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Arenaviruses other than Lassa virus

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Abstract

The family *Arenaviridae* includes 23 viral species, of which 5 can cause viral hemorrhagic fevers with a case fatality rate of about 20%. These five viruses are Junin, Machupo, Guanarito, Sabia and Lassa virus, the manipulation of which requires biosafety level 4 facilities. They are included in the Category A Pathogen List established by the Center for Disease Control and Prevention that groups agents with the greatest potential for adverse public health impact and mass casualties whether a situation characterized by a ill-intentioned abuse of natural or engineered arenavirus would be encountered. The aims of this article are to (i) summarize the current situation; (ii) provide information to help anticipating the effects to be expected in such a situation; and to (iii) emphasize the need for fundamental research to allow the development of diagnostic, prevention and therapeutic tools as countermeasures to weaponized arenaviruses.
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1. Virology

1.1. Introduction

Arenavirus particles are spherical to pleomorphic with a diameter ranging from 50 to 300 nm, with the average diameter of spherical particles being 120 nm. They possess a dense lipid-containing envelope covered with 8–10-nm-long club-shaped projections. Ribosomes (20–25 nm) are present inside the virions and are responsible for the "sandy" appearance of the virus by electron microscopy, hence the name arenavirus (arena: sand in Latin). The viruses are rapidly inactivated at 56 °C, at pH below 5.5 or above 8.5, or by exposure to UV and/or gamma irradiation. Arenaviruses possess a single-stranded bi-segmented RNA genomes. Each segment encodes two different proteins in two non-overlapping reading frames of opposite polarities, separated by an intergenic non-coding region with the potential to form one or more hairpin structures. The 5′- and 3′-ends of each segment are non-coding and the 19 terminal nucleotides of one extremity correspond to the reverse complementary sequence of the 19 nucleotides at the other extremity. The large (L) genomic segment (∼7200 nt) encodes the viral RNA-dependent RNA polymerase and a zinc-binding protein, the role of the latter not being fully elucidated. The small (S) genomic segment (∼3500 nt) encodes two structural proteins: the nucleoprotein (NP) and the glycoprotein precursor (GPC). The GPC is secondarily cleaved into the envelope proteins G1 and G2 (Southern, 1996).

1.2. Classification

The family *Arenaviridae* consists of a unique genus (*Arenavirus*) that currently contains 23 recognized viruses (Table 1) which have been classified according to their antigenic properties into two groups. The Tacaribe serocomplex (or New World group) includes viruses indigenous to the Americas. The Lassa–lymphocytic choriomeningitis serocomplex (LCM) (or Old World group) includes viruses indigenous to Africa and the ubiquitous LCM virus (Clegg et al., 2000; Moncayo et al., 2001). The geographic distribution of each arenavirus is determined by the range of its reservoir species. LCM virus is the only arenavirus to have a worldwide distribution due to its association with the ubiquitous *Mus musculus*. Other arenaviruses are distributed either in the New World or in Africa. To date, no arenavirus other than LCM virus has been isolated in Europe, Asia or Oceania. The most important viral antigens are those of the nucleoprotein and glycoprotein, with the nucleoprotein antigens being the most conserved among arenaviruses. The analysis of quantitative immunological relationships based on these antigens shows the basic split between Old World and New World viruses. Fine-scale

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Table 1

Virus	Acronym	Evolutionary lineage	Distribution[a]	Reservoir	Human significance	Cat.A
Junin	JUNV	NW-B	Argentina	*C. musculinus*	Yes	Yes
Machupo	MACV	NW-B	Bolivia	*C. callosus, C. laucha*	Yes	Yes
Guanarito	GTOV	NW-B	Venezuela	*Z. brevicauda*	Yes	Yes
Sabia	SABV	NW-B	Brazil	Unknown	Yes	Yes
Lassa	LASV	OW	Nigeria, Ivory Coast, Guinea, Sierra Leone	*Mastomys* sp.	Yes	Yes
Lymphocytic choriomeningitis	LCMV	OW	worldwide	*M. musculus*	Yes	No
Mobala	MOBV	OW	Centrafrican Republic	*Praomys* sp.	NR	No
Mopeia	MOPV	OW	Mozambique	*Mastomys natalensis*	NR	No
Ippy	IPPYV	OW	Centrafrican Republic	*Arvicanthus* sp.	NR	No
Flexal	FLEV	NW-A	Brazil	*Oryzomys* spp.	Yes	No
Pichinde	PICV	NW-A	Colombia	*O. albigularis*	NR	No
Parana	PARV	NW-A	Paraguay	*O. buccinatus*	NR	No
Allpahuayo	ALLV[b]	NW-A	Peru	*Oecomys bicolor*	NR	No
Pirital	PIRV	NW-A	Venezuela	*Sigmodon alstoni*	NR	No
Tacaribe	TCRV	NW-B	Trinidad	*Artibeus* spp. (bat)	Yes	No
Cupixi	CPXV[b]	NW-B	Brazil	*O. capito*	NR	No
Amapari	AMAV	NW-B	Brazil	*O. capito–Neacomys guianae*	NR	No
Oliveros	OLVV	NW-C	Argentina	*Bolomys obscurus*	NR	No
Pampa	PAMV	NW-C	Argentina	*Bolomys* sp.	NR	No
Latino	LATV	NW-C	Bolivia	*Calomys callosus*	NR	No
Whitewater arroyo	WWAV	NW-rec	Southwestern USA (NM, TX, UT, OK)	*Neotoma albigula, N. mexicana, N. micropus, N. cinerea*	Yes	No
Tamiami	TAMV	NW-rec	Florida, USA	*Sigmodon hispidus*	NR	No
Bear Canyon	BCNV[b]	NW-rec	California, USA	*Peromyscus* sp.	NR	No

Cat.A: virus included in the Category A Pathogen List as defined by the CDC, Cat.A arenaviruses are biosafety level 4 agents. NR: not reported; NW: New World; OW: Old World. Recombinant lineage as reported previously (Charrel et al., 2001a,b; R.N. Charrel, personal data).

[a] Listed countries are included on the basis of virus isolation, not serology-based data.

[b] Acronym is tentatively proposed because the corresponding virus is not currently recognized by the International Committee of Taxonomy of Viruses (Clegg et al., 2000).

identification of arenaviruses is achieved via neutralization tests, which depend on highly specific epitopes of the envelope glycoproteins (Peters et al., 1996a).

Genetic classification (at the present time mostly based on a partial sequence of the NP gene) is congruent with serological analyses (Bowen et al., 1996; Charrel et al., 2001a,b). It also indicates that the 23 arenaviruses represent 4 phylogenetic lineages. The Old World (Lassa–LCM serocomplex) lineage comprises five viruses (LCM, Lassa, Mopeia, Mobala and Ippy) and is deeply rooted to the three New World (Tacaribe serocomplex) lineages, designated A, B and C. Lineage A includes three North American viruses (Whitewater Arroyo, Tamiami and Bear Canyon) and five South American viruses (Pirital, Pichinde, Flexal, Parana, Allpahuayo). Lineage B includes seven South American viruses (Sabia, Junín, Machupo, Guanarito, Amapari, Tacaribe, Cupixi). Lineage C comprises three South American viruses (Oliveros, Latino and Pampa) (Table 1).

1.3. Virus transmission

Rodents are the natural hosts of the arenaviruses (Childs and Peters, 1993). Humans usually become infected through contact with infected rodents, or inhalation of infectious rodent excreta or secreta. Therefore, the dynamics of rodent populations are probably one of the major determinants of the epidemiology of human infection (Mercado, 1975).

Whenever a virus-natural host relationship has been characterized, one or a low number of closely related rodent species have been found to be the principal hosts of a given arenavirus. The only exception is Tacaribe virus, which is associated with bats (Table 1). The factors implicated in the evolution of arenaviruses are complex and probably include genetic recombination and re-assortment, and also "horizontal" transfer of virus strains between different populations of rodents (Charrel et al., 2001a; Charrel et al., 2002). However, it is now widely agreed that the diversity of arenaviruses is mostly the result of a long-term, shared evolutionary relationship (termed co-evolution or -speciation) between the viruses of the family *Arenaviridae* and the rodents of the family Muridae (Bowen et al., 1997). Chronic infection of the host (accompanied by a chronic viremia or viruria) appears, therefore to be crucial for the long-term persistence of arenaviruses in nature.

Due to this specific association, the geographic area where a particular arenaviral disease is found is limited by the distribution of the corresponding rodent host(s). Aerosols from infected rodent urine are probably the most important mechanism of disease transmission. Infectious aerosols are strongly suspected when there is no possibility

of direct contact between the infected person and the source or when large numbers of persons in an enclosed space are infected. Cases of aerosol-acquired LCM virus infection have been reported (Hinman et al., 1975; Biggar et al., 1975). For example, the large number of laboratory infections indirectly attests to the aerosol infectivity of arenaviruses, and this is further supported by direct studies of LCM and Junin virus infections by quantitative exposure of experimental animals to small particle aerosols (Danes et al., 1963; Stephenson et al., 1984; Lehmann-Grube, 1984). Endemic Junin virus infections principally affect corn-harvesting machinery drivers with no direct rodent contacts and are virtually certain to be aerosol-transmitted (Peters et al., 1996a).

1.3.1. Human-to-human transmission

There has never been any suggestion of person-to-person transmission of LCM virus. Hundreds of cases of Argentine and Bolivian hemorrhagic fevers have been cared for in the endemic areas without suspicion of secondary cases in medical personnel, despite the common occurrence of hemorrhages. However, Machupo virus has clearly been responsible for severe nosocomial outbreaks, in which all cases were associated with a single index case that had returned from an endemic region. The only hospital-based outbreak recognized resulted in four secondary cases followed by a tertiary case acquired from a necropsy incident; all but one died. Recently, an epidemic was reported in which seven members of the same family were infected, with a fatal outcome for six (CDC, 1994). The common features of reported nosocomial outbreaks are: (i) the index case was critically ill and died; (ii) aerosol spread was the most likely explanation for the route of infection of at least some of the secondary cases; (iii) lethality was high; and (iv) transmission ceased after the secondary or tertiary cases. Such outbreaks are sufficiently rare that the overall pattern of contagion in hospitals is low and the most dangerous route is parenteral exposure through improperly sterilized needles, autopsy accidents, or other failings in techniques (Peters et al., 1996b). This has led to the reasonable recommendation that ordinary mask, gown and glove isolation procedures should be used to protect medical staff; additional respiratory protection would be a useful precaution in some settings. Although common clinical laboratory tests performed on viremic blood samples in Argentina did not result in an increased risk to workers, it must be noted that both series of tests and series of workers exposed are small. Necropsy is extremely hazardous, and two scalpel accidents have led to a fatal outcome for the attending pathologists (Carey et al., 1972; Peters et al., 1974). It must be remembered that all arenaviruses manipulated in virology labs have been infectious to humans under circumstances implicating aerosol spread. The data demonstrating nosocomial and human-to-human transmission of several arenaviral diseases highlights the necessity for bio-containment precautions when dealing with arenavirus-infected patients and materials.

1.4. Human significance

1.4.1. Viral hemorrhagic fever: South American arenaviruses: Junin, Machupo, Guanarito, Sabia

All South American arenaviruses have been identified during the second half of the 20th century. However, arenaviruses are quite ancient, and it is certain that they have existed in nature for years as silent zoonotic foci. The hypothesis was raised that at least some of the epidemic diseases that caused catastrophic demographic collapse in Mexican populations during the 16th century may be due to arenaviruses (Marr and Kiracofe, 2000). The more likely candidate lasted between 1576 and 1580 and was named “Huey Cocoliztli” which can be translated by “great pestilence”. This outbreak also made casualties among Spaniards and Africans.

In the 1950s, a new severe hemorrhagic disease emerged in the Buenos Aires province of Argentina. It was named “Argentine hemorrhagic fever” (AHF), and found to be caused by the Junin virus (Parodi et al., 1958). Junin virus is hosted by rodents (*Calomys musculinus*) widely distributed in the region. The case-fatality rate of AHF is approximately 20% in the absence of specific therapy. Since the 1950’s, Junin virus is estimated to have caused ∼30,000 cases of symptomatic disease. The region at risk has been progressively expanding into north-central Argentina and almost 5 million of humans are today considered to be at risk for AHF (Enria and Feuillade, 1998). AHF is typically a seasonal disease, with a peak of frequency occurring during the corn harvesting season (March–June); during this period, 75% of the infected people are male agricultural workers who are contaminated by inhalation of infected aerosols produced from rodent excreta or from rodents caught in mechanical harvesters (Maiztegui, 1975). Since the late 1980s, the epidemiology of AHF has been dramatically modified by the development of a live-attenuated vaccine.

Bolivian hemorrhagic fever (BHF) was first described in Bolivia in 1959. The causative agent (Machupo virus) was isolated in 1963. Its natural host is the rodent *C. callosus*. The virus is responsible for large outbreaks with a case-fatality rate of about 20%. From 1962 to 1964, there was a series of outbreaks involving more than 1000 patients, of which 180 died. After 20 years without any reported cases (presumably mostly due to effective rodent control), a new series of 19 cases was reported in the 1990s. Nosocomial transmission of Machupo virus was clearly demonstrated (Peters et al., 1974), although most of the recorded infections were acquired by direct contact with *Calomys* rodents or by aerosol through infected excreta.

Venezuelan hemorrhagic fever (VHF) was recognized in 1989 in Venezuela, and is caused by Guanarito virus. The main rodent hosts of the virus are thought to be *Zygodontomys brevicauda* and *Sigmodon alstoni*. To date, ∼200 confirmed cases of VHF have been reported (de Manzione et al., 1998). The number of reported human cases has spontaneously dropped between 1992 and 2002, despite

the continuous circulation of virus in the rodent population during this period (Weaver et al., 2000). A new outbreak was detected in 2002; so far, 18 cases have been recorded, 2 of which had a fatal outcome (Promed, 2002). The reasons behind the disappearance and the secondary re-emergence of the disease are unknown.

Sabia virus was isolated in 1994 after it caused a fatal case of hemorrhagic fever in Brazil; subsequently, two additional cases were reported in laboratory workers. The natural rodent host of the virus has not been identified.

Junin, Machupo, Guanarito, and Sabia viruses, known to cause a severe hemorrhagic fever, are included in the Category A Pathogen List as defined by the CDC, and listed as Biosafety Level 4 (BSL-4) agents (CDC, 2000a).

The clinical picture of these South American hemorrhagic fevers is almost identical regardless of the virus responsible for the disease (Peters et al., 1996a; Harrison et al., 1999; de Manzione et al., 1998). The incubation period is typically 7–14 days with extreme cases extending from 5 to 21 days. Secondary infection to very high-load inoculum may result in the reduction of the incubation period to 2 days. The onset is gradual with fever and malaise, secondarily joined by myalgia, back pain, headache and dizziness. Hyperesthesia of the skin is common. The most important clinical manifestations are hemorrhagic and neurological. They can be present separately or combined.

Hemorrhagic manifestations: petechiae of the skin and haemorrhaging from the gums, vagina and gastrointestinal tract beginning around the fourth day of illness, herald the advent of hypovolemic clinical shock. Blood loss is usually minor, so the hematocrit generally increases as the capillary leak syndrome, the hallmark of the disease, becomes more severe. Bleeding and prothrombin time may be prolonged, and reductions of factors II and VII of the coagulation cascade have been noted. Renal function generally is delayed until shock occurs, but urinary protein may be high. Pronounced thrombocytopenia may occur.

Neurological manifestations, tremor of the hands and an inability to swallow or to speak clearly, may develop, and these can progress to grand mal convulsions, coma, and death in the absence of significant capillary leak or hemorrhagic signs. Death usually occurs 7–12 days after onset. Those who survive generally recover completely without permanent sequelae, although transient loss of scalp hair and Beau lines in digital nails are a common consequence of the high and sustained fever.

Symptoms that appear to be more specifically associated with one or other of the viruses have been reported (Vainrub and Salas, 1994). While the frequency of clinical and laboratory findings are identical for Junin and Machupo virus infections, there are clear differences with Guanarito virus infections: pharyngitis, vomiting and diarrhea are more frequently observed with Guanarito virus; in contrast, petechiae, erythema, facial edema, hyperesthesia of the skin and shock are more frequently observed in the case of Junin or Machupo infections. Fatal outcome of Junin virus infection is more frequently observed in pregnant women in the last trimester, and a high fetal mortality is associated with both Junin and Machupo virus infections (Johnson et al., 1967).

1.4.2. Central nervous system and congenital malformations

Lymphocytic choriomeningitis (LCM) virus is an agent of acute central nervous system disease (Barton and Hyndman, 2000), and is also responsible for congenital malformations (Barton et al., 1993).

1.4.3. Other features

Aerosol infections of laboratory workers have been repeatedly reported in laboratories where arenaviruses are manipulated. This underlines the requirement for very careful manipulation when handling these agents. Very little is known about the health consequences of infection with arenaviruses other than Junin, Machupo, Guanarito, Sabia and LCM virus. Pichinde virus has resulted in numerous seroconversions without any notable clinical significance (Buchmeier et al., 1974). Flexal and Tacaribe virus have caused febrile illnesses in laboratory workers (Peters et al., 1996a,b; Buchmeier et al., 1974). Flexal virus has resulted in two symptomatic laboratory infections and should be regarded as potentially dangerous (Peters et al., 1996a,b). Tacaribe virus has resulted in a single case of febrile disease with mild central nervous system symptoms (Peters et al., 1996a).

In 1999 and 2000, three fatal cases of illness were reported in California, and the association with Whitewater Arroyo virus infection (a recently described arenavirus indigenous to the southwestern US and hosted by *Neotoma* rodents), was invoked, based on PCR and sequencing results (CDC, 2000b). The patients infected with Whitewater Arroyo virus were healthy prior to the virus infection and there was no history of travel outside California during the 4 weeks preceding the illness. In one case the virus was probably acquired via the aerosol pathway during the removal of rodent droppings from the home. The onset was characterized by non-specific febrile symptoms including fever, headache and myalgia. All patients presented with acute respiratory distress syndrome and two developed liver failure and hemorrhagic manifestations. Death occurred within 8 weeks after the onset. The direct implication of the Whitewater Arroyo virus in the pathophysiology of these clinical cases remains to be formally established.

1.5. Diagnosis

1.5.1. Direct diagnosis

Virus isolation can be achieved by propagation in cell culture (particularly in Vero cells). Since the cytopathic effect is inconsistent, arenavirus-infected cells are usually detected by direct immunofluorescence tests. For South American arenaviruses causing hemorrhagic fevers, the delay for

isolation in Vero cells is 1–5 days, much faster than animal inoculation that requires 7–20 days before illness develops. Virus can be isolated from serum and throat washings collected 3–10 days after onset, less frequently from urine. Specifically, Machupo virus is recovered from only 20% of acute phase sera and even less frequently from throat washings or urine. It should be noted that the virus is not recovered from CSF of patients presenting CNS symptoms when infected by the South American agents. Viral RNA can be detected by RT-PCR from serum, plasma, urine, throat wash and various tissues. The sequencing of the amplified region can be used for identification of the implicated virus. RT-PCR-based diagnosis offers the advantage of reducing the delay to response and demonstrates a greater sensitivity compared to cell culture. Consequently, molecular tests based on the RT-PCR methodology are developed with increasing frequency for the diagnosis of arenaviral infections (Lunkenheimer et al., 1990). Specific primers designed to amplify certain arenaviruses have been proposed but their use for diagnostic purposes in epidemic conditions has still not been reported in the literature. For Junin and Machupo virus infection specifically, this technique is the only one to be sensitive enough to detect low viremia encountered during the early period when immune plasma therapy can still be used effectively (Lozano et al., 1995). The main drawback of molecular methods is the need for specific equipment to be fully applicable in the field conditions where an outbreak occurs.

Additional techniques for direct detection of viral presence in tissues are being actively developed; these include in situ nucleic acid hybridization techniques and antigen detection ELISA. The frequent circulation of two or more arenaviruses in the same geographic area has encouraged the development of RT-PCR systems that can either specifically amplify selected arenaviruses, or amplify all recognized arenaviruses by a combination of degenerate primers located in the conserved regions of the genome (Lozano et al., 1997; Bowen et al., 1996). Again, there are no reports of the use of these strategies in practical situations. At the present time, the diagnostic strategy based on degenerate primers is hampered by the lack of genomic data outside a partial region of the nucleoprotein gene (Bowen et al., 1996, 1997). Despite an apparent decrease in occurrence, LCM virus continues to circulate in rodents and to infect humans, as established by sero-epidemiological studies (Childs et al., 1991; Stephensen et al., 1992). The incidence of LCM virus as the etiologic agent underlying a substantial number of cases of aseptic meningitis and encephalitis has been established by serology-based diagnostic methods (Skinner et al., 1976; Meyer et al., 1960). To date, there is only one publication that discusses the use of molecular methods for the diagnosis of LCM virus infection (Park et al., 1997). Although there is no report in the literature of the diagnosis of an LCM virus infection via molecular assay techniques, the prospects of this technique are favorable. In regard of Junin virus, RT-PCR is to date the only method available for rapid diagnosis. For these reasons, several RT-PCR-based assays have been developed over a 20-year period (Bockstahler et al., 1992; Lozano et al., 1993, 1995). However, no report is available concerning the use of these techniques in an epidemic situation to manage the outbreak, or more specifically to detect Junin virus infection at an early stage in order to initiate the immune plasma therapy that has been proven to reduce mortality from 30 to 1% (Maiztegui et al., 1979). Such techniques should be progressively developed with reduced cost and increased ease of use.

1.5.2. Indirect diagnosis

Serological diagnosis is based on the detection of antibodies to the nucleoprotein and/or the envelope glycoproteins. It must be emphasized that blood obtained in the early convalescence stage may be infectious despite the presence of antibodies, and therefore should be handled accordingly. Immunofluorescence (IF) tests and ELISAs using lysates of infected cells principally permit the detection of antibodies to the nucleoprotein. Because this antigen is the most conserved among arenaviruses, cross reactions are frequent. However, indirect IF tests remain a simple, inexpensive, rapid and sensitive assay for the detection of arenavirus infections. Serological diagnosis is usually made by the demonstration of a fourfold rise in the titer of specific antibody in two sequential serum samples. A high IgG antibody titer or the presence of specific IgM is indicative of a probable case. Antibodies specific to South American arenaviruses appear 12–30 days after the onset, and this often correlates with clinical improvement (Peters et al., 1973). Seroneutralization tests allow the detection of antibodies to the envelope glycoproteins, which are far more specific than those directed to the nucleoprotein. Consequently, neutralization is the test of choice for differentiating viral strains of arenaviruses, for confirmation of unexpected results, and for detection of infection from the distant past. Based on data available for LCM virus infections, IF assays appear to be the method of choice for making a rapid diagnosis soon after infection. Neutralizing antibodies appear relatively late after infection, and therefore cannot be recommended for detection of seroconversion early in convalescence. However, because neutralizing antibodies persist for many years (presumably lifelong), they are well suited for confirmation of unexpected results and for detection of infection from the distant past. Many studies have determined that complement fixation tests are of little value for the serological diagnosis of arenavirus infections.

To summarize briefly, rapid arenavirus diagnosis should be based on IF or ELISA tests; positive sera should be confirmed by the assessment of seroconversion; precise identification of the viral species involved should be based on neutralization tests. Serological diagnostic assays have been tested in the conditions of an outbreak and proven to be effective.

In the endemic area of Argentina, asthenia and dizziness accompanied by petechiae and conjunctival congestion

have shown to be indicative for the diagnosis of AHF; the presence of leukopenia, thrombocytopenia and proteinuria further reinforces the suspicion of arenaviral infection based on clinical findings. The association of a platelet count $<100{,}000/mm^3$ and a white blood cell count <2500 or $<4000/mm^3$ was reported to have a sensitivity of 87 and 100%, respectively and a specificity of 88 and 71%, respectively (Harrison et al., 1999).

For South American viruses causing hemorrhagic fever, the differential diagnosis principally includes yellow fever, dengue hemorrhagic fever, viral hepatitis, leptospirosis, hemorrhagic fever with renal syndrome caused by hantaviruses, rickettsial diseases, typhoid, sepsis with disseminated intravascular coagulation, and in the case of CNS involvement, viral encephalitis.

2. Control, prevention and therapy

Arenaviral hemorrhagic fevers can be approached by different prophylactic and/or therapeutic strategies: (i) vaccination; (ii) administration of high-titer antibodies through convalescent serum; and (iii) treatment with antiviral drugs.

2.1. Control

During investigations of South American hemorrhagic fevers, several studies have been designed to address the possible link between annual outbreaks of human disease and levels of virus infestation in rodent populations (Maiztegui et al., 1986). The number of Junin virus-infected rodents was higher in a year with a large number of AHF cases than in a year when a small number of human cases were observed. High prevalences (5–57%) of Junin virus infection are reported from *C. musculinus* in epidemic areas of AHF (Sabattini et al., 1977), but no infection or lower levels of infection (Mills et al., 1991) are reported outside these locations. This suggests that the control of arenavirus in nature could be performed through the regulation of rodent populations. The prevalence of infected rodents clearly is a potentially crucial factor in determining the risk to humans, but there is remarkably little data that bears directly on this issue. Rodent control aimed at reducing the incidence of arenaviral disease in humans has been reported as successful only in the isolated case of Machupo virus in Bolivia (Kuns, 1965; Mercado, 1975). In this instance intensive house-to-house trapping of *C. callosus* was shown to coincide with a dramatic decrease in the incidence of BHF. In parallel, BHF cases seem to occur in a setting of high population levels and high infection rates of *C. callosus*, although there are no quantitative studies (Johnson et al., 1967). Naturally acquired human infections with LCM virus appear to reflect its zoonotic distribution in *M. musculus* in Europe (Blumenthal et al., 1968), England (Smithard and Macrae, 1951) and the US (Armstrong and Sweet, 1939). The infection is focal in *M. musculus* populations and preferentially spreads to humans in a rural setting or when rodents invade dwellings either to escape winter conditions or because of suboptimal neighborhood hygiene. This results in a fall-winter preponderance of LCM virus-induced aseptic meningitis (Adair et al., 1953; Meyer et al., 1960). Alternatively, LCM virus may contaminate humans through infection of an intermediate host that is capable of excreting large quantities of virus over prolonged periods. The hamster-associated cases and the report of human disease acquired from laboratory mice are good examples in this regard (Hinman et al., 1975; Deibel et al., 1975; Vanzee et al., 1975; Rousseau et al., 1997).

2.2. Prevention

AHF is the only South American arenaviral hemorrhagic fever for which extensive studies have been conducted to develop and evaluate a first candidate vaccine. Early attempts were based on the use of Tacaribe virus to elicit protection in guinea pigs and primates against lethal challenge with Junin virus Carballal et al., 1987a,b). Alternative attempts based on killed or live Junin virus proved unsuccessful until the production of a live-attenuated vaccine, named Candid 1, which was developed through a cooperative international effort (Barrera Oro and McKee, 1991). Protective efficacy of Candid 1 was assessed in non-human primates (*Macacus rhesus*) by obtaining full-protection against a virulent strain. Candid 1 is most likely to function by inducing a neutralizing antibody response (McKee et al., 1992) together with development of a virus-specific antibody-dependent cellular cytotoxicity (Peters et al., 1987). Immunogenicity and safety of Candid 1 were tested in *Rhesus* macaques by subcutaneous injection of increasing doses: no clinical and/or biological signs were noted; these favorable results led to the consideration of Candid 1 vaccine for phase I and II human trials (Maiztegui et al., 1998).

More recent work consisted of large-scale evaluation in an AHF endemic region. A prospective, randomized, double-blind, placebo-controlled trial was initiated among 6500 male agricultural workers in the AHF-endemic region. Among the 23 laboratory-confirmed AHF cases that were recorded, 22 occurred in patients which were given the placebo. Additionally, no serious side effects could be attributed to vaccination (Maiztegui et al., 1998). More than 150,000 at-risk persons living or working in the AHF-endemic area have received Candid 1, and since then a substantial reduction in reported AHF cases has been documented (Maiztegui et al., 1998). Whether Candid 1 vaccine may prove useful to cross-protect populations against BHF infection, and possibly against VHF, is unknown.

2.3. Therapy

Non-specific treatment of arenaviral hemorrhagic fever cases is based on monitoring and correcting fluid, electrolyte and osmotic imbalance. Hemorrhaging can be treated with

clotting factor and/or platelet replacement whereas the most serious symptoms usually are caused by capillary leakage.

2.3.1. Immune therapy

The case-fatality ratio of AHF which is ~20% without specific treatment drops to less than 1% when patients are treated by the administration of convalescent serum (Harrison et al., 1999). For Junin virus infection, immune serum therapy is effective when given within the first 8 days of illness (Enria et al., 1984; Maiztegui et al., 1979). There is experimental evidence suggesting that immune plasma may work through viral neutralization. Despite the recognized efficacy of convalescent plasma for treatment of Machupo virus infection, the low number of cases since the initial outbreak and the absence of a program for collection and storage of BHF immune plasma make a future problem of plasma shortage likely. For these reasons, antiviral-based approaches may have a more promising future for BHF treatment. Among AHF cases treated by immune therapy, one patient out of 10 secondarily developed transient CNS manifestations, such as headache and tremors.

2.3.2. Ribavirin
[1-beta-D-ribofuranosyl-1,2,4-triazole-3-carboxamide]

Ribavirin's primary target is supposed to be the enzyme IMP dehydrogenase that converts IMP to XMP (Streeter et al., 1973); viral RNA synthesis is reduced through a marked decrease of the level of GMP, GDP and GTP. This is supported by the demonstration that exogenous addition of guanosine, but not other nucleosides, reverses the antiviral and cytotoxic effects of ribavirin (Scholtissek, 1976). GTP pool level reduction results in a decrease of the ATP pool level since GTP acts as cofactor for the conversion of IMP to succinyl AMP by the adenylsuccinate synthetase. Ribavirin treatment leads to a rise of the intracellular pool level by (i) direct inhibitory effect on IMP dehydrogenase; (ii) indirect inhibition of adenosuccinyl synthetase secondarily to reduced GTP pool levels; (iii) direct interference with viral RNA synthesis (Eriksson et al., 1977); (iv) direct interference with the viral mRNA capping guanylation process; and (v) by direct interference with primer generation and elongation during viral RNA transcription.

The first animal experiments conducted to address the usefulness of ribavirin either for post exposure prophylaxis or for treatment were carried out with Lassa virus (Stephen and Jahrling, 1979; Jahrling et al., 1980). Further studies showing that ribavirin was efficient both in vitro and in vivo to prevent the death of experimental animal models challenged with different arenaviruses (Smee et al., 1993; Lucia et al., 1989; Huggins, 1989) fuelled similar experiments with Junin virus (Rodriguez et al., 1986). Its efficacy (in prophylactic and therapeutic situations) was successfully tested on primate models (Weissenbacher et al., 1986; McKee et al., 1988). Despite ribavirin's likely efficacy in AHF patients, there is little data reported in the literature (Enria et al., 1987). The main side effects of ribavirin include thrombocytosis and severe anemia that resolve after administration is stopped.

In 1994, two patients infected with Machupo virus were treated with ribavirin (Kilgore et al., 1997); both recovered without sequelae. Despite the fact that no statistically valid conclusion can be drawn from these cases, they plead for more extensive clinical studies to evaluate the usefulness of ribavirin for the treatment of BHF.

In 1995, a case of laboratory-acquired Sabia virus infection occurred in US; the researcher was treated intravenously by ribavirin 3 days after infection and recovered without any sequelae (Barry et al., 1995); further tests did not detect neutralizing antibodies. Because there is only one documented case of naturally acquired fatal Sabia virus infection, it is impossible to draw conclusions concerning ribavirin's role in recovery. However, in light of its broad spectrum of action against distinct viruses within the *Arenaviridae* family, it is probable that most, if not all, arenaviruses are susceptible to ribavirin.

Protocols for ribavirin administration were defined for Lassa virus infections. With regards to therapeutic use, ribavirin should be given intravenously as follows: a loading dose of 30 mg/kg, then 16 mg/kg every 6 h for 4 days, then 8 mg/kg every 8 h for 6 days (CDC, 1988). Concerning prophylactic use for at-risk contact persons, the recommended regimen for ribavirin is oral administration with 500 mg every 6 h for 7 days (CDC, 1988). Although there is no data specific for South American BSL-4 arenaviruses, therapeutic and prophylactic regimens described for Lassa virus may be recommended. The recent introduction of ribavirin in the treatment of hepatitis C chronic infections can be interpreted as a large-scale phase IV trial, which demonstrated a rare occurrence of side effects with daily doses of 800–1200 mg.

In light of the data available for Lassa virus, it is justified to replace the total isolation of patients by simple barrier nursing techniques to prevent transmission to hospital personnel (CDC, 1988). Contacts should be asked to report any fever or acute symptoms occurring during a period of 21 days.

2.3.3. Other compounds

Compounds other than ribavirin have been tested for their efficacy on selected arenaviruses. The antibiotic pyrazofurin, 3-(beta-D-ribofuranosyl)-4-hydroxypyrazole-5-carboxamide, markedly inhibited in vitro replication of Pichinde, Lassa and LCM viruses, but failed to protect mice and guinea pigs against a lethal challenge with Pichinde virus (Canonico et al., 1982). *S*-Adenosylhomocysteine (AdoHcy) hydrolase inhibitors were evaluated for their inhibitory effects on the replication of the arenaviruses Junin and Tacaribe in Vero cells; they inhibited arenavirus replication within the concentration range of 1–10 μg/ml, while not being toxic for cell morphology or cellular DNA synthesis at a concentration of 100–400 μg/ml; however, only (S)-DHPA, DHCA, C-c3 Ado and adenosine dialdehyde could be considered as truly selective inhibitors.

Several IMP dehydrogenase inhibitors such as ribamidine (1-beta-D-ribofuranosyl-1,2,4-tiazole-3-carboxamide), tiazofurin (2-beta-D-ribofuranosylthiazole-4-carboxamide), selenazofurin (2-beta-D-ribofuranosylselenazole-4-carboxamide), FICAR (5-fluoro-1-beta-D-ribofuranosylimidazole-4-carboxamide), EICAR (5-ethynyl-1-beta-D-ribofuranosylimidazole-4-carboxamide) have shown efficacy against arenaviruses (De Clercq et al., 1991; Kirsi et al., 1983; Huggins et al., 1984; Sidwell et al., 1985). Animal experiments were conducted with Pichinde virus, and showed that the mortality of challenged Golden Syrian hamsters (LVG/Lak strain) was significantly reduced by intraperitoneal injection of ribamidine, a derivative of ribavirin, at a dosage of 32, 100 and 320 mg/kg for 10 days; the virus titers were reduced by 100- to >10,000-fold depending on the organs (Smee et al., 1993). Mortality of Machupo virus-infected chimpanzees was reduced comparatively to the control animals. Similar results were observed with guinea pigs and hamsters challenged with Pichinde virus (Lucia et al., 1989) or Junin virus (Kenyon et al., 1986).

Very high and selective inhibition was observed with the sulfated polysaccharides dextran sulfate, lambda-carrageenan, fucoidan, heparin and pentosan polysulfate: they inhibited virus replication at a concentration of 0.1–2.8 μg/ml, whereas the compounds were not inhibitory to cell growth even at a concentration of 200 μg/ml. (Andrei and De Clercq, 1990). The nucleoside analog, 3′-fluoro-3′-deoxyadenosine (3′F3′dAdo) was evaluated for its activity against LCM and Pichinde virus in Vero cell culture. A 50% inhibitory effect on virus plaque formation was obtained at a concentration of 7.7 and >32 mM for LCM and Pichinde virus, respectively (Smee et al., 1992). Recent studies showed that phenotiazines (trifluoperazine and chlorpromazine) have in vitro efficacy against Junin, Tacaribe and Pichinde virus (Candurra et al., 1996); the same group also reported inhibition of Junin virus replication by two myristic acids (DL-2-hydroxymyristic acid and 13-oxamyristic acid) (Cordo et al., 1999).

Binary combinations of ribavirin and the C-nucleoside analog selenazofurin (2-beta-D-ribofuranosylselenazole-4-carboxamide) were found to be synergistic against Pichinde virus in cell culture, but experiments were not further developed for toxicity reasons (Huggins et al., 1984).

3. Arenaviruses as bioterrorism agents

To date, the greatest concern regarding bioterrorism agents at the governmental level has been shown by the USA, who have designated the Centers for Disease Control and Prevention (CDC) as the leading agency for issues surrounding bioterrorism. The CDC has formed a Bioterrorism Preparedness and Response Office (BPRO) to address several major issues, such as: (i) planning activities; (ii) improvement of surveillance and epidemiologic capacities; (iii) rapid laboratory diagnostic capability; (iv) enhanced communications; and (v) medical therapeutics stockpiling (CDC, 2000a).

One of the first issues addressed by the BPRO was the determination of those biological agents that could be used in the event of a bioterrorist attack. The five arenaviruses known to cause viral hemorrhagic fever were listed as Category A pathogens (as established by the CDC); this grouping includes agents with the greatest potential for adverse public health impact with mass casualties, thus requiring the improvement of surveillance, laboratory diagnosis and stockpiling of specific medications.

The characteristics that justified inclusion of South American BSL-4 arenaviruses in the Category A list were associated with (i) the public health impact of the disease caused by these viruses; (ii) the case-fatality ratio (15–30%); (iii) the high public perception of, and reaction to, viral hemorrhagic fevers; (iv) the relative ease of obtaining the virus (in AHF-epidemic areas, the proportion of infected rodents can reach 50%).

Conditions in which BSL-4 arenavirus infections are "naturally acquired" are characterized by a relatively low inoculum to each exposed individual and a low attack rate in the population living in rural environment. In contrast, it is likely that a bioterrorist attack with BSL-4 arenaviruses would target a highly populated urban area to attempt massive airborne exposure of the largest possible number of persons. The anticipated consequences of such substantial virus dissemination are the following:

First, a massive inoculum may result in reduced incubation period and high viremia. According to data from existing literature, the person-to-person rate of transmission of South American arenaviruses is low. However, specific conditions such as an index patient experiencing high viremia have been associated with a higher transmission rate and higher mortality. It follows that massive airborne inoculation may be associated with a higher frequency of secondary cases. Second, a large number of secondary cases due to nosocomial blood-mediated transmission may be observed because of the disturbance created by an overwhelming flow of patients into emergency facilities. Third, the conjunction of a massive inoculum with a high population density may be responsible for an infection rate much higher than that observed in outbreaks of naturally acquired cases in South American endemic regions.

3.1. Why are arenaviruses good candidates for weaponization?

- Large quantities of arenaviruses can be produced by propagation in cell culture.
- The contamination of large human populations is possible since infection occurs via the respiratory pathway. For example, in the case of Junin virus, 75% of cases occur in male agricultural workers contaminated by inhalation of infected aerosols produced from rodent excretas or from rodents caught in mechanical harvesters.

This mode of infection presents striking similarities with virus-containing aerosols that may be dispersed during a bioterrorist attack.

- Secondary cases resulting from human-to-human transmission are to be expected for the reasons discussed above.
- Arenavirus genomes display remarkable plasticity as demonstrated by the identification of genomes formed by inter- or intra-segmental recombination mechanisms. For example:
  - Reassortant arenaviruses have been experimentally produced by the co-cultivation of closely or distantly related arenaviruses (Lukashevich, 1992; Riviere and Oldstone, 1986; Riviere et al., 1986). Laboratory generation of reassortant virus consisting of the L RNA of Mopeia virus and the S RNA of Lassa virus has been reported (Lukashevich, 1992). This demonstrates that despite a genetic diversity of 28% at the amino acid level, genetic exchange is possible and can result in a viable virus. Notably, reassortant viruses were produced by co-cultivation in Vero cell monolayers, without the use of sophisticated equipment or complicated molecular techniques. Reassortant viruses of different strains of LCM virus can acquire the capability to cause lethal disease in mice, while, in contrast, parental strains do not cause lethal disease (Riviere and Oldstone, 1986). As recently demonstrated, reassortment can suddenly create a new virus that exhibits an increased human pathogenicity and/or specific biologic properties (comparatively to the parental strains). Specifically, a reassortant bunyavirus caused several cases of fatal hemorrhagic fever in human populations of Kenya and Somalia (Bowen et al., 2001).
  - In addition to experimental work, arenaviruses possessing a recombinant S RNA genomic segment have been recently identified and reported within the natural environment in North America, namely Whitewater Arroyo (Charrel et al., 2001a), Tamiami and Bear Canyon virus (Charrel et al., 2002). Importantly, recombinant arenaviruses are strongly suspected of being capable of infecting humans (Kosoy et al., 1996). Whether they cause disease in infected individuals is still not clearly established; however, three cases of fatal human infection associated with Whitewater Arroyo virus were reported in California (CDC, 2000b), and there is evidence that at least two rodent species that circulate in California are infected by viruses closely related to Whitewater Arroyo virus (Bennett et al., 2000).
  - Although documented evidence does not yet exist, the generation of ribavirin-resistant strains may theoretically be achieved by propagation in the presence of increasing concentration of ribavirin.
- Diagnostic capacities are very limited. There is no commercially available diagnostic kit based on either serologic or molecular techniques. There is a lack of properly evaluated molecular diagnostic assays for BSL-4 arenaviruses. In-house molecular assays have been described for Lassa and Junin viruses, but never used in natural epidemic situations. In addition, there is no operational technical platform adapted to perform the large scale molecular diagnosis of arenaviruses.
- Available genetic data for arenaviruses are far from complete. In particular, full-length genomic sequences are available for only a few arenaviral species. The only homologous sequences available for the majority of arenaviruses correspond to a small region (~600 nt) of the S genomic segment (nucleoprotein gene) that represents less than 6% of the total genomic information. The complete sequence of the S genomic segment has been determined for only 11/23 viral species, not including Machupo and Guanarito virus. The L genomic segment has been fully characterized for four viruses only, of which Lassa virus is the sole BSL-4 agent. There is no partial or complete genetic characterization of the L genomic segment of any of the four South American BSL-4 arenaviruses. Molecular diagnosis of arenaviruses is thus dramatically hampered by the lack of genetic information.

4. Future directions

To reduce the consequences of any ill-intentioned abuse of natural or engineered strain(s) of arenaviruses, it is necessary:

- To extend our knowledge of arenavirus genomics; this is the very first priority. To date there is no technical limitation to its completion. The time necessary is solely dependent upon the priority given to the task.
- To develop diagnostic and detection tools for arenaviruses by (i) extending technical capabilities which are already partially or completely described; this will allow the concurrent determination of priorities for the development and evaluation of tests that can be easily applied to large-scale diagnosis; (ii) the definition of a strategy intended to allow the rapid diagnosis and identification of natural and engineered arenaviral strains via new methods. At this point, molecular tests represent the most likely candidates. The development of a ready-to-use molecular diagnostic kit, that has undergone properly conducted standardization and evaluation, is essential.
- To reinforce therapeutic capacity (i) by stockpiling ribavirin for rapid response; and (ii) by addressing efficacy, development, evaluation and production of alternative molecules with priority given to compounds active through mechanisms that are distinct from those of ribavirin.
- To improve measures of prevention through the development of vaccines that could be used in the days following a bioterrorist attack to protect populations living outside the targeted area once the arenaviral nature of the agent is established.

- To better understand the mechanisms leading to the creation of stable modified arenaviruses (mutants, reassortants or recombinants).

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