EFFECT OF VIRUS ANTIBODY ON INFECTION OF MOUSE BRAIN BY MOUNT ELGON BAT VIRUS

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SUMMARY. Intranasal inoculation of mice aged 1–6 days with Mount Elgon bat virus produced an acute brain infection and death of all the mice at 5–7 days after inoculation. Virus multiplied in the olfactory bulbs and spread to the midbrain and then hindbrain, reaching titres of 10^9 – 10^{10} plaque forming units/g of wet tissue. Fatal disease was prevented by administration of virus antibody after infection, which was then restricted to the olfactory bulbs. Antibody was ineffective when infection was established in the midbrain; it reduced infectivity by about 99% in olfactory bulbs but by only a small fraction in midbrain and hindbrain. The findings may reflect differences in virus maturation and spread, and the accessibility of virus antibody to the olfactory bulbs, midbrain and hindbrain.

INTRODUCTION

There have been many studies of viral invasion, replication, and the role of the host defences, particularly the immune responses, in the pathogenesis of primary viral infections of the central nervous system (CNS) (Hotchin, 1967; Albrecht, 1968; Johnson and Mims, 1968; Nathanson and Cole, 1970). Mount Elgon bat virus (MEBV) is an antigenically distinct rhabdovirus which was isolated, in suckling-mouse brain, from the salivary glands of a bat, *Rhinolophus hildebrandti eloquens* (Metselaar *et al.*, 1969). The pathogenesis of the virus infection was subsequently studied in mice (Murphy *et al.*, 1970; Patel, 1979*a* and *b*). Virus administered intranasally or intracerebrally killed suckling mice readily but older mice became progressively resistant (Patel, 1979*a* and *b*); this age-related resistance was ascribed to maturation of host immune responses. In sucklings up to 13 days of age, virus administered intranasally invaded the olfactory bulbs via the olfactory nerve and grew to high titre in olfactory bulbs, midbrain and hindbrain, killing the mice (Patel, 1979*a*). Older sucklings were resistant to intranasal infection yet virus invaded and persisted in the olfactory bulbs until the appearance of an immune response (Patel, 1979*a*). In the experiments reported here, the effect of virus antibody administered after the intranasal infection of mice aged 1 day or 4–6 days was studied.

MATERIALS AND METHODS

Virus. A sixth passage of MEBV in suckling-mouse brain was prepared and titrated (Patel, 1978). The titre of a 10% (w/v) stock suspension stored at -70°C was $8.4 \log_{10}$ plaque forming units (pfu)/ml.

Virus antiserum. Adult female mice, strain LACA, that survived intracerebral inoculation of 10^6 pfu (Patel, 1979*b*) were given an intraperitoneal injection of 1×10^6 sarcoma-37 cells suspended in phosphate-buffered saline, pH 7.3 (PBSA, Oxoid) 2 weeks after the virus inoculation; ascitic fluid was collected 1 week later. Normal ascitic fluid was prepared in mice by intracerebral injection of 0.1 ml of a 10% (w/v) suspension of normal suckling-mouse brain.

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The virus-neutralising titre of the pooled immune ascitic fluid was 20 480, but activity was not detected in normal ascitic fluid in a plaque-count-reduction assay in chick cells (Patel, 1978).

Mice. Outbred category-4 LACA mice were used (Patel, 1979a). Sex-related differences in susceptibility to the virus had not been observed (Patel, 1979a) and equal numbers of male and female mice were used in all experiments.

Experimental design. Groups of 16 mice aged 1 day and 4–6 days were inoculated intranasally with 10^6 pfu of virus and given immune ascitic fluid intraperitoneally (0.1 ml/mouse) 30, 52 or 72 h later. Control groups were given normal ascitic fluid at 30 h. The survival time in each group was recorded during 50 days. In each group, two male and two female mice were killed before and after administration of immune or normal ascitic fluid and the infectivity in pooled olfactory bulbs, mid-brain and hindbrain was determined by plaque count in chick cells (Patel, 1978, 1979a). Other tissues were not assayed because they did not yield virus except for nasal mucosa, which became infected after the virus had multiplied in the olfactory bulbs (Patel, 1979a). The serum of normal sucklings aged 1 day or 4–6 days was tested for virus-neutralising antibody daily for 7 days after intraperitoneal injection of the immune ascitic fluid; titres were 1024–5160. Tests were not done for virus antibody in brain of these sucklings, neither were tests done to determine whether the mice that were protected by virus antibody manifested an anamnestic antibody response.

RESULTS

Infection in mice aged 1 day

In the control group, virus was first detectable in the olfactory bulbs 24 h after infection (figure, a); by 48 h the olfactory bulbs contained $5.5 \log_{10}$ pfu/g of wet tissue and the midbrain $4.0 \log_{10}$ /g of wet tissue; by 72 h the virus had spread to the hindbrain (figure, a). Mice began to die at 96 h when the brain contained $9-10 \log_{10}$ pfu/g of wet tissue (figure, a). Virus-neutralising antibody was not detected in pooled serum from four control mice tested daily for 4 days. Infected 1-day-old mice given virus antibody 30 h after infection yielded virus from the olfactory bulbs at 48 h after infection but this declined to less than $2.5 \log_{10}$ pfu/g (figure, b); all the mice in this group survived infection (see the table). Although virus antibody given 52 h after infection did not prevent fatal disease (see the table), it considerably reduced the amount of virus recovered from olfactory bulbs; it was less effective in reducing virus in the midbrain and hindbrain (figure, c).

Infection in mice aged 4–6 days

Virus infection of control sucklings resulted in death of the mice by 7 days. Virus was first recovered from the olfactory bulbs at 48 h, followed by midbrain at 72 h after infection and hindbrain a day later (figure, d); by 120 h, virus titres of $8-9 \log_{10}$ /g of wet tissue were found in olfactory bulbs, midbrain and hindbrain of these mice. If they were given virus antibody 30–52 h after infection, more than 90% of these sucklings survived and transient virus growth was

TABLE

The protection conferred by virus antibody on mice aged 1 day or 4–6 days infected intranasally with Mount Elgon bat virus

Time of antibody administration (h after infection)	Number surviving/number inoculated at age of	
	1 day	4–6 days
30	16/16	16/16
52	0/16	15/16
76	0/16	0/16

* Observation period was 50 days; all control mice died by 5–7 days.

Figure.—Effect of virus antibody on titre of Mount Elgon bat virus in brain; 1-day-old (a–c) and 4–6-day-old mice (d–g) were infected intranasally with 10^6 pfu followed by an intraperitoneal injection of immune ascitic fluid (0.1 ml/mouse) 30 (b and e) 52 (c and f) and 76 h (g) later (arrows). In each group two male and two female mice were killed at intervals and 10% (w/v) suspensions of the olfactory bulbs (●), midbrain (▲) and hindbrain (■) in ice-cold gelatine (0.5%, w/v) in PBSA were titrated for infectivity after pooling corresponding tissues. Control groups (a and d) were given normal ascites fluid 30 h after virus inoculation.

restricted to the olfactory bulbs (see the table and the figure, e and f), whereas those given virus antibody 76 h after infection died with considerable virus growth in the midbrain and hindbrain (see the table and figure, g).

DISCUSSION

Previous reports have shown that the brain is the target organ for MEBV in mice (Metselaar *et al.*, 1969; Murphy *et al.*, 1970; Patel, 1979*a* and *b*). This and the lack of viraemia (Patel, 1979*a* and *b*) closely parallels infection of experimental hosts by rabies and related viruses (Murphy, 1977). It has been established, in weanling and adult mice, that the immune response was responsible for their recovery from low-grade, midbrain and hind-brain infection (Patel, 1979*a* and *b*). The immune response was shown to be important in the recovery of a proportion of mice from an established brain infection by rabies (Lodmell *et al.*, 1969) and several other neurotropic viruses (Nathanson and Cole, 1970). The present experiment suggests differences in the protective effect of virus antibody at different sites in the brain. Whereas control suckling mice died from MEBV encephalitis before forming detectable virus-neutralising antibody (Patel, 1979*a* and *b*), the present experiment shows that virus antibody given intraperitoneally could

prevent the fatal disease even when virus had grown in the olfactory bulbs. The antibody appeared to prevent infection in the olfactory bulbs from extending into the midbrain and thence to the hindbrain but it was apparently ineffective after infection had been established in the midbrain. In the latter groups of mice, the antibody reduced infectivity by 99% in olfactory bulbs but by only a small fraction in midbrain and hindbrain. Thus, passively given virus antibody may have spread to the olfactory bulbs but not to the midbrain and hindbrain. The rate and extent of virus growth in the three areas of the brain were similar and could not account for the differences observed.

Although the mechanism of virus neutralisation is not clearly understood (Della-Porta and Westaway, 1978), if antibody can limit or arrest an existing CNS infection then the spread of virus is likely to be through extracellular space (Liu *et al.*, 1959; Albrecht, 1968; Nathanson and Cole, 1970). MEBV (Murphy *et al.*, 1970), rabies (Miyamoto and Matsumoto, 1967) and Klamath viruses (Murphy *et al.*, 1972) form cytoplasmic inclusions in mouse brain; these are presumed sites of virus maturation, differing perhaps from those of other rhabdoviruses that mature at the plasma membrane (Knudson, 1973). This raises the question of whether MEBV matures predominantly at the plasma membrane in olfactory-bulb neurones (Patel, 1979a). Studies of this, and the availability of antibody in the brain (Lodmell *et al.*, 1969) may give an insight into the pathogenesis of infection with MEBV and other neurotropic viruses such as rabies.

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