CHAPTER 17

Arenavirus Pathophysiology

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I. INTRODUCTION

Arenavirus infections are virtually without pathophysiology in the naturally infected rodent host. The animal normally experiences silent but persistent and lifelong infection, the viruses being adapted to this highly successful ecological niche by asymptomatic horizontal or vertical transmission at or near birth (Traub, 1935; McCormick, 1990). In adult rodents, however, infection does induce pathology and an acute self-limiting disease, ending in death or recovery, with virus clearance depending on the dose and route of inoculation and the genetic background of the rodent. Arenavirus infections in humans takes an altogether different course. Infections range from a febrile disease with aseptic meningitis with lymphocytic choriomeningitis virus (LCMV) (Armstrong and Sweet, 1939), to total collapse and death with circulatory and respiratory failure with the hemorrhagic fever viruses: Lassa fever (Rose, 1956; Buckley *et al.*, 1970; Frame *et al.*, 1970), Argentine hemorrhagic fever (AHF) (Ruggiero *et al.*, 1964a; Maiztegui, 1975), and Bolivian hemorrhagic fever (BHF) (Aribalzaga, 1955; MacKenzie *et al.*, 1964; Johnson *et al.*, 1965). A meningoencephalitis is characteristic of LCMV in humans, but most infections are apparently mild and self-limiting. In contrast, the arenavirus hemorrhagic fevers are often severe, generalized febrile diseases with multiorgan involvement and case/fatality rates of about 16% in untreated hospitalized patients. The hemo-

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static defect, which is their hallmark, is characterized by platelet dysfunction rather than coagulation cascade activation, with little evidence of major hepatorenal failure or organ destruction by direct viral replication. Tissue and pulmonary edema with hypovolemic shock and adult respiratory distress syndrome (ARDS) are prominent in severe disease, and nervous system involvement is a feature of both acute and convalescent stages of these infections.

Infection of humans by these viruses is an accidental event, reflecting minimal host/parasite adaptation and bearing little resemblance to the course of infection in rodents. However, except for LCMV, which may be acquired from house mice or pet hamsters and laboratory hamsters or nude mice almost anywhere in the world, human arenavirus infections are rural diseases. Since they usually occur in areas with limited facilities for medical care, details of the clinical and pathogenic features of the diseases are scarce. Human-to-human spread has been reported for Lassa fever in the community and in hospital settings (McCormick *et al.*, 1987a), whereas only a few cases of nosocomial transmission have been reported for Bolivian hemorrhagic fever (Peters *et al.*, 1974), and none for Argentine hemorrhagic fever.

II. INFECTIONS IN THE NATURAL RODENT HOST

All arenaviruses establish persistent infection in the natural rodent host following virus acquisition *in utero* or within a few days of birth. Most of the persistently infected animals have viremia and viruria throughout life (Traub, 1935; Webb *et al.*, 1975). This persistence is not only a highly efficient means of virus perpetuation in most of the rodent offspring, but it is also the primary source of contamination of the environment leading to human infections. These mice are relatively deficient in virus-specific immune lymphoctcytes, but are able to mount high titers of antiviral antibodies (Oldstone, 1987). Intracerebral inoculation of Lassa virus may be lethal to mice (Lukashevich, 1985).

A. LCMV in Mice

When immunocompetent adult mice are inoculated with LCMV by the intracerebral route, they develop an acute inflammatory leptomeningitis, choroiditis, and ventriculitis, leading to death within 6–10 days (Oldstone, 1987). High white blood cell counts are found in the CSF, with only a few red blood cells, although the the blood–brain barrier has been violated. The animals develop tremors with characteristic extensor spasms of the legs and finally convulse and die. The white cell infiltrate of the CSF comprises T cells (Thy-1^+ and Lyt-2^+), NK cells, and macrophages. Inflammation is mediated by class I MHC-restricted cytotoxic T

cells specific for LCMV antigens, apparently recognizing viral epitopes different from those which induce humoral antibodies, and which may vary depending on the genotype of the mouse (Allan *et al.*, 1987). This model can be reproduced in persistently infected immunosuppressed mice by reconstitution with virus-specific Lyt-2^+ cells from immunized animals.

When adult animals are inoculated peripherally, the outcome is variable. Viremia peaks about 4–5 days postinoculation and then rapidly declines. Antibodies are detectable by 4–5 days postinfection, and virus-specific cytotoxic T cells (CTL) appear about day 5, with highly activated macrophages, and reach peak activity around days 7–9 (Cole *et al.*, 1972; Marker and Volkert, 1973).

Classically the disease results from widespread immune-mediated damage to the meninges, choroid plexus, and ependyma, which are heavily infected with the virus and which become targets for effector T lymphocytes (Cole and Johnson, 1975). The processes of cellular immune responses are discussed in detail elsewhere (see Chapters 11 and 12).

In newborn mice less than 24 hr old, LCMV inoculation by any route, including intracerebral, results in silent but persistent infection (Oldstone, 1987). Virus replication in the brain soon weakens the ventricles and virus rapidly enters the circulation, reaching tissues, serum, and urine. In the central nervous system in these animals, virus is primarily expressed in neuronal cells, in contrast to the acutely infected adults, in which the virus replicates mainly in the leptomeninges and lining of the ventricles (Cole *et al.*, 1971; Southern *et al.*, 1984). Ultrastructural studies of persistently infected neural tissue show localization of antigen to neurons in the cortex, limbic system, and hypothalmus and the anterior horn of the spinal cord and Purkinje cells in the cerebellum (Rodriguez *et al.*, 1983; Monjan *et al.*, 1975). The infection is not associated with any morphological changes, and expression is mainly of the nucleocapsid protein. The viral antigens appear to be mostly associated with ribosomes (Rodriguez *et al.*, 1983). The persistent infectious state can also be achieved in adult mice if they are immunosuppressed by thymectomy, irradiation, antilymphocytic serum, or immunosuppressive drugs. Transfer of virus-specific immune lymphocytes to either neonatally infected or adult immunosuppressed, persistently infected mice results in clearance of the virus (Oldstone *et al.*, 1986). The ultimate site of persistence appears to be renal tissue (Ahmed *et al.*, 1987). In a recent outbreak in laboratory personnel, inoculation of persistently LCMV-infected tumor cell lines into nude mice resulted in a number of human infections (Dykewicz *et al.*, 1992). The nude mouse provides an immunological host comparable to the neonatal mouse, and virus persistence may be expected (Frei *et al.*, 1988).

There is now considerable evidence from studies in LCMV-infected mice that replication of the virus in a specialized cell can result in selec-

tive disruption of cell function while incurring no structural injury (Oldstone *et al.*, 1984a,b). Growth hormone deficiency, caused by reduction in transcription initiation for the growth hormone gene, results in low serum glucose levels and down-regulation of the gene for thyroid-stimulating hormone, but not "housekeeping genes" (Klavinskis and Oldstone, 1989). These effects are apparently dependent on the strain of infecting virus and mapped to the small (S) segment of the viral RNA (Riviere *et al.*, 1985b). The virus has also been show to be diabetogenic, and viral persistence in this model has been demonstrated in the beta cells of the islets of Langerhans without obvious cytolysis (Oldstone *et al.*, 1984b). In the nonobese diabetic (NOD) mouse model, virus infection averts insulin-dependent diabetes, and this maps to the small (S) segment of LCMV (Oldstone *et al.*, 1991).

B. Lassa Virus in *Mastomys*

The only known reservoir of Lassa virus in West Africa is *Mastomys natalensis*, one of the most commonly occurring rodents in Africa (Wulff *et al.*, 1977; McCormick *et al.*, 1987a). At least two species of *Mastomys* (diploid types with 32 and 38 chromosomes) inhabit West Africa, and both have been found to harbor the virus. All species are equally susceptible to silent persistent infection in the same way that LCMV infects mice. Experimental inoculation of laboratory-reared neonatal *Mastomys* leads to persistent excretion of Lassa virus in urine, but adult animals clear virus from serum and urine within three–weeks. Studies of wild-caught *Mastomys* show that over half the captured animals in some foci may be chronically infected, and antibody and virus may be present at the same time in about one-third of these (McCormick, personal observations).

C. Junin and Machupo Viruses in *Calomys*

The major rodent hosts for Junin virus are *Calomys* species. Both of the South American viruses may cause illness and death in newborn mice or may induce persistence (Sabattini *et al.*, 1977; Webb *et al.*, 1975). The rodents are affected by the virus, with up to 50% fatality among infected suckling animals and stunted growth in many others. Machupo virus renders its major natural host *Calomys callosus* essentially sterile, with the young dying *in utero*. Machupo virus also induces a hemolytic anemia in its rodent host with significant splenomegaly, often an important marker of infected rodents in the field. Transmission from rodent to rodent is horizontal, not vertical, and is believed to occur through contaminated saliva and urine. Both viruses induce a humoral

immune response, which may include neutralizing antibody, in the face of persistent infection.

III. INFECTIONS IN LABORATORY RODENTS

A. LCMV in Hamsters

In adult hamsters LCMV behaves much as in adult mice, with variable outcome depending on the virus strain used, the WE strain giving the highest mortality (Hotchin *et al.*, 1975). Some hamster strains were more susceptible than others to developing high serum virus titers, severe disease, and death. Virus was cleared by the fourth week postinoculation in survivors. There were no differences in outcome when the virus was inoculated intraperitoneally or into the footpad. Immunosuppressed hamsters had a higher frequency of severe disease and death when inoculated with LCMV (WE or Armstrong strains) and, unlike mice, did not develop persistent infection (Peters *et al.*, 1987). Histopathological studies show that the virus is pantropic in lethally infected animals, though it appears to be concentrated in or around blood vessels, with little necrosis or inflammatory infiltrate or significant organ damage. There is marked wasting and profuse diarrhea, with up to 40% loss of body weight. This model has interesting similarities with the processes observed in humans and primates infected with African and South American arenaviruses. In another study, neonatally infected hamsters and cyclophosphamide-treated adult animals inoculated with the less pathogenic LM_4 strain of LCMV appeared to develop subclinical persistent infection, as do mice (Hotchin *et al.*, 1975). This study also reported persistence in immunocompenent "golden" hamsters inoculated at 30 days of age. From these limited data it seems both hamster genotype and virus strain are important in determining outcome.

B. LCMV in Guinea Pigs

The lethal dose of LCMV is also highly dependent on virus strain, ranging from less than 1 plaque-forming unit (pfu) for the WE strain to more than 10^6 pfu for the Armstrong strain. Reassortants of the WE and Armstrong strains of LCMV have been used to map the lethality of WE to the S RNA segment (Riviere *et al.*, 1985a). As in hamsters, the virus is pantropic, with high titers of virus and little evidence of histopathological damage. Endothelial cells and mesangial cells in the kidneys are sites of abundant replication, and large amounts of virus in the transitional epithelium of the bladder provide an obvious source for viruria (Parker *et al.*, 1976; Peters *et al.*, 1987).

C. Lassa Virus in Guinea Pigs

Guinea pigs have been used as an experimental laboratory model for Lassa fever with variable results. Outbread guinea pigs exhibit a range of responses with 20–30% survival following a mild or moderate febrile episode. Those that die have pathological evidence of myocarditis, pulmonary edema, and hepatocellular damage (Walker *et al.*, 1975; Callis *et al.*, 1982; Jahrling *et al.*, 1982). Lethality for inbred guinea pig strains may vary with virus strains. However, some strains that do not kill adult inbred guinea pigs are uniformly lethal for 3- to 5-day-old animals and for pregnant guinea pigs, which abort infected fetuses. Animals destined to die develop earlier viremia and reach higher persisting viral titers.

D. Junin Virus in Guinea Pigs

Outcome with Junin infection is again related to high viremia and depends on the infecting strain (Kenyon *et al.*, 1988). In the guinea pigs, virus is mainly viscerotropic, with no evidence of an immunopathological mechanism, so this laboratory animal is a reasonable model for human disease (Oubina *et al.*, 1984). These animals develop florid hemorrhagic disease with extensive necrosis of lymphatic tissue (Weissenbacher *et al.*, 1975; Molinas *et al.*, 1978). There are occasional reports of neurological disease as a later development in animals with lower viremia, but with high-titer virus replication in the brain (Contigiani and Sabattini, 1977). As in primates, a late neurological syndrome is also reported associated with effective administration of immune serum (Kenyon *et al.*, 1986). The authors of this study suggest that the brain provides a site where virus may evade the antibody and subsequently replicate to generate symptoms typified by prominent rear-limb paralyses. Although this type of lesion suggests ischemic or thrombotic events in the spinal cord, apparently neurotropism is frequent in any animals surviving Junin virus infection long enough to allow it to reach the CNS, and indeed, guinea pigs develop encephalitis with recoverable virus from the brain if death is delayed by treatment with immune plasma. Transplacental transmission of Junin virus in guinea pigs has also been demonstrated, with variable outcome depending on the stage of pregnancy (Sangiorgio and Weissenbacher, 1983). Some fetuses died and were aborted with hemorrhagic manifestations and recoverable virus, and a few survived. Only in early preganancy was the outcome favorable for both mother and fetus, as has been observed in human Lassa virus infections (Price *et al.*, 1988).

In experimental Junin virus infections of guinea pigs depleted of polymorphonuclear monocytes (PMN), the disease was apparently more severe, with higher virus titers and lung pathology suggesting pulmonary distress syndrome (Gonzalez *et al.*, 1987). The implications of these

data are as yet obscure, but they do suggest a role for PMN in the pathology of arenavirus infections. It is unclear whether they are responsible for generation of or protection from disease processes.

IV. ARENAVIRUSES IN NONHUMAN PRIMATES

A. LCMV in Monkeys

The disease produced by the WE strain of LCMV in rhesus and cynomolgus monkeys is fatal within about 2 weeks, with a course similar to that described in monkeys and humans for Lassa fever (Peters *et al.*, 1987). The course is relentless fever and viremia reaching 10^7 to 10^8 pfu/ml by death. In a report of six cynomolgus monkeys infected with this strain of LCMV, an initial leukopenia was followed by a leukocytosis, primarily neutrophilia as previously observed in Lassa fever–infected primates and humans. All monkeys had intradermal hemorrhage (petechiae and ecchymoses) and *epistaxis*. At autopsy, large effusions were found, as well as high titers of virus in all tissues, including vascular components in the brain. Serum aspartate amino transferase (AST) levels were elevated as in Lassa fever. Monkeys similarly infected with the Armstrong strain of LCMV failed to show illness, despite a seroconversion to the virus. Development of antibodies with ability to neutralize LCMV was markedly delayed in the Armstrong infection.

B. Lassa Virus in Monkeys

Infection of rhesus and cynomolgus monkeys by Lassa virus causes fever after 5 days, significant anorexia, and progressive wasting (Jahrling *et al.*, 1980). This laboratory model has proved extremely useful in studying the pathogenesis of fulminating viral infections and in testing potential vaccines for Lassa fever. In rhesus and cynomolgus monkeys the infection almost invariably ends after 10–15 days in death from vascular collapse and shock, with mild to moderate hemorrhage affecting primarily mucosal surfaces (Fisher-Hoch *et al.*, 1987).

Although Lassa virus is pantropic, pathological findings are limited to mild hepatic focal necrosis without significant inflammatory response, some evidence of pulmonary interstitial pneumonitis, chiefly interstitial edema, and occasional focal adrenal cortical necrosis (Walker *et al.*, 1982a). Although the liver is the most affected (Edington and White, 1972; Winn *et al.*, 1975; McCormick *et al.*, 1986b), biochemical measures of liver function and the extent of tissue necrosis are inadequate to account for death due to hepatic failure. A marked discrepancy in AST:ALT ratios, as in Marburg and Ebola infections, is found. Since

elevation of alanine amino transferase (ALT) is the closer marker of hepatocyte failure, it is possible that the AST levels are not due solely to damage to hepatocytes. This conclusion is further supported by the observation that prothrombin coagulation times are only marginally prolonged, and blood glucose is within the normal range (Fisher-Hoch *et al.*, 1987).

Absence of significant disturbances in coagulation, low titers of fibronogen degradation products (FDPS), and absence of evidence for increased platelet and fibrinogen consumption make disseminated intravascular coagulation (DIC) unlikely as a primary pathological process (Fisher-Hoch *et al.*, 1987; Lange *et al.*, 1985). In primates thrombocytopaenia is rarely seen, though petechiae have been observed in two animals challenged during vaccine studies (Fisher-Hoch, personal observation). Platelet function, on the other hand, is markedly depressed, and as in humans, a circulating inhibitor of platelet function has been observed (Cummins *et al.*, 1989a; Fisher-Hoch, personal observation). There is also evidence for disturbance of endothelial function in that prostacylin production in *postmortem* vascular samples is depressed compared with material from normal control animals (Fisher-Hoch *et al.*, 1987). Subtle changes in vascular function obviously occur, and appear to be rapidly reversible. Presumably these are sufficient to account for the failure of integrity of the intravascular compartment, leading to edma of the face, shock, and the effusions regularly observed at autopsy.

Early lymphopenia followed by rising relative and absolute neuthrophilia has been reported in primates (Fisher-Hoch *et al.*, 1987). *In vitro* lymphocyte proliferation tests during the acute phase of the illness show impaired responses to nonspecific mitogens, suggesting the function of lymphocytes is also inhibited, a fact that may be associated with the observation that no inflammatory infiltration of tissues is observed. The viral glycoprotein G2 has been observed to be associated with circulating neutrophils, but not with lymphocytes. The significance of this finding is uncertain, but in view of the capacity of the platelet inhibitor of Lassa fever to affect neutrophil function (Roberts *et al.*, 1989), some consideration needs to be given to the role of neutrophils in the pathogenesis of severe Lassa fever.

Serum antibodies to Lassa virus do not usually neutralize Lassa virus *in vitro* in a classical replication inhibition, serum dilution neutralization assay (Jahrling *et al.*, 1982; Wulff and Lange, 1975; Fisher-Hoch *et al.*, 1989). In one study of primates vaccinated with a vaccinia recombinant vaccine expressing the Lassa glycoproteins that survived challenge with Lassa virus, neutralizing antibody by a fixed-serum, varying-virus-dilution technique could only be demonstrated in a few samples between 21 and 97 days postchallenge, at a time when the IFA antibody was as much as 1:250,000, and when all animals had long cleared virus from their serum. Antibodies to viral proteins G1, G2, and N can be detected in dying unprotected animals following challenge. None of the

prechallenge specimens from vaccinated, protected animals had any measurable neutralizing activity *in vitro* (Fisher-Hoch *et al.*, 1989). Neutralizing antibody does not play any role in clearance of virus from serum in acute infection, and it seems unlikely to play a major role in protection from challenge.

C. Junin and Machupo Viruses in Monkeys

Junin and Machupo virus infection of primates, from rhesus monkeys to marmosets, closely approximates the disease in humans, with fever, anorexia, weight loss, and gastrointestinal symptoms (Eddy *et al.*, 1975a; Kastello *et al.*, 1976; Avila *et al.*, 1987; Weissenbacher *et al.*, 1979). As the disease progresses, the animals develop a rash, flushing, thrombocytopenia, and petechiae with bleeding, especially in the mucous membranes. The animals die with cachexia and severe dehydration and at postmortem have hemorrhages and lymphocyte depletion from nodes, spleen, and bone marrow. In addition, the animals appear to reflect the same biological response as that of humans to virus-determined factors (McKee *et al.*, 1985). Thus a strain that produces primarily hemorrhagic disease in humans elicits the same in monkeys. Likewise, a primarily neurotropic strain in humans also produces a similar disease in rhesus monkeys. Studies in neotropical primates, such as *Callithrix jacchus*, also reveal an acute hemorrhagic disease with early, severe thrombocytopenia (Molinas *et al.*, 1983). Both the coagulation and complement cascades were activated.

Machupo virus infection has been studied in a variety of Old World monkeys (Eddy *et al.*, 1975a,b; Scott *et al.*, 1978; Kastello *et al.*, 1976; Terrell *et al.*, 1973; Peters *et al.*, 1987; McLeod *et al.*, 1976). The same variation in pathology observed with Junin is observed with Machupo. Some of the monkeys develop progressive fever, weight loss, hemorrhage, and eventually shock and death after 3–4 weeks. These animals demonstrate progressive thrombocytopenia, anemia, lymphocytopenia, and neutropenia. Inconstant and highly variable degrees of pathological change were also noted in the liver, myocardium, bowel, adrenals, and lymphoid tissues. A second group of animals survived the initial infection only to develop a late neurological disease manifested by tremors, ataxia, nystagmus, and paresis with rare survival. At this stage there is no viremia, and the brain shows lymphocytic infiltration and vasculitis. The acute hemorrhagic disease in monkeys is more like that observed in humans, whereas the neurological disease is much less frequently observed in human infections.

Treatment of Junin virus–infected primates with immune serum, as in guinea pigs and humans, even though successful in treating the acute hemorrhagic disease, predisposes to a late neurological syndrome involving hind limb paralyses (Avila *et al.*, 1987). Similar observations

have been made in Machupo-infected monkeys (Eddy *et al.*, 1975b). Acute-phase phenomena may be due to direct viral replication in the brain (encephalitis or meningitis), but there are no data to suggest this is the case (Weissenbacher *et al.*, 1987). The etiology of these events is obscure, though obviously the apparent induction of lesions by use of immune serum indicates that some immune-mediated phenomenon is involved, strongly supported by the observation of lymphocytic infiltration with vasculitis in Junin-infected monkeys. Whether the treatment induces the disease or whether successful salvage of an otherwise fatal infection uncovers the neurological involvement is unclear. The cerebellar syndrome described in Machupo infections resembles that observed in patients with a variety of viral infections, including, in particular, varicella zoster, which is known to be neurotropic. On the other hand, localization to hind limb paralyses suggests spinal cord injury, which could be vascular. The only coherent unifying hypothesis would be that focal vasculitis, possibly mediated by immune complexes, may be involved in both syndromes.

V. ARENAVIRUS INFECTIONS IN HUMANS

A. Lymphocytic Choriomeningitis

LCMV illness in humans follows an incubation period of 1–3 weeks (Armstrong and Sweet, 1939; Hinman *et al.*, 1975). There has never been a reported case of person-to-person transmission, however. In a recent outbreak, inoculation of persistently LCMV-infected tumor cell lines into nude mice led to a number of infections in laboratory workers. In this study there is very close statistical association with regular contact with the nude mice and their bedding (Dykewicz *et al.*, 1992). Data from this and other studies show that close exposure to aerosol of animal excreta may be a route of infection.

LCM virus infection in humans may be asymptomatic, mild, or moderately severe with central nervous system (CNS) manifestation requiring hospitalization. In the largest studies of human infections reported, 33 of 94 (35%) infections were asymptomatic, 47 (50%) were mild to moderate febrile illnesses without significant CNS manifestations, and 14 (15%) had typical LCM (Deibel *et al.*, 1975; Hinman *et al.*, 1975.). Although rarely fatal (Smadel *et al.*, 1942; Warkel *et al.*, 1973), the disease can be severe with a prolonged convalescence. Severity of illness may depend on both dose and route of infection as well as the host immunogenetic background.

Typical lymphocytic choriomeningitis, from which the virus derives its name, begins with fever, malaise, weakness, myalgia, and headache, which is often severe, retro-orbital, and associated with photophobia. Myalgia is marked in the lumbar region. Anorexia, nausea, and

dizziness are common. As many as 50% of patients may have combination of sore throat, vomiting, and arthralgias, with chest pain and pneumonitis occurring less frequently (Biquard *et al.*, 1977; Farmer and Janeway, 1942). Alopecia, orchitis, and transient arthritis of the hands have also been reported. The white blood cell count is often 3000/mm^3 or less with a mild thrombocytopenia.

Physical examination shows pharyngeal inflammation, usually without exudate, and in more severely ill patients, meningeal signs including nuchal rigidity. About one-third of patients with CNS manifestations will develop encephalopathy, while the rest exhibit primarily aseptic meningitis (Meyer *et al.*, 1960). An interstitial pneumonia has also been described in two atypically fatal human cases and in nonhuman primate postmortem studies (Smadel *et al.*, 1942). Convalescence is prolonged, with persistent fatigue, somnolence, and dizziness.

In LCMV infections, cerebrospinal fluid (CSF) from patients with meningeal signs contains several hundred white cells per cubic centimeter, predominantly lymphocytes (>80%), with mildly increased protein and occasionally low sugar levels. Virus is often found in spinal fluids taken during acute disease (Vanzee *et al.*, 1975). In mice experimentally infected with LCMV, intrathecal levels of B-cell-stimulating factor 2 (BSF-2) and interferon-gamma have been correlated with the development of meningitis, and there is evidence that intrathecal BSF-2 also rises in CSF from patients with acute viral meningitis. This suggests that one component of the pathology of meningitis in mice and humans is invasion of the CNS by lymphocytes and plasma cells and their attendant cytokines (Frei *et al.*, 1988).

There are few published descriptions of the pathology of LCMV infection in humans. In one report of a fatal case with primarily neurological manifestations, there was evidence of perivascular infiltration of macrophages in multiple areas of the brain (Warkel *et al.*, 1973). Antigen was observed in the meninges and cortical cells by IFA, consistent with viral replication in the CNS. Neurological sequelae to LCMV infection are unusual but have been reported (Meyer *et al.*, 1960). Some experimental and indirect epidemiological evidence of hydrocephalus in newborns following maternal LCMV infection has been reported (Casals, 1977; Farmer and Janeway, 1942; Sheinbergas, 1975).

Less well-substantiated neurological associations with LCMV infection in humans are reported in a study from the U.S.S.R. of 12 patients with amyotrophic lateral sclerosis (Tkachenko *et al.*, 1984). The long incubation period of this disease is markedly at variance with the acute nature of the disease in all the animal models currently known. Three had antibody to LCM virus, and antigen was detetected in serum from two patients and tissue from one at postmortem by ELISA and solid-phase radioimmunoassay techniques. If the virus is indeed responsible for the pathology, this would represent a further instance of fatal outcome from LCMV infection. Confirmation of these observations in

independent studies would obviously throw new light on the potential of LCMV to induce a wide range of human pathology, including chronic neurological disease. Also of interest is a single report of unilateral deafness caused by LCMV infection (Ormay and Kovacs, 1989). Since this is a well documented late complication of Lassa virus infections in humans, it may be that arenaviruses have tropism in humans for the auditory nerve or organ of Corti, or that some other processes resulting in local ischemia lead to localized nerve injury.

B. Lassa Fever in Humans

Lassa fever virus normally infects humans via mucosa or cuts or abrasions. The incubation period of 1–3 weeks that follows infection suggests a silent primary replication site as yet unknown, though in all probability this is within the reticuloendothelial system. Subtle onset with generalized symptoms including high fever, joint pain, back pain, and severe headache does not distinguish this infection from many other viral syndromes (McCormick *et al.*, 1987b). The characteristic dry cough and exudative pharyngitis suggest some upper and lower respiratory tract involvement early in the disease, but though virus may be isolated from the pharynx, this is variable and at low titer (Johnson *et al.*, 1987) and viral pneumonia is not a feature of Lassa fever. It does not appear that the upper respiratory tract is a major site of viral replication or excretion despite the severe sore throat.

Route and titer of infecting dose may be important determinants of outcome. Case fatality in hospitalized patients with Lassa fever is about 16% (McCormick *et al.*, 1987b). However, in recent outbreaks in Nigeria, much higher death rates have been observed (Tomori *et al.*, personal communication). Whether this is due to variation in virulence with different virus strains or the high dose and route of inoculation in the Nigerian outbreak (by sharing of needles and syringes in hospitals administering parenteral drugs) remains to be seen.

The degree of organ damage in fatal human infections is mild; sharply at variance with the clinical course and collapse of the patient (Walker *et al.*, 1982b). Liver damage is variable, with concomitant cellular injury, necrosis, and regeneration (McCormick *et al.*, 1986a). Nevertheless serum AST levels over 150 IU/liter are correlated with poor outcome, and an ever-increasing level is also associated with increased risk of death (McCormick *et al.*, 1986a,b). ALT is only marginally raised, and the ratio of AST:ALT in natural infections and in experimentally infected primates is as high as 11:1 (Fisher-Hoch *et al.*, 1987). Furthermore, prothrombin coagulation times and glucose and bilirubin levels are near-normal, excluding biochemical hepatic failure and suggesting that some of the AST may be nonhepatic in origin.

It is clear that the outcome in Lassa fever is associated with the

degree of virus replication (McCormick *et al.*, 1987b). An increasing viremia is associated with an increasing case fatality. In addition to the liver, high virus titers occur in brain, ovary, pancreas, uterus, and placenta, but no significant pathological or functional lesions are observed. Since these patients also have high viremia, the titer in these organs may reflect their blood content rather than specific parenchymal replication of the virus. Immunofluorescence studies suggest that though parenchymal replication occurs, it is limited. Individual hepatocytes or groups of cells contain viral antigen, as may a few endothelial cells, but there is little evidence for extensive replication in other parenchyma such as neuronal tissue or alveolar cells. Electron microscopy does not show extensive cellular damage. Indeed, there are few clues to the pathogenesis of Lassa fever in standard pathological studies.

On admission to the hospital the hematrocrit of Lassa fever patients is often elevated (mean of 50.6/100 ml), presumably owing to dehydration (McCormick *et al.*, 1987a). Severe cases progress with vomiting and diarrhea. Some patients develop severe pulmonary edema and adult respiratory distress syndrome, gross head and neck edema, pharyngeal stridor, and hypovolemic shock (Fisher-Hoch *et al.*, 1985). This pattern is consistent with edema due to capillary leakage rather than cardiac failure and impaired venous return. Endothelial cell dysfunction has been demonstrated in experimentally infected primates dying of Lassa fever, in that there is apparently a marked decrease in prostacyclin production by endothelial cells (Fisher-Hoch *et al.*, 1987). Loss of integrity of the capillary bed presumably causes the leakage of fluids and macromolecules into the extravascular spaces and the subsequent hemoconcentration, hypoalbuminemia, and hypovolemic shock. Proteinuria is common, occurring in two-thirds of patients. The blood urea nitrogen (BUN) may be moderately elevated, probably owing to dehydration.

Edema and bleeding may occur together or independently. There is no characteristic skin rash in Lassa fever and petechiae and ecchymoses are not seen. In only about 15–20% of patients is there frank bleeding, manifest as oozing gums, epistaxis, gastrointestinal or vaginal bleeding, and conjunctival hemorrhages. The case fatality of patients with hemorrhage is 50%. Since there is minimal disturbance of the intrinsic and almost none of the extrinsic coagulation system, and there is no increase in fibrinogen breakdown products, disseminated intravascular coagulation (DIC) is excluded (Fisher-Hoch *et al.*, 1988). Furthermore, platelet and fibrinogen turnover in experimental primate infections are normal (Lange *et al.*, 1985; Fisher-Hoch *et al.*, 1987).

Though platelet numbers are only moderately depressed, in severe disease their function is almost completely abolished by a circulating inhibitor of platelet function (Cummins *et al.*, 1989a). The origin of this inhibitor is not known; however, it cannot be reproduced with viral material nor can it be blocked by antibodies to Lassa virus. In the platelet it blocks dense granule and ATP release and thus abolishes the secondary

wave of *in vitro* aggregation while sparing the arachidonic acid metabolite–dependent primary wave. Its inhibition is probably by interference with calcium channel mechanisms and phosphatidyl inositol secondary messenger pathway systems. In a few Lassa fever cases there is later relative or absolute neutrophilia, (Fisher-Hoch *et al.*, 1988). Polymorphonuclear leukocyte (PMN) counts as high as $30 \times 10^3/mm^3$ have been recorded in very sick or terminally ill patients. The inhibitor of platelet function also interferes with the generation of the FMLP-induced superoxide generation in PMN (Roberts *et al.*, 1989).

Acute neurological manifestations are common in Lassa fever. These range from isolated unilateral or bilateral deafness, with or without tinnitus, to moderate or severe diffuse encephalopathy with or without general seizures (McCormick *et al.*, 1987a). The encephalopathic complications generally carry a poor prognosis, while the deafness usually occurs just as recovery is underway. Manifestations during the acute phase range from mild confusion and tremors to grand mal seizures and decerebrate coma. Focal fits are not seen. CSF examination usually shows a few lymphocytes, but otherwise is normal, with low virus titers where blood-free samples have been reliably drawn. Furthermore, brain pathology is minimal both in humans and in primates infected with Lassa virus, so it is unclear whether cerebral involvement is a result of direct infection. Observations of cardiac pathology have been limited to hemorrhage and a lymphocytic infiltrate in the pericardium, and occasional interstitial myocarditis. The severe retrosternal or epigastric pain seen in many patients may be due to pleural or pericardial involvement, and late in the disease about 20% of patients have pleural or pericardial "rubs" (grating noises heard as the heart beats) (McCormick *et al.*, 1987a). Up to 70% of electrocardiogram (ECG) observations made on 32 patients in a recent study showed a range of abnormalities. These do not suggest a consistent cardiac pathology associated with Lassa virus infection, such as myocarditis or pericarditis (Cummins *et al.*, 1989b). The changes included nonspecific abnormalities in the ECG wave patterns (ST-segment and T-wave abnormalities, ST-segment elevation, generalized low-voltage complexes); all changes reflected electrolyte disturbance, but none correlated with clinical severity of infection, serum transaminase levels, or eventual outcome. Thus ECG changes were common in this limited study, but usually unassociated with clinical manifestations of myocarditis.

Lassa fever is particularly severe during the third trimester of pregnancy (Price *et al.*, 1988). Studies have shown that the overall case fatality in pregnant women infected by Lassa virus is about 20%, and very high levels of virus replication have been found in placental tissue in third trimester patients. A fourfold reduction was noted in case fatality among women in all trimesters who were spontaneously or therapeutically aborted compared to those who were not (odds ratio for fatality with pregnancy intact is 5.47 compared to those with uterine evacua-

tion). The excess mortality in the third trimester may be due to the relative immunosuppression of pregnancy, which peaks at that time. Lassa fever is also devastating to the fetus.

Fetal/neonatal loss is 87%. Lassa virus is known to be present in the breast milk of infected mothers, and neonates are therefore at risk of congential, intrapartum, and postpartum infection with Lassa virus. Lassa fever also occurs in children, in whom the disease is similar to that in adults. However, a "swollen baby syndrome," consisting of widespread edema, abdominal distention, and bleeding, has been associated with serological evidence of Lassa virus infection of young children in a report from Liberia (Monson *et al.*, 1987). This syndrome has not been seen in adjacent areas of Sierra Leone. It would be of interest to compare viruses isolated from such cases to confirm the etiology and possible involvement of particular strains of virus.

Rare complications of Lassa fever include uveitis and orchitis. Virus replication occurs extensively in the adrenal glands, but no functional studies of the adrenal system have been done, though adrenal insufficiency during convalescence has been seen (McCormick, unpublished observation). The most significant sequel of Lassa fever is acute VIIIth nerve deafness, (Cummins *et al.*, 1990a). The onset is invariably during the convalescent phase of illness, and its development and severity are unrelated to severity of the acute disease. Nearly 30% of patients with Lassa fever infection suffer an acute loss of hearing in one or both ears. The mean auditory threshold of these patients is 55 dB (normal < 25 dB), and the mean disability is over 20%. About half the patients show a near or complete recovery over the 3–4 months after onset, but the other half continue with permanent, significant sensorineural deafness. Many patients also exhibit cerebellar signs during convalescence from severe disease, particularly tremors and ataxia, but this usually resolves with time. As with the late neurological syndrome of the South American hemorrhagic fevers, it is unclear whether this deafness is due to damage by neurotropic viruses, thrombosis, vasculitis, or focal hemorrhage. A late event observed in a few patients has been polyserositis (Hirabayashi *et al.*, 1988), but its pathology is obscure. A single case report describes an interesting complex of hemorrhagic pericarditis and cardiac tamponade with pleural effusions and ascites 6 months after acute Lassa fever. Repeated cultures failed to isolate virus from effusion fluids, but these specimens contained high titers of Lassa-specific IgG and numerous lymphocytes, suggesting an immune-mediated mechanism.

The immunological response to Lassa virus infection is complex. There is a substantial macrophage response, with little if any lymphocytic infiltrate. There appears to be a brisk B-cell response with a classic primary IgG and IgM antibody response to Lassa virus early in the illness. This event does not, however, coincide with virus clearance, and high viremia and high IgG and IgM titers often coexist in both humans and primates (Johnson *et al.*, 1987; Fisher-Hoch *et al.*, 1987). Indeed,

virus may persist in the serum and urine of humans for several months after infection, and possibly in occult sites, such as renal tissue, for years. Acute lesions in Lassa virus infections are minimal, and without significant lymphocyte infiltration. There may be some impairment in the T-cell arm of the immune response during the acute infection, as has been seen in nonhuman primates. *In vitro* studies have shown that Lassa-virus is able to replicate in a continuous monocytic cell line, and this capacity is apparently enhanced by Lassa-specific antibody (Lewis *et al.*, 1988).

Neutralizing antibodies to Lassa virus are absent in the serum of patients at the beginning of convalescence, and in most people they are never detectable. In a minority of patients some low-titer serum-neutralizing activity may be observed several months after resolution of the disease and clearance of the virus (Jahrling *et al.*, 1980). The biological significance of this observation is not understood. Passive protection with antibody to Lassa virus has been demonstrated in animals given selected antiserum at the time of, or soon after, inoculation with virus, but clinical trials of human plasma have shown no protective effect (McCormick *et al.*, 1986a). Thus the clearance of Lassa virus appears to be independent of antibody formation and presumably depends on the cell-mediated immune (CMI) response. That the major immune response in human Lassa virus infection may be CMI-dependent is supported by recent experience with experimental Lassa vaccines in primates (Fisher-Hoch *et al.*, 1989; see also Chapter 15). A vaccinia virus recombinant vaccine expressing the surface glycoproteins of Lassa virus confers partial protection from Lassa challenge without eliciting neutralizing antibodies.

On the other hand, a second recombinant virus expressing the Lassa virus nucleoprotein produced a good IgG response by immunofluorescence. However, this vaccine is ineffective in protecting primates from lethal challenge with Lassa virus and may even accelerate the disease course (Fisher-Hoch, unpublished observations). During natural virus replication, nucleoprotein is produced in excess of glycoprotein, and antibody measured against this product *in vitro* is dominant. The role of this antibody in human infection could be important in the generation of disease.

These studies led to the conclusion that the cellular immune response must be critical in virus clearance and protection against disease possibly by limiting the extent of viral replication. Reinfection following natural Lassa infection does occur in humans, but it does seem that clinical disease does not ensue (McCormick, unpublished observation). It is possible that the apparent paralysis of the CMI component of the immune system during acute disease could also be associated with the host derived platelet inhibitory factor mentioned earlier. Perhaps this factor is itself an aberrant product of the acute immune response with very broad inhibitory effects.

C. Argentine and Bolivian Hemorrhagic Fevers

Argentine hemorrhagic fever (AHF) and Bolivian hemorrhagic fevers (BHF) are clinically similar diseases caused by arenaviruses related to Lassa virus (Aribalzaga, 1955; Rugiero *et al.*, 1964a,b,c; Maiztegui, 1975; MacKenzie *et al.*, 1964; Weissenbacher *et al.*, 1987; Johnson *et al.*, 1965; Peters *et al.*, 1974). After an incubation period of about 12 days, both AHF and BHF have insidious onset of a nonspecific illness consisting of malaise, high fever, severe myalgia, anorexia, lumbar pain, epigastric pain and abdominal tenderness, conjunctivitis, and retroorbital pain, often with photophobia. Reports of subclinical or asymptomatic infections are rare. There is no lymphadenopathy or splenomegaly. Although there may be a pharyngeal enanthem (mucous membrane erruption), there is no sore throat or cough. These viruses presumably involve the respiratory tract to a lesser degree than Lassa virus. Another important difference is that there is marked erythema of the face, neck, and thorax, and petechiae may be observed.

In severe cases involvement of the gastrointestinal and nervous systems is manifest with nausea, vomiting, tremors, and convulsions. Intense proteinuria, microscopic hematuria with subsequent oliguria, and uremia are frequent. Localization of renal damage is shown by severe structural damage in the distal tubular cells and collecting ducts with relative sparing of the glomeruli and proximal tubules (Cossio *et al.*, 1975), confirmed by clinical observations of normal glomerular filtration rates, renal plasma flow, and creatinine clearance values (Maiztegui, 1975). Renal failure has been reported (Agrest *et al.*, 1969). Fatal cases demonstrate vascular collapse with hypotensive shock, hypothermia, and pulmonary edema. There is some electrocardiographic evidence of myocarditis (Ruggiero *et al.*, 1964b). Case fatality may be as high as 30% in clinically diagnosed BHF, and 16% in laboratory confirmed hospitalized patients with untreated AHF. In contrast to Lassa fever, bleeding is frequent, especially in AHF, and may be manifest as gingival hemorrhages, epistaxis, metrorrhagia (inappropriate menstrual bleeding), petechiae, ecchymoses, purpura, melena (stool darkened with altered blood), and hematuria (Maiztegui, 1975). Histological observations include large areas of intra-alveolar or bronchial hemorrhage. Gross examination of organs at necropsy shows petechiae on the organ surfaces, and ulcerations of the digestive tract have been described. Nearly half the patients with South American hemorrhagic fevers have hemorrhagic manifestation, most commonly epistaxis and/or hematemesis (Melcon and Herskovits, 1981). Platelet counts under 100,000 are invariable, and bleeding and clot retraction times are concomitantly prolonged. Though reductions of levels of factors II, V, VII, VIII, and X and of fibrinogen are observed, alterations in clotting functions are minor. Despite some reports of the presence of fibrinogen degradation products and absence of fibrinolysis, DIC, though it has been occasion-

ally reported, is apparently not a significant feature (Agrest *et al.*, 1969; Molinas and Maiztegui, 1981; Weissenbacher *et al.*, 1987). Recently a circulating inhibitor of platelet aggregation has been described, as in Lassa fever (Cummins *et al.*, 1990b).

Nevertheless, bleeding is not the cause of shock and death. As in Lassa fever, pulmonary edema is common in severely ill patients, and intractable shock accounts for the majority of deaths. Persistent hypovolemic shock in the face of intravascular volume expanders suggests that this is due to the loss of endothelial function and leakage of fluid into extravascular spaces. Clinical observations led to the conclusion that vascular endothelial dysfunction and subsequent circulatory failure are also important in AHF and BHF (Rugiero *et al.*, 1964a). Microscopic examination shows a general alteration in endothelial cells and mild edema of the vascular walls, with capillary swelling and perivascular hemorrhage.

Fifty percent of AHF and BHF patients have acute neurological symptoms, such as tremors of the hands and tongue, progressing in some patients to delirium, oculogyrus, and strabismus. Meningeal signs and cerebrospinal fluid abnormalities are rare. As in Lassa fever, the pathology of central nervous system involvement is obscure, and there is, again, no evidence for direct infection. A late neurological syndrome has also been described, consisting mainly of cerebellar signs (Melcon and Herskovits, 1981; Maiztegui *et al.*, 1979; Enria *et al.*, 1986).

Virus titers in serum are not as high as in Lassa fever, but the infection is also apparently pantropic (Weissenbacher *et al.*, 1975). Outcome may, again, be related to virus titer in blood or tissues, though definitive studies demonstrating this have not been published. Electron microscopy studies have shown intracytoplasmic and intranuclear inclusions and marked nonspecific cellular damage in all organs examined. Immunofluorescence studies suggest that viral antigen but no immunoglobulins or C3 are associated with these damaged cells. Clinical studies reveal activation of the complement system, but no evidence of immune complex formation (de Bracco *et al.*, 1978). Cellular damage is probably mainly due to direct viral replication, rather than immune processes (Maiztegui, 1975; Maiztegui *et al.*, 1975).

The role of antibody in Junin and Lassa virus infections appears to be different (Cossio *et al.*, 1975; de Bracco *et al.*, 1978). There may be leukopenia with the thrombocytopenia (Rugiero *et al.*, 1964b). The antibody response to Junin virus may be very effective in clearing virus during acute infection and may also be sufficient to protect against future infections. Antibody, especially neutralizing antibody, is detectable at the time the patient begins to recover from the acute illness, and the therapeutic efficacy of immune plasma in patients with Junin infection is directly associated with the titer of neutralizing antibody in the plasma given (Enria *et al.*, 1984). Although elements of a CMI response to Junin virus have been shown, its importance in virus clearance and

subsequent protection is not known. Interferon levels in patients with AHF may be very high (up to 64,000 IU/ml), and these high levels correlate with severity of disease and with outcome (Levis *et al.*, 1984, 1985).

VI. SUMMARY

The pathophysiology of arenavirus infections in rodents, nonhuman primates, and humans is discussed. Arenaviruses naturally infect rodent hosts in which the viruses are normally persistent but silent, with minimal histopathology. Transmission is *in utero* or at birth. In the rodent infected as an adult, acute, self-limiting disease may occur. Nonhuman primates develop severe, hemorrhagic disease and death, with pathology resembling that in humans when experimentally inoculated with AHF, BHF, or Lassa virus, and sometimes also with LCMV.

In humans, LCMV infection produces a lymphocytic choriomeningitis of varying severity, but usually mild and, though convalescence is prolonged, self-limiting and without sequelae. The virus replicates in the CNS where there are dense infiltrations of lymphocytic cells. Lassa fever and AHF and BHF have case fatality of about 16% in hospitalized patients, characterized by tissue and pulmonary edema with prominent hypovolemic shock and adult respiratory distress syndrome (ARDS). The hemostatic defect is characterized by platelet dysfunction rather than coagulation cascade activation, with little evidence for major hepatorenal failure or organ destruction by direct viral replication.

Bleeding with characteristic severe thrombocytopenia is more common in AHF and BHF than Lassa fever. In Lassa fever, despite normal platelet counts, an inhibitor of platelet function has been demonstrated that may affect the function of other cells, such as lymphocytes and endothelial cells. DIC appears to be confined to the terminal phases of all these diseases. Acute encephalopathy is seen in severe Lassa fever without evidence of direct viral invasion of the CNS, and in all three hemorrhagic fevers, late neurological syndromes are observed. In Lassa fever, as many as one-third of patients may develop a sensorineural hearing deficit in the early phase of convalescence, which is some cases may be total and permanent.

Lassa fever is particularly severe in the third trimester of pregnancy, possibly owing to the immunomodulation of pregnancy. Neutralizing antibodies are produced in AHF, and convalescent serum is effective treatment, whereas in Lassa fever neutralizing antibodies are, if present at all, low titer and evolve late in convalescence. Cell mediated immunity is probably critical in virus clearance and resistance to reinfection.

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