INFECTION AND IMMUNITY, June 1983, p. 995–1003
0099-2240/83/060995-09$02.00/0

Vol. 40, No. 3

Pathogenesis and Immune Response of Vaccinated and Unvaccinated Rhesus Monkeys to Tick-Borne Encephalitis Virus

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Received 14 October 1982/Accepted 4 March 1983

The rhesus monkey was used as a model for diseases caused by viruses of the tick-borne encephalitis virus complex to study the efficacy and safety of a commercial killed vaccine. Animals infected intravenously developed a subclinical infection with no histopathological lesions but with transient clinical chemical changes that included elevated transaminase, dehydrogenase, and creatine kinase activities and that declined as an immune response developed. The immune response was detected as neutralizing antibody in serum and serum antibody to several viral proteins. Antibodies to viral envelope protein and two other infected cell-specific polypeptides were also detected. Intranasal infection resulted in a disease resembling that in humans, except that no pyrexia was observed. Clinical chemical changes similar to those in intravenously infected monkeys developed, but most animals died before an immune response was mounted. Using this model, we have demonstrated that a commercial vaccine protects animals against a wild-type virus isolate and that it elicits an effective immune reaction without any evidence of an immune enhancement phenomenon or adverse side effects as judged by clinical observation, clinical chemistry, and histopathology.

The viruses of the tick-borne encephalitis (TBE) complex of flaviviruses cause several diseases of humans and animals which may be transmitted by bites from infected ticks and include Russian spring-summer encephalitis, Central European TBE (CTE), and, in the United Kingdom, louping ill. This report describes attempts to establish the rhesus monkey as a nonhuman primate model for TBE for evaluating the efficacy and safety of a commercially available TBE vaccine in protecting against field isolates of the virus. As this group of viruses causes chronic and clinically inapparent infections, in addition to the well-known acute form of the disease (1), we have combined clinical observation, clinical chemistry, histopathology, and serology to monitor the disease and assess the quality of the vaccine. Such studies have become increasingly important in recent years as serious problems have arisen with the use of some killed measles vaccines (20), and it has also been shown that some rabies vaccines do not protect against certain strains of wild-type rabies virus (23).

In this study, we have employed classical histopathological and clinical chemistry techniques to detect chronic and inapparent infections. In addition, we have used both virus neutralization and radioimmune precipitation (RIP) to detect virus-specific antibody. The latter technique is particularly useful to determine the viral polypeptides to which a vaccine preparation elicits antibody. This has been shown to be important since early killed measles vaccines only elicit antibody to the hemagglutinin and not to the fusion protein and thus gave rise to the vaccine-associated syndrome of atypical measles (3). Moreover, atypical measles has been shown to lead to a considerable production of antibodies against the M protein, a situation which does not occur in normal infections or in people vaccinated with live measles vaccine (14, 28).

In this report, we demonstrate that intranasal (i.n.) infection of adult rhesus monkeys with a member of the TBE complex of flaviviruses mimics the human disease in many respects. In addition, we show that a commercial killed vaccine elicits an immune response indistinguishable from that of a laboratory infection with live virus and that no adverse side effects occur as the result of vaccination.

MATERIALS AND METHODS

Animals. Sixteen adult rhesus monkeys (*Macaca mulatta*) of both sexes, weighing 1.8 to 5.1 kg, were used. The animals were housed and fed as described previously (10). For infection and all sampling procedures, the animals were anesthetized by intramuscular injection of ketamine hydrochloride (Vetalar; Parke, Davis & Co., Pontypool, Gwent, United Kingdom).

Viruses. The virus isolate used to infect animals, TTE virus, was obtained from the Moredun Institute, Edinburgh, United Kingdom, and had originally been isolated from the brain of a sheep infected during an outbreak of TBE in Turkey (13). The original isolate was passaged several times in sheep brain and once in mouse brain in Edinburgh and then three or four times in suckling mouse brain in our laboratory. The reference strain of CTE virus was obtained from infected ticks collected by C. Kunz (University of Vienna, Austria) and subsequently passaged in mouse brain and plaque purified in primary chick embryo fibroblasts (CEF) by J. Keppie of our laboratory. The CTE virus used in this study was derived from clone H-Neudörfl and was passaged three times in suckling mouse brain.

Fruh-Sommer meningoencephalitis vaccine. Vaccine for Fruh-Sommer meningoencephalitis (FSME) was a generous gift from IMMUNO GmbH, Vienna, and was originally derived from clone H-Neudörfl. The vaccine was prepared from virus grown in primary CEF suspension cultures, inactivated with Formalin, and purified by zonal centrifugation.

Animal infection. Monkeys were infected with 3×10^8 to 5×10^8 PFU of a standardized challenge stock of the Turkish strain of TBE virus. One animal was infected by intraperitoneal injection, 5 by intravenous (i.v.) injection, and 10 by i.n. instillation of drops of virus suspension from a syringe.

Animal specimens. Blood (10 ml) was removed from a femoral vein before infection and at intervals after infection and was processed as described by Hambleton et al. (12). The separated filtered serum samples were stored at −20°C.

Cerebrospinal fluid (CSF) was taken from monkeys by cisternal puncture before infection and 9 to 11 days postinfection, filtered, and stored at −20°C as described by Hambleton et al. (10). Portions of unfiltered CSF were examined microscopically for the presence of leukocytes.

Monkeys were killed at intervals from 4 days to 14 weeks after infection and in extremis by i.v. injection of sodium pentobarbitone, and necropsy was carried out immediately. The brain and spinal cord were removed, and portions of tissue from each region were taken for virus isolation. The remainder of the central nervous system (CNS) was fixed in buffered 10% neutral Formalin, as were portions of liver, spleen, lung, kidney, and small intestine. After processing by standard methods and embedding in paraffin wax, sections of all tissues were cut and stained by hematoxylin and eosin. Selected sections of the CNS were also stained by phosphotungstic acid hematoxylin, by luxol fast blue-cresyl violet, and by the Glees and Marsland modification of the Bielschowsky technique for neurones.

Analytical methods. Concentrations of Ca, Cu, Fe, Mg, K, Na, and Zn were determined with either flame or flameless atomic absorption spectrophotometry (9, 11), and the activities of alkaline phosphatase (AP), alanine aminotransferase, aspartate aminotransferase (ASAT), lactate dehydrogenase (LDH), α-hydroxybutyrate dehydrogenase (α-HBDH), and creatine kinase (CK) and levels of creatinine, uric acid, and total protein were determined with a reaction rate analyzer for serum and CSF specimens as described by Hambleton et al. (10, 12). Serum amino acid levels were determined with an amino acid analyzer as described by Hambleton et al. (12).

Hematology. Total erythrocyte and leukocyte counts were measured with a Coulter counter (Coulter Electronics, Harpenden, United Kingdom) as described by Hambleton et al. (11).

Preparation of radiolabeled antigens. Primary CEF suspensions were prepared as described by Belton and Garriock (5). CEF monolayers were inoculated with the desired virus isolate at a multiplicity of infection of 100 and were incubated on a rocking platform at 35°C. After 1 h, the inoculum was removed, and fresh 199 medium, buffered to pH 7.4 with 50 mM HEPES (N-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid), was added. The cultures were incubated for 20 h at 35°C, and the medium was replaced with Earles balanced salts solution, buffered to pH 7.4 with 50 mM HEPES. After 2 h, the medium was removed, and fresh buffered Earles salts solution containing [^{35}S]methionine (50 μCi/ml, >600 mCi/mmol) was added. At the end of a further 2-h incubation period, the cells were drained, and 1 ml of buffer (0.15 M NaCl, 10 mM Tris-hydrochloride [pH 7.4], 1 mM EDTA, 0.01% [wt/vol] sodium azide, 1% Nonidet P-40, 500 U of Aprotinin per ml, 0.2 mg of PMSF [phenylmethylsulfonyl fluoride] per ml) was added. The cells were frozen at −20°C.

Immune precipitation. The serum samples were analyzed by RIP with 200 μl of clarified lysate and 2 μl of the required test serum as described previously (28).

Virus assay. Quantitative assay of the virus content of challenge suspensions, serum samples, and homogenized tissue samples from infected animals was performed by plaque assay in CEF in agar suspension (6). Neutralization titers of serum samples from infected animals were determined by a plaque reduction test with a standardized virus suspension incubated for 2 h at 37°C with dilutions of test serum. The mixture was then assayed as before, and the neutralization titer was expressed as that dilution of serum producing a 50% reduction in plaque counts.

Polyacrylamide gel electrophoresis. Samples were run for 2 h at 250 V on 15% discontinuous sodium dodecyl sulfate gels and denaturing conditions as described previously (27).

Preparation of reference sera. Hyperimmune sera were raised in 4- to 5-kg rabbits as follows. Each animal was inoculated intramuscularly with 2×10^8 PFU of virus in 1 ml of phosphate-buffered saline mixed with an equal volume of Freund incomplete adjuvant. Animals were boosted 7 days later with a further 2×10^8 PFU of virus without adjuvant and were bled 10 days later. All animals were checked for virus-specific antibodies before inoculation; all had neutralization titers of <8.

Reagents. Aprotinin and PMSF were obtained from Sigma London Chemical Co., Poole, United Kingdom. L-[^{35}S]methionine and ^{14}C-methylated protein molecu-

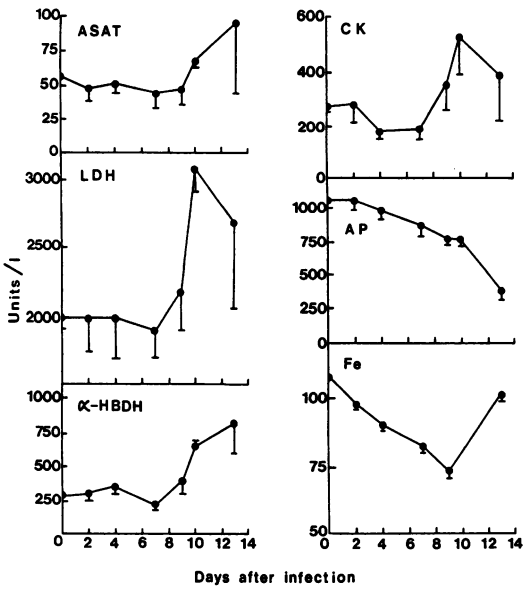

FIG. 1. Changes in some serum enzymes of five rhesus monkeys infected by i.v. injection with TTE virus (3×10^8 to 5×10^8 PFU). Serum samples were obtained before and at intervals after infection. Symbols show the mean values for several animals; bars indicate the standard errors of the mean.

lar weight markers were obtained from Amersham International, Amersham, United Kingdom.

RESULTS

Intraperitoneal infection. No clinical or biochemical responses were detected in a rhesus monkey infected by the intraperitoneal route. Virus was not detected in blood or CSF up to 2 weeks postinfection, nor was there any detectable antibody in either body fluid.

Infection by the i.n. route. (i) Clinical findings. In 6 of 10 monkeys infected i.n., there was sudden onset of clinical neurological signs between days 10 and 15. These consisted of tremors of the arms, neck twisting, incoordination, posterior paresis, and, occasionally, convulsions. This progressed to coma and death 12 to 24 h after the first onset. At no time did the affected animals have pyrexia or subnormal temperatures. Of the remaining animals, two were sacrificed early on, and two remained asymptomatic and survived.

(ii) Clinical chemistry: blood components. Changes in the activities of some serum enzymes were apparent from about 10 days after infection (Fig. 1). ASAT activity increased from day 10 (Fig. 1), whereas alanine aminotransferase activity was significantly raised in only one animal (preinfection value, 17.5 U/liter; postinfection values, 35.4 and 50.5 U/liter on days 9 and 13, respectively). Dehydrogenase and CK activities were similarly elevated 10 days postinfection (Fig. 1). In contrast, AP activity decreased progressively in all animals (Fig. 1) to about 40% of the preinfection level 13 days postinfection. Serum iron levels decreased progressively in all animals during the first 9 days after infection; thereafter, the values returned to more normal levels (Fig. 1). No changes in any other whole blood or serum components were detected.

(iii) Clinical chemistry: CSF components. The activities of ASAT and LDH were both elevated by 300% 9 days postinfection (preinfection values ± standard errors of the mean, 15.9 ± 2.9 and 158 ± 7.1 U/liter, respectively); α-HBDH activity was elevated in one animal 9 to 10 days postinfection by about three times the preinfection value of 89.7 U/liter. At this time, protein levels were also elevated three- to fourfold compared with preinfection levels of 270 ± 62 mg/liter. No changes were observed in the activities of alanine aminotransferase, CK, and AP (normal values were 12.3 ± 2.6, 6.5 ± 1.8, and 6.2 ± 2.4 U/liter, respectively). Leukocyte numbers were normally low (10^4/cm^3), and no increases were observed after infection.

(iv) Histopathology. Lesions were not found in the CNS of monkeys killed during the first 10 days of infection. In animals with inapparent infection (i.e., no clinical signs) killed up to 28 days postinfection, there were only occasional perivascular cuffs in the cerebral cortex and cerebellum, and these were small and involuting. Lesions in monkeys dying or killed in extremis between days 12 and 15 after i.n. infection were extensive and severe. They involved principally the cerebellum, posterior brain stem, and cervical spinal cord, but milder lesions were also present in the cerebral cortex and midbrain. Changes included localized meningitis, perivascular lymphocytic cuffs, diffuse and focal microgliosis, and neurone degeneration. The cerebellum was particularly severely affected, and there was widespread degeneration and loss of Purkinje cells. In monkeys surviving a few days longer, there was also proliferation of Bergmann glia. Perivascular cuffs were most numerous in the cerebellar peduncles, pons, and medulla; in affected blood vessels, there was endothelial damage. In the cervical cord, there were also large areas of microgliosis and necrosis of motor neurones.

(v) Appearance of virus in tissues and body fluids. Virus (10^2 to 5×10^2 PFU/ml) appeared transiently in the blood of 6 out of 10 infected animals, including those without clinical signs, 7 to 9 days postinfection. Concomitant with disappearance from the blood, virus was detected in the CSF of three of five infected animals tested about 11 days postinfection; this correlated with

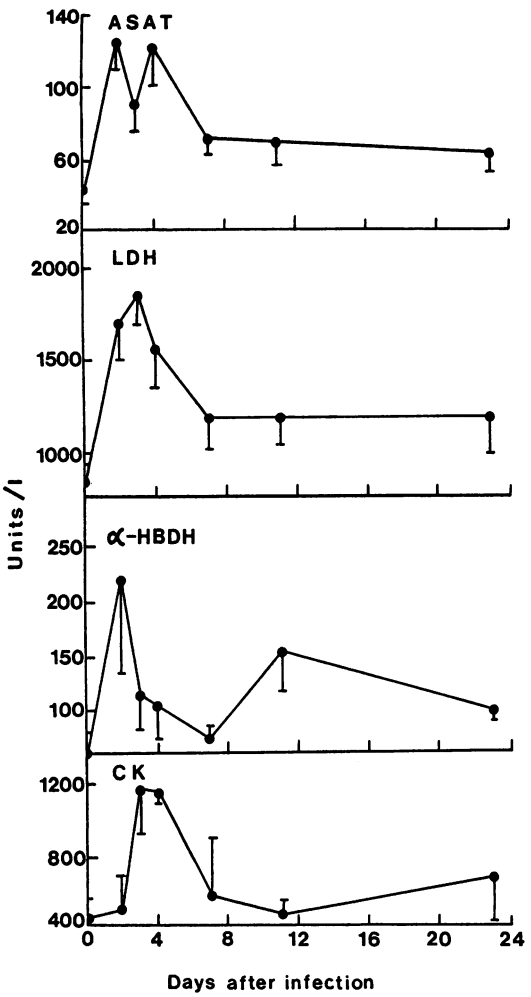

FIG. 2. Changes in some serum components of 10 rhesus monkeys infected by i.n. instillation with TTE virus (3×10^8 to 5×10^8 PFU). Serum samples were obtained before and at intervals after infection. Symbols show the mean values for several animals; bars indicate the value of the standard errors of the mean.

the sudden onset of clinical neurological signs between 10 and 15 days postinfection in the 50% of monkeys so affected. Virus titers were low ($<10^2$ PFU/ml) in those animals that did not exhibit clinical symptoms but were considerably higher (up to 10^5 PFU/ml) in those that did. In the latter group of animals, virus was recovered from all regions of the brain taken for examination, with titers often $>10^6$ PFU/g of tissue. Those animals that survived the experimental period showed no evidence of infective virus in any regions of the brain, even when virus had been isolated from the CSF.

(vi) Antibody response to viral antigens. Those animals infected by the i.n. route that demonstrated clinical symptoms did not develop detectable levels of neutralizing antibody at any stage of the disease. In addition, no virus-specific polypeptides were detected by RIP in serum from any animal up to 21 days postinfection. However, low levels of neutralizing antibody (titer, 1/100) were detected after 14 days in serum from only one of the two asymptomatic animals that survived.

Infection by the i.v. route. (i) Clinical findings. The five animals infected by this route showed no clinical symptoms.

(ii) Clinical chemistry: blood components. Changes in blood chemistry resembled those following i.n. instillation but generally were apparent sooner after infection. Thus (Fig. 2), ASAT, LDH, α-HBDH, and CK activities were all elevated 2 to 4 days postinfection. Thereafter, however, the activities of these enzymes decreased to levels approaching those observed before infection. No other changes in blood components were observed.

(iii) Clinical chemistry: CSF components. Postinfection samples were obtained from two monkeys only. In both of these, LDH activity was elevated 11 days postinfection by two- to threefold (preinfection value, 74 ± 18 U/liter). No other changes were observed.

(iv) Histopathology. Two of the animals were killed 14 weeks after infection, and histological examination of the CNS was carried out. No residual inflammatory lesions were present, nor was there evidence of degenerative changes in the CNS.

(v) Presence of virus in body fluid. Low levels ($<5 \times 10^2$ PFU/ml) of virus were recovered from the blood for the first two days after infection, but thereafter no infectious virus could be detected in blood or CSF throughout the course of the experiments.

(vi) Antibody response to virus-specific antigen. No virus-neutralizing activity was found in any serum samples taken during the first week of infection. However, from day 11 onwards, high titers of neutralizing antibody (1/100 to 1/600) developed in all animals and were maintained for at least 3 weeks. When antiserum samples from those animals were examined by RIP, no activity against any viral polypeptides was seen until day 7, but by day 11, serum samples from all animals contained antibodies which precipitated three discrete, virus-specific polypeptides (molecular weights, 62,000 [62K], 53K, and 21K; Fig. 3), and an additional polypeptide (37K) was precipitated by serum from day 23. Antibodies against these polypeptides persisted in the blood of all animals for at least 3 weeks after infection with the virus. However, although high titers of virus-specific antibody were detected in the blood of all animals both by neutralization and

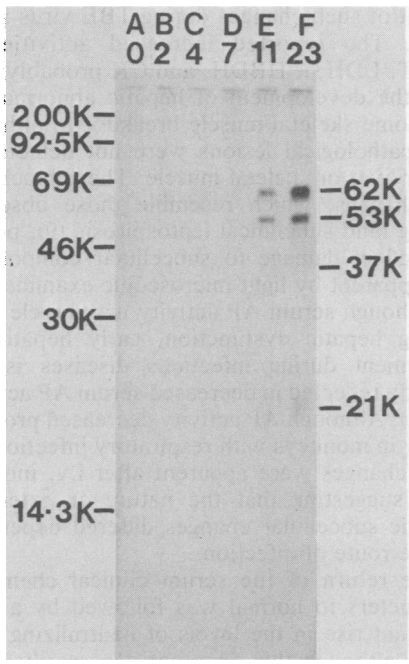

FIG. 3. RIP of proteins from TTE virus-infected CEF cells, precipitated by serum samples from monkeys experimentally infected by the i.v. route. Serum samples were taken before infection (A) and at various times after infection (B through F). The positions of molecular weight markers are shown on the left, and virus-specific polypeptides are designated by their molecular weights on the right.

by RIP, no antibody was found by either method in the CSF.

Inoculation of animals with commercial TBE vaccine (FSME vaccine). Experiments were conducted to demonstrate the efficacy of the FSME vaccine against a wild-type virus isolate and to search for any side effects that might accompany vaccination. This isolate (TTE virus) was chosen because it has a high degree of cross-neutralization with the CTE virus isolate, from which the vaccine was derived, and also antisera raised against either virus can precipitate the same polypeptides that the homologous serum can (see below). Two rhesus monkeys were used, one seronegative animal that had never been exposed to TTE virus and one animal convalescing from an i.v. infection as described above.

Throughout the course of vaccination and on subsequent challenge with live virus, no adverse clinical effects were apparent in either animal. No immune enhancement phenomena were observed, nor were any of the histopathological or clinical chemical changes associated with the i.n. virus infection detected.

Within 1 month of inoculation with a single dose of TBE virus vaccine, serum from the previously unexposed animal contained specific antibody which was capable of precipitating all of the four polypeptides (62K, 53K, 37K, and 21K) seen in immune precipitates with sera from an i.v. infection (Fig. 4) and showed a good neutralizing titer against TTE virus (1/70); these features persisted for at least 3 months. Repeat vaccination did not alter these immune properties of the serum. Similarly, the immune response of the animal which had previously recovered from a TTE virus infection showed no change in the pattern of polypeptides precipitated by serum (Fig. 4), although virus neutralization titers were boosted after subsequent vaccination or challenge with infectious virus.

Antigen relationships between CTE and TTE viruses. Hyperimmune serum raised against CTE virus is capable of neutralizing TTE virus to high titers (Table 1). Similarly, hyperimmune TTE virus-specific serum cross-neutralizes with CTE virus. To investigate the molecular basis of this cross-neutralization, hyperimmune antise-

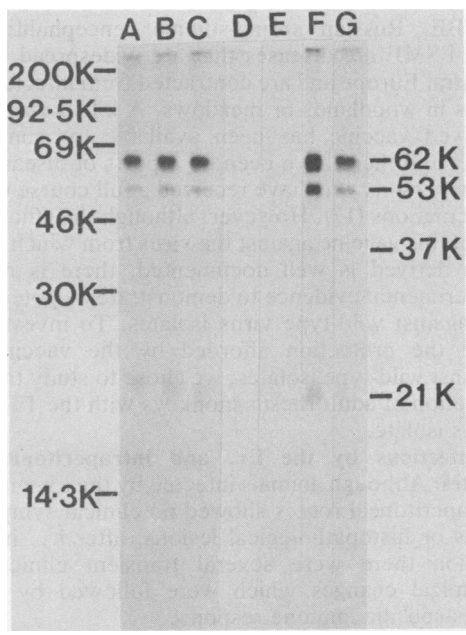

FIG. 4. RIP of proteins from TTE virus-infected CEF cells, precipitated by serum samples taken from monkeys before and after vaccination. (A through C) Late convalescent-phase serum from an animal taken 4 months after an experimental i.v. infection (A), immediately before vaccination (B), and 1 month after vaccination (C). (D through G) Serum from an animal which failed to show any signs of infection, taken after i.v. inoculation with TTE virus (D), immediately before vaccination (E), and 1 month (F) and 2 months (G) after vaccination. Molecular weight markers and virus-specific polypeptides are marked as in Fig. 3.

TABLE 1. Neutralization of TBE virus by hyperimmune rabbit serum

Antiserum against:	Neutralization titer			
	Dilution with[a]:		%[b]	
	TTE virus	CTE virus	TTE virus	CTE virus
TTE virus	20	1,600	100	358.3
CTE virus	3,200	12,800	23.48	100

[a] Dilution of serum that neutralized 50% of the plaques in a standard infectivity assay.

[b] Percentage of the titer obtained with the homologous antigen.

rum to CTE virus was examined by RIP. Antigenic cross-reactions can be demonstrated with two virus-specific polypeptides (Fig. 5). At present, it cannot be formally demonstrated which polypeptide contains sites that elicit neutralizing antibody, but as the 62K polypeptide is found on the viral envelope and a similar protein from CTE virus can be shown to elicit neutralizing antibody in vitro (16), it is an obvious candidate.

DISCUSSION

TBE, Russian spring-summer encephalitis, and FSME are diseases that are widespread in Central Europe and are contracted from infected ticks in woodlands or meadows. A killed virus-derived vaccine has been available for some time, and there have been no reports of disease from persons who have received a full course of vaccinations (17). However, although the efficacy of this vaccine against the virus from which it was derived is well documented, there is no experimental evidence to demonstrate its potency against wild-type virus isolates. To investigate the protection afforded by the vaccine against wild-type isolates, we chose to study the infection of adult rhesus monkeys with the TTE virus isolate.

Infections by the i.v. and intraperitoneal routes. Although animals infected by the i.v. and intraperitoneal routes showed no clinical symptoms or histopathological lesions, after i.v. infection there were several transient clinical chemical changes which were followed by a virus-specific immune response.

The observed changes in serum enzyme activities were similar in both i.n. and i.v. infections. However, in i.v. infected animals, the indications of systemic disease were transiently evident 2 to 4 days after infection, whereas after respiratory infection this phase was not apparent until 10 days, i.e., at about the time of onset of the histopathological lesions in the brain.

Although changes in blood chemistry often occur in acute infections, there appears to be no published information on the nature and the extent of such changes during TBE virus infections. The reported increased activities of ASAT, LDH, α-HBDH, and CK probably indicate the development of hepatic abnormalities and some skeletal muscle breakdown, although histopathological lesions were not detected in either liver or skeletal muscle. These biochemical changes, which resemble those observed during mild subclinical leptospirosis (9), probably reflect damage to subcellular components not apparent by light microscopic examination.

Although serum AP activity may be elevated during hepatic dysfunction, early hepatic involvement during infectious diseases is frequently reflected in decreased serum AP activity (8, 11). Although AP activity decreased progressively in monkeys with respiratory infection, no such changes were apparent after i.v. inoculation, suggesting that the nature or extent of hepatic subcellular changes differed depending on the route of infection.

The return of the serum clinical chemistry parameters to normal was followed by a concomitant rise in the levels of neutralizing antibody, although this does not necessarily mean that the mounting of a successful immune response prevented significant tissue damage.

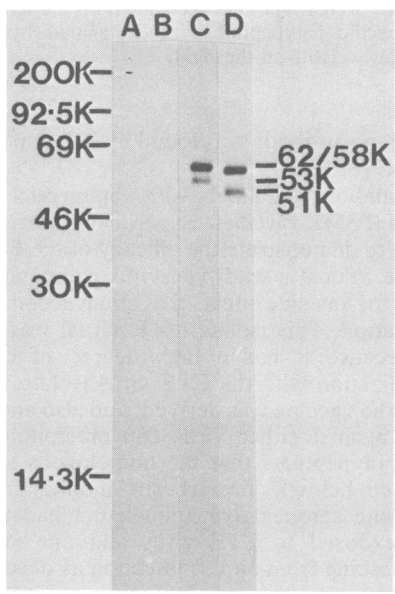

FIG. 5. Antigenic relationships between CTE and TTE viruses, shown by RIP of radiolabeled antigens from infected cells. (A) TTE antigens by normal rabbit serum; (B) CTE antigens by normal rabbit serum; (C) TTE antigens by hyperimmune rabbit serum raised against CTE virus; (D) CTE antigens by hyperimmune rabbit serum raised against CTE virus. Molecular weight markers and virus-specific polypeptides are marked as in Fig. 3.

Antiserum specimens from TTE virus infected animals, examined by RIP, were shown to precipitate four virus-specific polypeptides. At present, it cannot be formally demonstrated which polypeptide contains sites which elicit neutralizing antibody, but as the 62K polypeptide is the only envelope component of the virus detected so far and a similar protein from CTE virus which cross-reacts with this polypeptide has been shown to elicit neutralizing antibody (16), the presence of antibodies against this polypeptide is thought to account for the neutralizing activity found in the serum samples of these animals. The 21K polypeptide is thought to correspond to the major protein found in virion cores, as they have similar molecular weights, but the identities of the 53K and 37K species are not known at present.

Infection by the i.n. route. This experiment followed the course of the human disease much more closely; clinical disease occurred in 6 of 10 infected animals and was usually fatal, but pyrexia was absent. In humans, fever is invariably present and mortality is very low, although there is a high prevalence of chronic neurological impairment (2). Our findings are similar to those of Morris et al. (18) with Russian spring-summer encephalitis virus in monkeys. These workers also found that a high proportion of animals did not develop viremia or clinical disease, and this aspect seems to mimic the course of the human disease, in which some people have obviously experienced infection with the virus (as they have virus-specific antibodies in their blood) but have never reported any signs of disease (24). Pathological examination of those monkeys that survived the inapparent infection showed no evidence of degenerative changes in the CNS such as those described by Zlotnik et al. (31).

The decreased serum iron levels observed in i.n. infected monkeys were rather less pronounced than decreases induced by other viral or bacterial infections of humans and animals (4, 8, 11, 29). Decreased serum iron levels are generally characteristic of the early stages of acute pyrexic infections (25, 29), but elevated body temperatures were not detected in this study. Infection-induced pyrexia in monkeys may lead to an increased serum phenylalanine-to-tyrosine ratio, but no such change was observed, and so it is unlikely that there was an undetected transient pyrexia.

Disease-related changes in CSF components are not well documented, although changes in amino acids (21) and elevated dehydrogenase and transaminase activities (19, 30) occur in human meningitis. Dehydrogenase activity in the CSF was elevated in those i.n. infected animals which subsequently developed neurological signs and had histopathological lesions; this might indicate that viral involvement of the CNS was occurring at this time.

The distribution of the histological lesions in the brain and the clinical chemical results suggests that virus may have spread from the site of the infection to the brain via the blood rather than by direct spread from the nasal mucosa. This hypothesis is supported by the findings of Godman and Koprowski (7) who demonstrated with another flavivirus (West Nile virus) that the macrophage population of mice appears to be important in the spread of the disease. Since TBE is naturally acquired by tick bites, it would have been expected that direct i.v. infection would cause disease, but it did not. The reasons for this apparent anomaly are obscure.

Unlike the animals infected i.v., those infected by the i.n. route failed to mount a significant immune response. As it has been known for some time that these viruses replicate in macrophages (26) and that this activity is responsible for the phenomenon of immune enhancement (22), it is possible that when inoculated by the i.n. route, the virus can become established before the immune defenses can operate and may even replicate in and destroy the cells of the immune system.

Vaccination in adult monkeys. (i) Side effects. In neither a seronegative nor a seropositive animal were any adverse clinical symptoms or changes in clinical chemistry or histopathology observed throughout the course of vaccination or when the animals were challenged with virus. Similarly, there was no evidence of immune enhancement when the seropositive animal was vaccinated or on repeated vaccination or virus challenge of the seronegative animal.

(ii) Antigenic relationship between CTE and TTE viruses and the ability of the CTE virus-derived vaccine to protect animals from challenge with TTE virus. Hyperimmune serum raised against CTE virus is capable of neutralizing TTE virus to high titers. Similarly, hyperimmune serum against TTE virus neutralizes CTE virus. The very high titers against CTE virus in the anti-TTE virus serum could represent the presence of a particular population of TTE virus-specific antibodies, which have a much closer fit with CTE virus antigens than with the original TTE virus antigens against which they were raised. Similarly, antigenic cross-reactions between two virus polypeptides of CTE and TTE viruses were demonstrated by RIP. It is known that the V3 or envelope polypeptide elicits neutralizing and hemagglutination inhibition antibody (16) and that this protein from different virus isolates has a high degree of homogeneity (15). Therefore, the cross-neutralization between CTE and TTE viruses may be a function of antigenic similarity between their respective

V3 polypeptides (i.e., the 62K polypeptide of TTE virus and the 58K polypeptide of CTE virus).

Although the 21K polypeptide is a major component of the virion core, no antibodies against it were seen in hyperimmune rabbit sera. However, as these sera were raised against purified, formalin-fixed virions, probably only intact virus particles were presented to the immune system, whereas in a natural infection, infected cells and cell debris would be present.

The FSME vaccine elicited an immune response in a seronegative animal within 1 month of vaccination. Serum from this animal precipitated all of the four virus-specific polypeptides seen in immune precipitates from convalescent-phase serum samples, and all showed high virus-neutralizing titers. Further doses of vaccine or virus challenges in either the previously seronegative or seropositive animal did not alter the pattern of polypeptides precipitated, although rises in antibody titers were observed.

Although antibodies to both the envelope and the putative core polypeptide were present in serum samples from both the seropositive and the seronegative animals, neither elicited antibody against the V1 polypeptide, i.e., the protein which is thought to constitute the inner shell of the virus particle. This antibody was absent, even though both animals were given an extensive course of treatment with vaccine and were challenged with live virus. A similar situation has been shown to occur with measles virus. Antibody against the matrix protein is seldom detected in either convalescent-phase serum samples or in patients in a hyperimmune state (14, 28). However, significant levels of antibody to this protein can routinely be demonstrated in patients who have had an adverse reaction to killed measles vaccine (14). Therefore, as none of the animals vaccinated in the experiments described here produced antibody against a similar protein from TTE (V1), there is no evidence of any similar adverse immune response to the vaccine, a conclusion which is in agreement with the clinical findings, the histopathology, and the clinical chemistry.

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