dose 10^8 (■); 10^6 (●); 10^5 (○); 10^4 (△) pfu. Full lines: no interferon treatment; dotted line: 3×10^6 units of interferon given at time 0 post-infection with 10^5 pfu of EMC virus. Each curve corresponds to one monkey. (B) Interferon. Appearance of endogenous interferon after i.m. injection of 2 baboons with 10^5 pfu of EMC virus. Blood samples were taken daily for 10 days.

presence of EMC virus. The levels of virus in the heart tissue depended on the dose of virus. Since in the heart extract the virus concentration was 5–10-fold higher than in blood, one may conclude that the virus developed in the tissue itself and was not due to contamination with residual blood. At death, the virus titer in brain was $10^{4.4}$ to $10^{4.7}$ pfu/g.

Pathology

In the EMC virus-infected baboons that were not treated with interferon, clinical symptoms, paleness, anorexia and apathy appeared only 1 day before death. Post-mortem examination revealed pale to cyanotic mucous membranes, some congestion in the abdominal organs, frothy fluids in the bronchi, various degrees of lung congestion and oedema. In some cases whitish streaks in the myocardium were observed and there was pleural and pericardial fluid.

The mean features in histological sections were oedema of the lung, interstitial myocarditis with diffuse granulocytic infiltrate, degeneration and some calcification of myofibrils; some perivascular hemorrhages were seen in cerebellum, cortex and white matter. Meningeal hemorrhages were also seen. There was a moderate degree of subchronic myocarditis and pericarditis. Extensive myocardial areas were degenerating.

Protective effects of interferon

In order to assess the effect of interferon on EMC virus infection, we first determined the levels of endogenous interferon appearing as a result of the EMC virus infection. Figure 2B illustrates the kinetics of appearance of endogenous interferon in a baboon infected with 10^5 pfu of the virus. In this baboon, viremia appeared on day 5, and the animal died on day 8, when the titer of the virus in the blood was 10^6 pfu/ml. On day 6, the level of endogenous interferon was still undetectable, and it rose to about 100 units/ml on the day preceeding death. Thus, the endogenous interferon appeared too late to affect the course and outcome of the infection.,

Figure 3 shows serum interferon levels following intramuscular interferon injections. Interferon was injected at doses of 3×10^6 units intramuscularly (i.m.), twice daily for 5 days, beginning at the time of viral inoculation or later at times specified in Figure 3. The interferon reached a peak within 3–4 h after injection. Blood levels then declined to about 25 units/ml. This level persisted as long as interferon was administered.

The prophylactic and therapeutic effects of interferon were tested in 4 experiments involving 16 baboons, with 10^6 pfu of EMC virus. The first injection of interferon was given immediately after the virus inoculation or 4, 6, 8 or 24 h thereafter. After this first injection interferon was administered twice daily, every 12 h for 5 consecutive days. All treatment schedules suppressed the infection and prevented death. Even when the administration of the first dose of interferon was delayed till 24 h post-infection, interferon was still effective in preventing illness and death, but not always viremia. Effects of interferon administration beyond 24 h after virus inoculation were not tested. Interferon given only once, at time 0 to a baboon receiving 10^5 pfu of EMC virus, completely aborted viremia and disease (Fig. 2A).

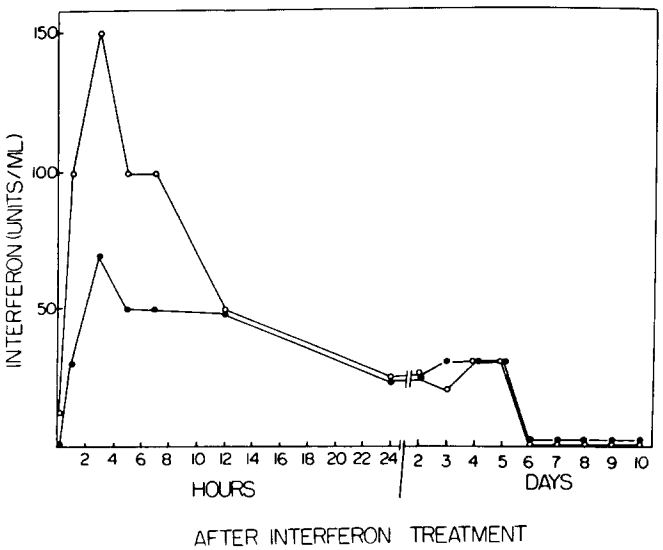

Fig. 3. Human leukocyte interferon pharmacokinetics in EMC virus-infected baboons. Blood samples were taken from two EMC virus-infected baboons 1, 3, 5, 7, 12 and 24 h after the interferon treatment.

The efficacy of antiviral substances, including interferon, has already been tested in various animal species, but only a little in primates. Generally, early initiation of treatment has been found to be a crucial requirement to obtain significant protective effects. With low virus doses initiation of treatment may be delayed until after the injection takes place. Thus, Heremans et al. [8] showed that in mice injected with low doses of Mengo virus, late treatment (3 days post-infection) with a suitable dose of interferon could protect 70% of the animals from death. Goeddel and coworkers [7] and Weck [23] studied a lethal EMC virus infection in squirrel monkeys. Natural human leukocyte interferon as well as bioengineered α- and β-interferon were all effective in preventing death but only when the first injection of interferon was given before and not after the viral infection. In the baboon EMC virus system leukocyte interferon administration was effective in preventing death, even when the interferon treatment was initiated as late as 24 h post-infection. This protective effect of interferon was apparent at blood concentrations ranging from 50 to 100 units/ml serum 2–7 h after injection of 25 units/ml during the 5-day course of treatment.

Another primate model system that we employed involved the 17D vaccine strain of yellow fever virus and baboon monkeys [8]. In that system, the only evidence of viral infection was the appearance of antibodies to the 17D virus, but there was no viremia or any clinical symptoms. Interferon administered within 30 min of infection prevented the appearance of the antibodies. The results of the study described here suggest that EMC virus infection in baboons provides a more useful system for testing human interferons as well as other antiviral substances.

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