

Experimental biology and pathogenesis of Junin virus infection in animals and man*

M. C. WEISSENBACHER, L. B. DE GUERRERO, & M. C. BOXACA

A fatal disease resembling Argentine haemorrhagic fever of man has been produced in guinea-pigs and mice by inoculation with Junin virus. Infected guinea-pigs show macroscopic and microscopic haemorrhagic lesions, marked bone marrow changes, decreased leukocytes and platelets in the peripheral blood, and impairment of immunological response. This response permits differentiation between pathogenic (XJ) and attenuated (XJ Cl₃) strains. Guinea-pigs inoculated with the XJ Cl₃ strain develop an inapparent infection accompanied by slight haematological changes, the appearance of antibody, and protection against challenge with the pathogenic strain. The attenuated strain has been used successfully as an immunizing antigen in 636 human volunteers. Guinea-pigs infected with Tacaribe virus show cross-protection against Junin virus, with the presence of heterologous neutralizing antibodies. Suckling mice infected with Junin virus develop a typical viral encephalitis; the pathogenicity of the virus decreases with increasing age of the mice. Experiments with thymectomized mice and with mice treated with antithymocyte serum suggest that the pathogenicity of Junin virus in this host is related to the integrity of the thymus-dependent immune system. There is evidence that humoral antibodies do not play any role in the development of the encephalitic lesions but rather protect mice against Junin virus infection. A recent serological survey among laboratory workers and inhabitants of the endemic area has demonstrated the presence of inapparent infection with Junin virus.

INTRODUCTION

In the early 1950s a new disease, called Argentine haemorrhagic fever, appeared in the north-west of the province of Buenos Aires, Argentina; by 1958 the etiological agent had been isolated by Parodi et al. (1) and its identity was confirmed later by Pirotsky et al. (2). It has been named Junin virus. Much has been learned about the disease, including its geographic distribution and the characteristics of the nucleic acid of the virus, but much remains to be done. The first studies were concerned with clinical manifestations in man and with some physicochemical properties, such as the stability of infectivity at different pH levels and on exposure to heat, lipid solvents, radiation, neutral red, and other agents. They were followed by research on: virus infectivity *in vitro* and *in vivo*; the size, morphology, and replication of the virus and its relationship to other

viruses; the effect of inhibitors; the natural and experimental host range; tissue culture susceptibility; persistent infection of cells; interferon production; pathogenicity for man; epidemiology; transmission; diagnostic techniques; passive and active immunization; mechanisms of pathogenesis for different hosts; etc. The results of these studies have been discussed in 3 reviews (3, 4, 5). Advances that further clarify concepts on the pathogenesis of Argentine haemorrhagic fever (AHF) in natural and experimental hosts will be dealt with in this paper.

ARGENTINE HAEMORRHAGIC FEVER IN GUINEA-PIGS

Junin virus produces in guinea-pigs an experimental haemorrhagic fever resembling in some aspects the disease it causes in man. Many studies have been carried out in this host because of its high susceptibility to the virus, the clear postmortem signs, and its sensitivity to differences in pathogenicity between various strains of Junin virus. Until a better system

* From the Cátedra de Microbiología y Parasitología, Facultad de Medicina, Paraguay 2155, Piso No XI, Buenos Aires, Argentina.

is developed, the guinea-pig has been chosen as "marker" to differentiate between pathogenic (XJ) and attenuated strains of Junin virus (XJ Cl₃).

Infection with the prototype pathogenic XJ strain

Subcutaneous, intramuscular, intraperitoneal, intranasal, intracerebral, and oral routes are possible routes of infection; the least successful is the oral one. Overt signs of disease, such as fever and loss of weight, appear on the 5th and 8th day after infection, respectively. The animals invariably die, although the time of death depends upon the infecting dose; with an inoculation of 100 LD₅₀ or more, death occurs in hypothermic shock between 11 and 15 days after infection. Boxaca et al. showed that persistent viraemia is present until death. Virus is also localized in the lymph nodes, bone marrow, liver, lungs, heart, spleen, and adrenals; virus is excreted in the urine but not in the stools. The platelet count showed a dramatic decrease and the number of megakaryocytes in the bone marrow was less than normal. A marked leukopenia was also observed; it began at the expense of the lymphocytes and ended with an almost complete disappearance of neutrophils in the final stages (6). Alterations in the coagulation factors led to increasing deficiencies in clot retraction and a lengthening of the coagulation time. Electrophoretic analysis of infected serum showed that total proteins, albumin, and immunoglobulins, decreased while β -globulins increased. The serum complement level was also diminished. Increases in plasma lipids, cathepsin levels, acid protease, and acid and alkaline phosphatase activity have been reported.

Pathological changes appear late in the course of the disease, the most noticeable being the microscopic and macroscopic haemorrhagic lesions: petechiae, localized ecchymosis or haemorrhages in subcutaneous tissue, the large and small bowel, adrenal glands, lymph nodes, stomach walls, lung, peritoneum, or thoracic cavity. The pathology of the bone marrow ranges from moderate necrosis on the 9th day after infection through severe necrotic lesions to massive necrosis on the 14th day. There is a focal lymphoid necrosis with abundant nuclei detritus, increased phagocytic activity, and depletion of the lymphocyte population in the spleen, lymph nodes, and lymphoid tissue of the bowel and lungs. Focal necrosis with secondary bacterial infection is found in the gastrointestinal tract (Elsner, B., unpublished observations, 1975).

Ultrastructural studies of the bone marrow and

peripheral leukocytes of guinea-pigs on the 12th day after infection showed a progressive destruction (up to 85%) of the cells. Viral particles morphologically identical with those described as arenavirus were found in the platelet demarcation channels from 10 to 14 days after infection (Carballal et al., personal communication, 1975).

Immunological competence was also impaired; Parodi et al. (7, 8) demonstrated a decrease in primary and secondary responses to human and sheep red cells. Nota et al. (9, 10) showed a decreased reaction in the Arthus phenomenon and in the active and passive Jones-Mote type hypersensitivity. It was suggested that a direct action of the virus on the immunologically competent cells was responsible for this effect. Nejamkis et al. (11) demonstrated that phagocytosis of peritoneal macrophages remained unaltered in animals infected with the XJ strain of Junin virus. Weissenbacher et al. (12) showed that homologous immune serum given 24 h before or 5 days after infection with Junin virus protected around 55% of the guinea-pigs, while immune serum given 9 days after the infection had no effect. Surviving animals were challenged 60 days later with a second dose of the virus. Those receiving the immune serum 24 h before the infection died with the typical AHF signs, while those receiving the treatment after 5 days resisted the challenge. This leads to the conclusion that immune serum given 24 h before inoculation with Junin virus prevents the spread of the virus, and that the animals do not develop an active immunity. In the second case, when serum was given 5 days after the infection, the animals with active viral multiplication survived, developing their own antibodies which protected them 60 days later.

Infection with XJ Cl₃ attenuated strain

By cloning a high passage of the prototype strain in a continuous line of rabbit kidney cells, the attenuated strain XJ Cl₃ was obtained (13). Guinea-pigs inoculated intramuscularly with Junin virus XJ Cl₃ strain developed an inapparent infection detectable only by slight haematological alterations and the appearance of antibody. Guerrero et al. (13) observed that guinea-pigs did not show any clinical changes during the 30 days following inoculation. Virus was detected only in the spleen and lymph nodes between 8 and 15 days after inoculation. In contrast to what happens in guinea-pigs infected with the pathogenic XJ strain, the total white cell, lymphocyte and polymorphonuclear cell counts re-

mained within normal values (6), only the platelets showing a brief but significant decrease around 11 days. Frigerio et al. (14) found that the immunological function is not altered: there is no decrease in the levels of circulating antibody or in the number of rosette-forming cells with sheep erythrocytes when compared with noninoculated animals. Complement-fixing antibodies against Junin virus appeared 10 to 20 days after inoculation, lasting for 60 days and decreasing afterwards; neutralizing antibodies developed somewhat later, about 20 days after inoculation, but persisted in high titres for more than 2 months. Guinea-pig infection with XJ Cl₃ elicited an active immunity that protected the animals from challenge with the pathogenic XJ strain (13). Recent studies carried out on a larger number of animals showed repeatedly that a few guinea-pigs (less than 5%) died between 17 and 30 days after inoculation without signs of AHF. Several attempts to isolate Junin virus from their organs have so far been unsuccessful.

Cross-protection between arenaviruses

In 1964, Parodi & Coto (15) reported that guinea-pigs inoculated with live Tacaribe virus (which is not pathogenic for guinea-pigs) were resistant to challenge with high doses of Junin virus. Tauraso & Shelokov (16) confirmed these results and found that sera from the guinea-pigs immunized with Tacaribe virus contained high levels of homologous neutralizing and complement-fixing antibodies. Levels of heterologous complement-fixing antibodies were consistently lower, but a progressive increase in titre was demonstrated from the 14th to the 49th day; by this time, negligible amounts of neutralizing antibodies against Junin virus were detected. Coto et al. (17) showed that partial resistance did not appear before the 7th day and complete immunity only after 2 weeks. Infective virus was isolated, but not consistently, from the lymph nodes. In these papers, the authors raised the question whether this protective effect was due to a true immunization or to interference between the two viruses.

In a recent cross-protection study (18) between Junin and other arenaviruses, the total protection conferred by Tacaribe against Junin virus was confirmed. It was also shown that high doses of Amapari virus elicited partial protection. Furthermore, in a small number of animals, complete protection against Junin virus was seen in guinea-pigs previously infected with Machupo virus. No protective effect of a previous inoculation with Pichinde or

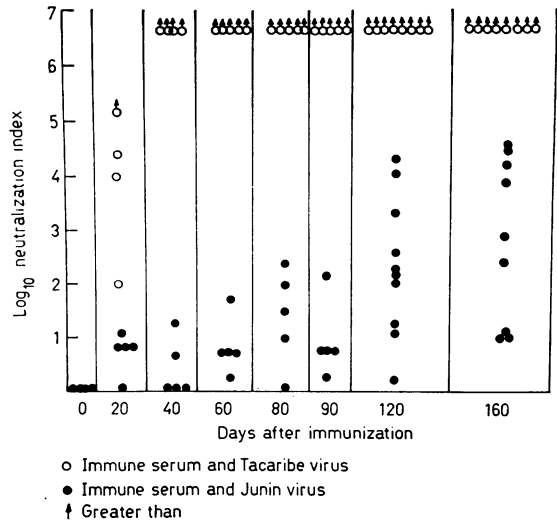


Fig. 1. Neutralization test in sera from guinea-pigs immunized with Tacaribe virus.

Tamiami viruses could be demonstrated. Weissenbacher et al. suggested that the resistance conferred by Tacaribe against Junin could be due to the presence of increasing titres of heterologous neutralizing antibodies found in the sera of guinea-pigs from 20 days to 5 months after immunization with 5 doses of Tacaribe virus, as can be seen in Fig. 1.

ARGENTINE HAEMORRHAGIC FEVER IN MICE

Infection of mice with Junin virus produced the typical picture of encephalitis, similar to that described for many other viruses. Sick mice showed tremors, lateralization of walking, and spontaneous or induced convulsions, followed frequently but not regularly by flaccid paralysis of the hind legs. Those signs appeared 7–12 days after inoculation and led to death around 5 days later. The experimental haemorrhagic fever in mice, as described above, appeared to be independent of the strain of Junin virus used (Boxaca et al. tested 66 strains, including the attenuated XJ Cl₃); it was also independent of the strain of mice (Rockland, Balb C, CFW, C₃H, CF₁) and of the route of inoculation (intracerebral, intraperitoneal, or subcutaneous), but dependent on host age. The maximum death rate (95–100%) was found when mice were infected between 1 and 10 days of age. Older mice showed a drastic reduction

in mortality accompanied by changes in the clinical picture of the disease: death was generally sudden and frequently occurred without any overt sign. When mice 48 h old received 10^8 LD₅₀, a 95–100% mortality was observed between 9 and 20 days after inoculation. Surviving animals remained healthy without any sequelae, but with high circulating antibody titres, indicating that infection was 100%. Virus was isolated from the brain 48 h after inoculation, followed by a progressive dissemination with increasing virus titres that coincided with the onset of clinical disease (19). Total leucocyte and lymphocyte counts showed a tendency to drop 10 days after inoculation and this leuko-lymphopenia persisted until death of the animal.

Studies performed in infected mice up to 30 days old showed that virus multiplied in the brain, reaching almost the same titres at the 5th day after inoculation regardless of the age of the host at the time of inoculation; but in older groups the virus titre dropped to undetectable levels by 10 days after inoculation, coinciding with a decrease in mortality.

Interferon response was generally very poor and the role of interferon was difficult to ascertain because of its presence in the brain, blood, and organs of mice of all age groups, irrespective of the mortality observed (19). However, treatment with an interferon inducer, polyinosinic polycytidylic acid, produced a significantly longer survival time as compared with controls, without change in the overall death rate.

Circulating antibody response was detected in surviving animals of all age groups. Complement-fixing antibodies started to appear 15 days after inoculation and neutralizing antibodies not before 20 days after inoculation, persisting for at least 200 days (19). Recently, after blind passages, it has been possible to demonstrate the persistence of virus in the brain and kidneys of surviving animals for at least 200 days after inoculation. On the other hand, virus but not circulating antibodies could be detected in litters of the surviving mice, suggesting a vertical transmission of the infection (20).

The principal histopathological lesions found in the brain of infected newborn mice sacrificed at 12–15 days after infection were: choroiditis, glial hyperplasia, vasculitis, and perivasculitis, characterized by infiltration of mononuclear inflammatory lymphocyte-type cells. Furthermore, regressive changes were observed in the neuronal cells and astrocytes, accompanied by a discrete microglial reaction (21). Using an immunofluorescence tech-

nique, viral antigen was found in the cortical neural cytoplasm and in the wall of the small vessels of the meninges.

Ultrastructural studies carried out by Lascano & Berria (22) in mouse brain showed that neurons and astrocytes released virus particles by a budding mechanism. In the neurons, viral multiplication took place in the perikarion as well as in the cytoplasmic processes, including areas near the synapses. Viral particles were observed emerging from pericapillary neurons and astrocytes.

Several authors have suggested that the lesions observed in mouse brain are secondary to the destruction of infected cells acting as targets for sensitized lymphocytes. The role of immunological reactions of the cellular type and the role of circulating antibodies in the course of the acute fatal encephalitis produced in the experimental Argentine haemorrhagic fever of mice, have been extensively studied. Delayed hypersensitivity-type phenomena were studied by means of thymectomy, antithymocyte serum, and antimetabolites. Thymectomy, which mainly impairs immunological phenomena of the cell-mediated type was able to protect several strains of infected newborn mice (Rockland, CFW, and Balb C), preventing the development of neurological symptomatology and death, while virus multiplied in brain, reaching the same titres as in non-thymectomized mice. Survival of the thymectomized mice permitted the demonstration of the persistence of virus in brain, although the amount decreased continuously until 7 weeks after inoculation. Histological examination of the brain from thymectomized, infected mice sacrificed at 10–13 days showed only a moderate hyperaemia without inflammatory signs, in contrast to control mice which showed the histopathological picture of AHF. The effect of thymectomy can be counteracted by the inoculation of immune mouse spleen cells (23). Normal suckling mice inoculated with spleen cells of adult immune mice and infected with Junin virus died 3–4 days before infected controls (Nota et al., personal communication, 1975).

Similar protection to that described for Junin virus was observed in thymectomized mice infected with Machupo, Tacaribe, or Pichinde virus; however, thymectomized mice infected with Amapari virus showed the same percentage mortality as non-thymectomized animals, with similar clinical symptoms and histopathological changes (21). It was evident, therefore, that the intense host response of lymphocytic brain infiltration and the associated

tissue damage were related to the mortality of the disease.

All these studies strongly suggest that the pathogenicity of Junin virus in mice is related to the integrity of the thymus-dependent immune system; this view is supported by the fact that infected mice survived after treatment with antithymocytic serum, which is known to be a potent inhibitor of delayed type hypersensitivity. Animals treated with this immunosuppressor showed an increase in survival rate with no impairment of virus multiplication in the brain. In surviving mice, neutralizing and complement-fixing antibodies appeared after 40 days, coinciding with a decrease of virus titre in the brain. Histological examination of the brain showed that infected animals pretreated with antithymocytic serum had only minimal or no lesions in their brains (24). It was concluded that Junin virus encephalitis in suckling mice might have an immunological basis similar to that described in experimental allergic encephalitis and that immunosuppressive treatment allowed mice to overcome the period of susceptibility to Junin virus.

Infected suckling mice treated with cyclophosphamide showed a survival probability ranging from 25% to 54%; antibodies were found 60 days after inoculation and virus persisted in the brain and kidneys for longer periods (25). The mechanism of this protection is difficult to explain, since this immunosuppressor mainly impairs humoral antibodies.

It has been shown that treatment with bacterial endotoxin in suckling mice infected with Junin virus can either induce protection or accelerate the onset of the disease, resulting in earlier death (26). The importance of circulating antibodies in the course of the disease was studied in suckling mice by passive immunity naturally acquired from immune mothers or by inoculation of homologous hyperimmune serum. Partial protection against Junin virus was observed in the progeny of immune female mice as a result of the passive transfer of specific antibodies from mother to litter. Litters infected immediately after birth showed a lower survival rate (22%) than mice infected after 9 or 13 days of life (71% and 88% respectively); this agrees with the finding that maternal antibodies were not detectable in the circulating blood of the young in the first days after birth, but rose rapidly from the 8th to the 20th day of lactation. By exchanging litters at the time of birth between immunized and non-immunized mothers, a greater survival rate (22%) was obtained in mice allowed to suckle from their own immune

mother, while offspring of normal mice that suckled from immune mothers showed only an 8% survival. These findings suggest that protection against Junin virus infection was transferred from mother to young by placenta and milk, the latter being the most important mechanism of antibody transfer. Infected suckling mice could also be passively protected by specific immune serum administration (27).

Adult mice are resistant to Junin virus infection, but an 87% mortality was observed in adult mice 15–20 days after infection when they were treated with cyclophosphamide, this effect being attributed to the depression of humoral response mediated by this immunosuppressor (Giovanniello et al., personal communication, 1975). When considered together, these results suggest that humoral antibodies do not play any role in the development of the encephalitic lesions; on the contrary, they seem to protect mice infected with Junin virus.

Field studies carried out by Sabattini et al. (28) have shown that *Calomys musculus* is a natural host for Junin virus. This rodent exhibited a persistent viraemia and excreted virus by the oral and urinary routes. The authors were not able to detect manifest disease in the field mouse *Calomys* when it was captured in the area where AHF is endemic, in spite of the fact that it was possible to isolate virus from the animal. Experimental studies were performed in a laboratory colony of *Calomys* free of virus: animals of different ages infected intracerebrally with Junin virus developed viraemia during the first week and the virus was found in their organs for 9 months after infection; however, during this period, the mice did not show any of the signs of acute or chronic disease. All of them showed an increase in CF antibody titres except those infected at 3 days of age, which had a very low serological response. Recent studies have demonstrated (by virus isolation and serology) the presence of LCM virus infection in *Mus musculus* in a Junin virus endemic area.

ARGENTINE HAEMORRHAGIC FEVER IN MAN

Different types of infection with Junin virus in man are known and these have different manifestations:

- Natural infection in the endemic area (with or without clinical evidence of the disease).
- Infection acquired by laboratory accident (with or without clinical evidence of the disease).

Table 1. Neutralizing and complement-fixing antibodies in sera from laboratory personnel working with Junin virus

	Risk groups					Total
	High risk			Moderate risk	Low risk	
	With previous clinical disease	With previous vaccination	Without clinical disease or vaccination			
Number of sera tested	7	10	20	12	23	72
Number positive in CF test	0	0	0	0	0	0
Number positive in neutralization test	6	8	5	0	0	19
% positive in neutralization test	86	80	25	0	0	26

- Infection by volunteer inoculation of pathogenic strains of Junin virus (2 cases, both with typical signs of the disease).
- Infection by volunteer inoculation of an attenuated strain of Junin virus in the form of a vaccine (636 cases with inapparent infection or slight clinical manifestations).

The infection with typical signs of AHF in man is the subject of the paper by Maiztegui (29). Human cases of inapparent infection acquired in the endemic area, by a laboratory accident, or by inoculation of an attenuated strain of the virus are described below.

In our experience, human infection with Junin virus was always correlated with the occurrence of clinical disease and inapparent infection was never detected, either in the endemic area, or in the laboratory; however, recent serological surveys have demonstrated the presence of subclinical infection. Sera from 72 laboratory workers were studied for neutralizing and complement-fixing antibodies. Laboratory personnel were divided into 3 groups according to the risk of infection: high-risk group (those working with the virus), a moderate risk group (those working in the same laboratory area but not with Junin virus), and a low-risk group (those working in a different laboratory area of the same building in which Junin virus was being studied). As shown in Table 1, no neutralizing or complement-fixing antibodies were detected in the sera of people from the low-risk or moderate risk group. Among the 37 persons in the high-risk group, 7 had previously acquired haemorrhagic fever in the

laboratory, 10 had been vaccinated with an attenuated strain of Junin virus, and 20 had had neither apparent disease nor vaccination against this virus. Five persons (25%) out of this last group were found to have neutralizing antibodies against Junin virus, showing that this virus can also provoke inapparent infections. Of the 7 clinical cases of AHF, 6 showed high levels of neutralizing antibodies many years after contracting the disease. A long-lasting persistence of neutralizing antibodies was also demonstrated in 8 of the 10 persons who had been vaccinated with an attenuated strain of Junin virus and in those with inapparent infections.

In almost all cases, high-risk personnel had repeated exposures to the virus after active immunization by vaccination, inapparent infection, or disease, but no clinical evidence of reinfection was detected, demonstrating the high efficacy of any type of active immunization against Junin virus. Severe laboratory infections acquired by 19% of laboratory workers at high risk of contamination with Junin virus suggest the need for specific active immunization, at least for this group.

Among 268 sera collected from people without apparent disease in the area where AHF is endemic, 7 had neutralizing antibodies against Junin virus. Extensive seroepidemiological surveys are required in order to determine the prevalence of subclinical infection in the endemic area, as well as in laboratory personnel working with Junin virus. The data also suggest that the neutralization test may profitably be used for those serological studies because of its high specificity and the long-lasting persistence of neutralizing antibodies (30).

The finding that XJ Cl₃ strain was attenuated for guinea-pigs justified the assumption that it would also be attenuated for man and could be used as a vaccine against AHF. For this purpose an antigen was prepared from infected mouse brain. Seven volunteers (all having some professional connexion with Junin virus research) were successively inoculated with this antigen. One, two, and three years later, groups of increasing numbers of volunteers (64, 159, and 406 respectively) from the endemic area were also inoculated. The volunteers were submitted to several controls during the first period following inoculation. The clinical findings were as follows: 25% presented no symptomatology; 30% subjective symptomatology, such as asthenia, myalgia, or headache; and 45% had subjective symptomatology and fever with enlarged lymph nodes. The clinical picture lasted no more than 4 days and was followed by complete recovery. It was similar to that which accompanies yellow fever vaccination. The laboratory findings showed that 21% of the volunteers had

no haematological changes, 50% had leukopenia, 9% had thrombocytopenia, and 20% had both. Viraemia was not found in the few cases studied (31). After 1–2 months, 93% of the volunteers had high titres of neutralizing antibodies against Junin virus; CF antibodies appeared in less than 20%, with titres not exceeding 1:4. Among 31 cases studied after one year and 10 cases studied after 5 years, 74% and 80%, respectively, showed significant levels of circulating neutralizing antibodies.

Recapitulating, AHF due to Junin virus infection is a fatal disease in 10% of patients. The disease has been reproduced in the guinea-pig and in the mouse. An attenuated viral strain has been used successfully for vaccination in 636 volunteers. It has been discontinued for the last 5 years because of the mouse-brain substrate used for its preparation. The cross-immunity observed between Tacaribe and Junin virus may provide a new approach to the prevention of Argentine haemorrhagic fever.

RÉSUMÉ

BIOLOGIE ET PATHOGENÈSE EXPÉRIMENTALES DE L'INFECTION À VIRUS JUNIN CHEZ L'ANIMAL ET CHEZ L'HOMME

Le virus Junin, agent étiologique de la fièvre hémorragique d'Argentine, a été isolé pour la première fois sur un malade en 1958. On a isolé depuis lors de nombreuses souches de ce virus sur l'homme et les rongeurs sauvages, et une forme mortelle de la maladie, caractérisée par des pathologies différentes, a été reproduite chez le cobaye et la souris: on observe une symptomatologie hémorragique chez le cobaye tandis que la souris présente des troubles neurologiques. Le cobaye est atteint d'une forme de fièvre hémorragique argentine qui rappelle les aspects hémorragiques de la maladie chez l'homme. Des hémorragies macroscopiques ou microscopiques sont observées dans les tissus sous-cutanés, le gros intestin et l'intestin grêle, les glandes surrénales, les ganglions lymphatiques, les parois stomacales, les poumons, etc., et il y a leucopénie et thrombocytopénie dans le sang périphérique. Le cobaye présente aussi une forte nécrose de la moelle osseuse et une altération de ses réactions humorales et cellulaires aux antigènes d'autres maladies. La virémie persiste jusqu'à la mort, et l'on trouve le virus dans les ganglions lymphatiques, la moelle osseuse, le foie, les poumons, le cœur, la rate et les glandes surrénales. On observe aussi des altérations des facteurs de coagulation. En traitant les animaux infectés par des sérums immuns homologues on peut obtenir une survie de 50%.

Une souche atténuée du virus Junin (XJ Cl₃) a été obtenue par clonage de la souche pathogène proto-

type XJ sur des cellules rénales de lapin en lignée continue. L'inoculation de cette souche au cobaye provoque une infection inapparente qui ne se manifeste que par de légères altérations hématologiques et par la présence d'anticorps; il se crée chez l'animal une immunité active qui le protège de l'atteinte par la souche pathogène. On a également obtenu, chez le cobaye infecté par le virus Tacaribe, une protection croisée contre le virus Junin qui peut s'expliquer par un accroissement des titres d'anticorps hétérologues neutralisants.

L'infection par le virus Junin provoque chez la souris non sevrée une encéphalite virale typique qui aboutit à la mort dans presque 100% des cas. Les signes typiques de cette encéphalite ne semblent pas dépendre de la souche de virus utilisée, de la souche de l'animal d'expérience ou de la voie d'inoculation, mais de l'âge de l'hôte. Chez les souris âgées, le taux de survie est plus élevé et le tableau clinique de la maladie est différent. Chez les souris survivantes, le virus peut être isolé, mais pas toujours, par des passages aveugles et il paraît être transmis verticalement.

Les résultats d'expériences faites sur des souris thymectomisées et sur des souris traitées par du sérum antithymocytaire semblent prouver que le pouvoir pathogène du virus Junin chez cet hôte est en rapport avec l'intégrité du système immunitaire dépendant du thymus.

Des études effectuées sur des souris non sevrées infectées et présentant une immunité passive acquise soit

naturellement de mères immunes, soit à la suite d'un traitement par un sérum hyperimmun, font penser que les anticorps humoraux ne jouent aucun rôle dans le développement des lésions encéphaliques: au contraire, ces anticorps semblent jouer un rôle de protection.

Le fait que l'action de la souche XJ CL₃ est atténuée chez le cobaye a fait supposer qu'elle produirait aussi un effet atténué sur l'homme. On a vacciné 636 volontaires

contre la fièvre hémorragique argentine avec la souche atténuée du virus Junin; le vaccin a été bien toléré et l'on n'a observé que peu de symptômes cliniques. Des anticorps neutralisants ont été produits dans 93% des cas.

Une enquête sérologique récemment effectuée parmi des travailleurs de laboratoire et des habitants de la zone d'endémie a permis de déceler des cas d'infection subclinique.

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DISCUSSION

EDDY: Can you tell us how long before challenge with Junin virus you gave the heterologous cross-protecting virus?

WEISSENBACHER: With Tacaribe, Amapari, or Tamiami virus, 30 days; with Tacaribe virus you may have protection after the third week.

EDDY: Do you have any idea how long this protection lasts, and have you attempted the passive transfer of heterologous neutralizing antibody?

WEISSENBACHER: In Tacaribe-inoculated animals, the last challenge we made was at 6 months, and there was complete protection against 10^6 lethal doses of the XJ strain of Junin. We have not yet attempted passive immunization.

NATHANSON: In the guinea-pig model of the haemorrhagic disease, does immunosuppression with antilymphocyte serum or some other means provide protection?

WEISSENBACHER: In guinea-pigs it is not possible to protect using antilymphocyte serum, which actually appears to decrease the survival time by 2-3 days.

NATHANSON: Did you have independent evidence that your antilymphocyte serum was really a potent suppressive serum?

WEISSENBACHER: I did not perform this experiment myself, and I do not remember whether the workers had evidence of adequate immunosuppression.

CASALS: You mentioned the use of an attenuated strain of XJ virus as a vaccine. Is anything being done to improve this attenuated vaccine?

WEISSENBACHER: We did not progress with the attenuated strain last year because of the problems associated with licensing a vaccine produced in mouse brain.

NATHANSON: Is it clear whether or not the vaccinated individuals have persistence of infectious virus at a minimal level?

WEISSENBACHER: In a few cases, we have tried to detect virus in the blood, but we were unable to do so. The test was performed by inoculating 1-2 ml of blood intramuscularly into guinea-pigs.

NATHANSON: Did the vaccinated volunteers develop neutralizing antibody?

WEISSENBACHER: Yes, they had high titres of neutralizing antibody.