UNIVERSIDAD DE ANTIOQUIA
1803

Tales of mice and men: Natural History of Arenaviruses

Revista Colombiana de Ciencias Pecuarias

Juan D Rodas[1] MV, Ph.D, Maria S Salvato[2], Ph.D.

[1] Facultad de Ciencias Agrarias, Escuela de Medicina Veterinaria, Grupos de investigación en Ciencias Veterinarias "Centauro" y Grupo Inmunovirología, Universidad de Antioquia.

[2]Institute of Human Virology, University of Maryland at Baltimore
juandavid.rodas@gmail.com

(Recibido: 16 de febrero, 2006, aceptado: 6 de octubre, 2006)

Summary

Nowadays, Arenaviruses are among the most feared viruses due to their potential as weapons for bioterrorism purposes. This potential is based on their increasing diversity and the fact that they are carried by rodentswhose biologic success compares only wit insects and humans. The prototype of this family is Lymphocytic Choriomeningitis Virus which has been and excellent tool for a myriad of discoveries in immunology. Arenaviruses have been known for over 70 years but the number of members of the family is growing thanks to their insidious subsistence in third world countries and to the nature of their genome, that makes of them sorts of skilful machines for evolution This review collects some of the work of the authors about the best-known features described for this group of viruses, among the many still-to-be discovered characteristics of this puzzling, and hard-to-study, group of zoonotic viruses.

Key words: *hemorrhagic fever, LCM, rodent-borne viruses.*

Discovery and early characterization of arenaviruses

The arenaviruses are enveloped, single-stranded RNA viruses (meaning, with Ribonucleic acid as a genome), that are primarily carried by rodents and occasionally transmitted to humans. Old World and New World arenaviruses have been isolated on the African and American continents respectively and the members of each group are shown in tables 1 and 2. The arenaviruses are important clinically as human pathogens and experimentally as models for persistent infection and cellular immune responses (141).

The prototype virus, lymphocytic choriomeningitis virus, was first isolated from a human diagnosed with St Louis encephalitis; tissue homogenates passaged through monkeys and mice caused fever and aseptic meningitis (7). A year later, a filterable agent was isolated from the cerebrospinal fluids of two patients; this agent elicited similar symptoms in mice (132). At the same time, Traub (157) discovered a contaminant virus in the mouse colony of the Rockefeller Laboratories. Viruses were exchanged between the three laboratories and their identity was confirmed by neutralization in vitro and cross-protection in vivo (133). Lymphocytic choriomeningitis virus

(LCMV) has frequently been found as a contaminant of laboratory mice, rats and hamsters in North America and Europe, and may have entered North America via mice from Europe. Studies of murine LCMV infection have contributed to a wealth of information on the mechanisms of viral persistence and the interactions of viruses with host immune systems.

In 1956, the non-pathogenic Tacaribe virus was isolated from Caribbean fruit bats (49), and since then it has been the only arenavirus not isolated from rodents. With the agricultural expansion in South America, two pathogenic arenaviruses emerged: Junin, isolated from humans with Argentine haemorrhagic fever (125) and Machupo, isolated from humans with Bolivian haemorrhagic fever (80). Collaboration between field workers, the Yale Arbovirus Laboratories and the Rockefeller Laboratories documented the morphology and antigenicity of the 'Tacaribe group' of viruses. A non-pathogenic member of this group, Pichinde virus, was isolated during a trapping program in Colombia, and has since served in many biochemical studies (156).

It was not until the late 1960s that the morphological similarities between LCMV and the Tacaribe group of viruses were noted: both were enveloped viruses with a granular or sandy appearance. Serological tests later confirmed the relationship and they were named arenaviruses after the Latin arena for 'sandy' (40, 78, 138). When Lassa fever virus emerged in Africa, it was quickly identified as an arenavirus based on morphological and serological criteria (116). Arenaviruses are considered 'emerging pathogens' because new isolates are coming to our attention with great frequency (36).

Table 1. Species in the Genus.

Old World Arenaviruses	Reservoirs	Available sequences	Accession number	Acronyms
- *Ippy* (Dak AN B 188d)	*Arvicanthis sp.,* Central African Republic	N gene (partial)	U80003	IPPY
- *Lassa* (GA391)	*Mastomys sp.,* West Africa	S segment	X52400	LAS
(LP)		N gene (partial)	U80004	
(Josiah)		S segment L segment	J04324 U73034	
-*Lymphocytic choriomeningitis* (Armstrong)	*Mus musculus,* Europe, Americas	S segment L segment	M20869 J04331, M27693	LCM
(WE)		S segment	M22138	
- *Mobala* (3099)	*Praomys sp.*, Central African Republic	N gene (partial)	U80007, U80008	MOB
(3076)		N gene (partial)	AF012530	
- *Mopeia* (AN 21366: also referred to as 800150)	*Mastomys natalensis,* Mozambique, Zimbabwe	S segment	M33879	MOP
(AN 20410)		N gene (partial)	U80005	

Table 2. New world arenaviruses.

New World Arenaviruses	Reservoirs	Available sequences	Accession number	Acronyms
- *Allpahuayo* (CLHP-2472)	*Oecomys bicolor*, Oe. Paricola, Peru	S RNA (complete)	AY012687	ALL
- *Amapari* (BeAn 70563)	*Oryzomys capito*, Neacomys guianae, Brazil	N gene (partial)	U43685	AMA
-*Bear Canyon* (A0060209)	*Peromyscus californicus* U.S.A.	S RNA (complete)	AF512833	BCN
- *Cupixi* (BeAn 119303)	*Oryzomys sp.* Brazil	S RNA (complete)	AF512832	CPX
- *Flexal* (BeAn 293022)	*Oryzomys spp.*, Brazil	N gene (partial)	U43687	FLE
- *Guanarito* (INH-95551)	*Zygodontomys brevicauda,* Venezuela	N gene (partial) N gene (partial)	L42001 U43686	GTO
- *Junín* (MC2)	*Calomys musculinus*, Argentina	S segment	D10072	JUN
(XJ)		GPC gene N gene	U70799 U70802	
- *Latino* (10924)	*Calomys callosus*, Bolivia	N gene (partial)	U43688	LAT
- *Machupo* (AA288-77)	*Calomys callosus*, Bolivia	N gene	X62616	MAC
- *Paraná* (12056)	*Oryzomys buccinatus*, Paraguay	N gene (partial)	U43689	PAR
- *Pichinde* (3739)	*Oryzomys albigularis*, Colombia	S segment	K02734	PIC
- *Pirital* (VAV-488)	*Sigmodon alstoni*, Venezuela	N gene (partial)	U62561	PIR
(VAV-499)		N gene (partial)	U62562	
- *Oliveros* (RIID 3229)	*Bolomys obscurus*, Argentina	S segment	U34248	OLV
- *Sabiá* (SPH114202)	Natural host unknown, Brazil	S segment	U41071	SAB
- *Tacaribe* (p2b-2)	*Artibeus spp.*,	S segment	M20304	TCR
(T.RVL.II 573)	Trinidad	L segment	M65834, J04340, M33513	
- *Tamiami* (W10777)	*Sigmodon hispidus*, Florida, U.S.A.	N gene (partial)	U43690	TAM
- *Whitewater* Arroyo (AV 9310135)	*Neotoma albigula*, New Mexico, U.S.A.	N gene (partial)	U52180	WWA

Physical characteristics of arenaviruses

Arenaviruses are spherical with a mean diameter of 110-130 nanometers (nm), and a dense lipid envelope which is covered by club-shaped projections of 8-10 nm in length. Variable number of electrodense ribonucleoprotein particles (RNP) of 20-25nm can be found within viral particles (see Figure 1). The genome consists of two single stranded, RNA molecules, L (for Large) and S (for Short), of length about 7.5 kb and 3.5 kb respectively. The 3' terminal sequences (19-30 nucleotides, nt) are similar between the two RNAs and between different arenaviruses and are largely complementary to the 5' end sequences. Although the RNA genomic species are thought to be present in virions in the form of circular nucleocapsids (450 to 1300 nm in circumference), the genomicRNA is not covalently closed. Preparations of purified virus may also contain RNAs of cellular origin that include ribosomal RNAs. The viral m RNA (messangerRNA) species are associated with NP (149, 150).

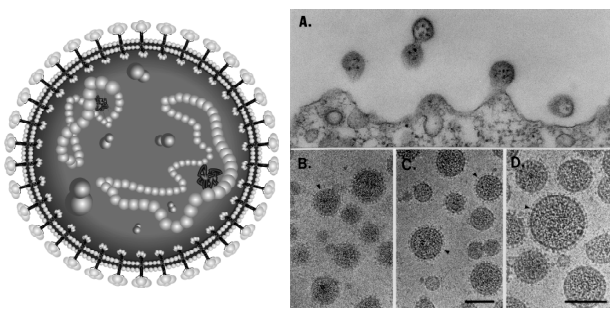


Figure 1. Diagrammatic representation of virion structure

L, L protein (RNA dependent RNA polymerase); NC, nucleocapsid; R, ribosome. (Andrew Featherstone and Christopher Clegg, CAMR). (Right) Electron microscopic images of lymphocytic choriomeningitis virus. A. Thin section showing several virions budding from the surface of an infected BHK-21 cell. B - D. Cryo-electron microscopic images of purified unstained virions frozen in vitreous ice, taken at –1.5, –3 and –4 microns de focus. Arrowheads indicate glycoprotein spikes which are composed of a transmembrane GP2 and globular GP1 head arranged in a tetrameric configuration. Bars indicate 100 nm. (Ronald Milligan, John Burns and Michael Buchmeier, Scripps Research Institute). Figure on the left taken form the International Committee for Taxonomy of Viruses and photo on the right, obtained from reference 143.

The most abundant structural protein is the nucleoprotein (N or NP), a non-glycosylated polypeptide (63 kDa) found tightly associated with the virus genomic RNA in the form of a ribonucleoprotein complex or nucleocapsid structure. A minor component is the L protein, an RNA polymerase (200 kDa). A putative zinc binding protein (Z, 11-14 kDa) is also a structural component of the virus (142). Two glycosylated proteins (GP1, GP2; 34 and 44 kDa respectively) are found in all members of the family and are derived by posttranslational cleavage from an intracellular precursor, GPC (75-76 kDa) (28, 30).

Other minor proteins and enzymatic activities have been described associated with virions including poly (U) and poly (A) polymerases, and a protein kinase that can phosphorylate N. It is thought unlikely that these are virally encoded. Lipids represent about 20% of virion dry weight and are similar in composition to those of the host plasma membrane. Carbohydrates in the form of complex glycans on GP1 (five to six sites in LCMV) and GP2 (two sites in LCMV) represent about 8% of virion dry weight (28, 120).

Genome organization and virus replication

The L and S RNAs of arenaviruses each have an ambisense coding arrangement (see Figure 2). The L RNA encodes the L protein in its viral-complementary sequence, and the Z protein (< 0.5 kb) in its viral-sense 5' end. The N protein is encoded in the viral-complementary sequence corresponding to the 3' half of the S RNA, while the viral glycoprotein precursor (GPC) is encoded in the viral-sense sequence corresponding to the 5' half of S. The mRNAs are capped and contain 1-5 non-templated nucleotides of heterogeneous sequence at their 5' ends. The mRNAs are not polyadenylated. The transcription mechanism is not fully elucidated. Initiation of transcription may involve cap-snatching or *de novo* cap synthesis. The 3' termini of the mRNAs have been mapped to locations in the intergenic regions (64, 149).

The process of infection (see Figure 3), involves attachment to cell receptors, entry via the endosomal route, uncoating and mRNA transcription in the cytoplasm of infected cells. There have been a number of recent studies

describing the participation of α-dystroglycan as a viral receptor for the viruses within this viral family. However, differences in affinity and avidity have also been detected for different virus species, suggesting that additional proteins are needed for this step of the virus life cycle (31, 88)

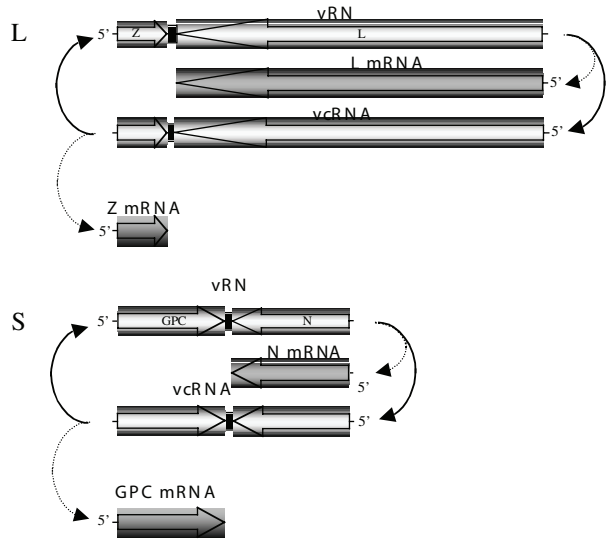


Figure 2. Organization, transcription and ambi-sense coding strategy of the arenavirus L and S RNAs.

Regions encoding the L, Z, GPC and N proteins are shown as boxes with arrowheads indicating the direction of translation. The intergenic regions separating the open reading frames are indicated by black boxes. The subgenomic RNAs which function as messengers are shaded grey. RNA transcription and replication processes are indicated by dotted and solid arrows respectively. vRN means viral RNA nucleic acid, vcRNA means viral copy of RNA for small (S) and large (L) segments and L, Z, N and GPC mRNA are the RNA messengers for the L, Z, N and GPC genes encoding the polymerase, the zinc-binding, the nucleo and the glyco proteins, respectively (After Victor Romanowski, Universidad Nacional de la Plata). This figure was generously provided to the second author of this review for Dr. Romanowski.

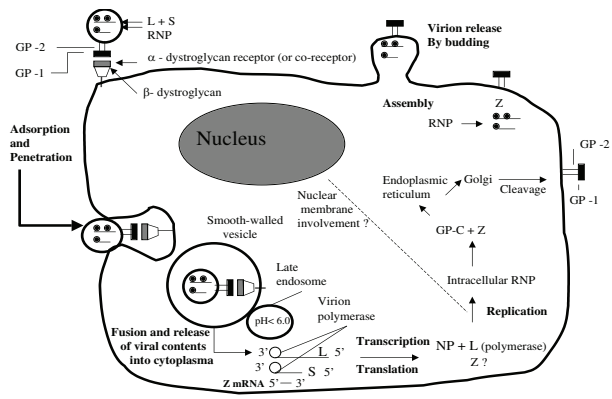


Figure 3. Arenavirus life cycle.

L, large RNA segment; S, small RNA segment; RNP, ribonucleoprotein; (modified from Southern *et al*, 1996 and taken reference 143).

Because of the ambisense coding arrangement, only N and L mRNAs can be synthesized from the genomic S and L RNAs respectively, by the virion polymerase, prior sythesis of GPc and Z. The products of N and L genes are presumed to be involved in the synthesis of full-length viral complementary species, which serve as templates for the synthesis of GPc and Z mRNAs and the synthesis of full-length viral RNAs. The process of RNAreplication, which may involve a "slippage" mechanism during initiation, and read-through of transcription termination signals, is not completely clear. However, the presence of full-length viral-complementary genomic RNAs and viral subgenomic mRNA species in virus preparations may affect this perceived temporal order of RNA and protein synthesis (140, 142).

The viral envelope glycoproteins are synthesized in cells as a single mannose-rich precursor molecule, which is proteolytically cleaved and processed to contain complex glycans during transport to the plasma membrane. Virions mature by budding at sites on the surface of cells. There are still many questions to be answered regarding replication, transcription and expression of arenaviruses during their life cycle using in vitro and in vivo conditions (28). Due to the recent success with the reverse genetic systems for negative strand RNA viruses (15, 25, 27, 90, 118, 145) and the obvious benefits of having such a great tool to answer many of these unsolved questions regarding viral transcription and replication as well as the molecular mechanisms underlying arenavirus persistence and pathogenesis, we tried to develop an infectious clone for LCMV (unpublished results).

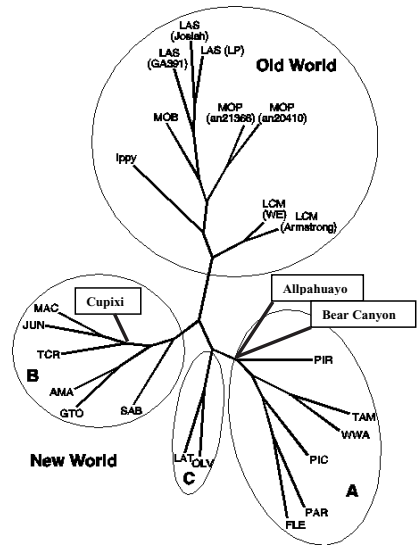
Classification of arenaviruses

Arenaviruses have been classified according to host, geographical location, antigenic cross-reactivity and nucleic acid sequence homologies. Type-specific antigens on the 44 kDa GP1 of LCMV are involved in virus neutralization. Cross-neutralization tests have demonstrated partially shared antigens

between Tacaribe virus and Junin virus and cross-protection has been demonstrated against Junin virus following prior infection by Tacaribe virus, or against Lassa virus following infection by Mopeia virus. Major complement-fixing antigens are associated with the viral N proteins, which were used to define the Tacaribe complex of arenaviruses. Monoclonal antibodies react with common epitopes on the N and Gp2 proteins of all arenaviruses, one of these was described by Buchmeier (aminoacids 374-378) with no reports of its function (29). A second (aminoacids 289-301) apparently plays an important role in viral entry as a fusion region (44, 71, 72).

By monoclonal and polyclonal antibody analyses, the African arenaviruses are distinguishable from the New World arenaviruses. Fluorescent antibody studies show that antisera against New World viruses, as well as those against African viruses, react with LCMV. Cytotoxic T-lymphocyte epitopes have been identified on the nucleoprotein and glycoproteins of LCMV. The number and location of epitopes vary depending on the virus strain and host major histocompatibility complex (MHC) class I molecules (121, 122, 169).

Nucleic acid sequences from the N genes of all the known arenaviruses have provided the basis for phylogenetic analysis, which supports previously defined antigenic groupings and further defines virus relationships within them (see Figure 4). Sequence data derived from other regions of the genome, where available, is largely consistent with this analysis. Among the Old World viruses, Lassa, Mopeia and Mobala viruses are monophyletic, while Ippy virus and LCMV are more distantly related (23). The New World viruses can be divided into three groups on the basis of the sequence data. In group A are Pirital, Pichinde, Parana, Flexal, and a recently found Allpahuayo (Perú) virus from South America (112) , together with Tamiami, Whitewater Arroyo and newly added Bear Canyon viruses from North America. Group B contains the human pathogenic viruses Machupo, Junin, Guanarito, and Sabia and the non-pathogenic Tacaribe, Amapari, and Cupixi (Brazil) viruses (33, 34, 65). Latino and Oliveros viruses form a small separate group (group C)(22). The division of the arenaviruses into Old World and New World groups, as well as the subdivision of New World arenaviruses into three groups, is strongly supported by bootstrap resampling analysis. It is important to note that the trait of human pathogenicity appears to have arisen on at least two independent occasions during arenavirus evolution (24) .



Abbreviations

LAS: Lassa
LAT: Latino
MOP: Mopeia
OLV: Oliveros
LCM: Lymphocytic Choriomeningitis
PIR: Pirital
MAC: Machupo
TAM: Tamiami
JUN: Junin
WWA: White Water Arroyo
TCR: Tacaribe
PIC: Pichinde
AMA: Amapari
PAR: Parana
GTO: Guanarito
FLE: Flexal
SAB: Sabia

Figure 4. Phylogenetic relationships among the Arenaviridae.

Partial N gene nucleotide sequences corresponding to nt 1770-2418 of Tacaribe virus S RNA sequence (GenBank accession no. M20304) were aligned (PILEUP, adjusted manually) and analysed by maximum parsimony using PAUP (Michael Bowen, C.J. Peters, Stuart Nichol, CDC). BCN, and ALL recently join group A and CPX joined group B. Taken from reference 143.

It is apparent that recombination has influenced the evolution of several RNA viruses, including arenaviruses. There is published evidence that the Whitewater Arroyo, Tamiami, and Bear Canyon virus S segments are the product of recombination between ancestral arenaviruses from different lineages. In fact, the nucleocapsid and glycoprotein genes of the Whitewater Arroyo virus, the

Tamiami virus, and the Bear Canyon virus have divergent phylogenetic histories (6, 33, 34). Separate analysis of full-length amino acid sequences using maximum parsimony or neighbor-joining methods show that the nucleocapsid protein genes of these three viruses are related to those of Pichinde virus and Pirital virus (New World lineage A), while the glycoprotein genes are more closely related to those of Junin, Tacaribe, and Sabia viruses (New World lineage B). Recombination seems to have also played a role in the genome of Río Carcarañá virus, but sequence data are incomplete. By way of comparison, amino acid (AA) distances observed between Amapari and Cupixi viruses (GPC gene = 31.4%, NP gene = 14.6%) and between Junin and Machupo viruses (GPC gene = 30.0%, NP gene = 14.4%) are the lowest observed between distinct arenaviruses species. Río Carcarañá virus from Argentina has recently been described by Ghiringelli *et al* (68) and is not yet considered a species.

This thesis employs the Old World LCMV strains, WE and Armstrong, in a monkey model for hemorrhagic fever. These viruses are 60% homologus to Lassa fever virus (Josiah strain) and 86% homologus to each other. Their differences are critical in defining virulent outcomes for LCMV WE and benign outcomes for LCMV-ARM in this model.

Arenaviral host-range, tropism and transmission

Arenaviruses replicate in a broad range of mammalian hosts and in almost every tissue of the host, reaching high titers in brain, kidney, liver and secondary lymphoid organs.

Virus replication is restricted in lymphocytes, macrophages and terminally differentiated neurons, probably because of the absence of host cell factors (21, 42, 128). Arenaviruses are often propagated in adherent cell lines such as BHK-21 cells, mouse L cells or Vero cells.

The reservoir hosts of almost all the arenaviruses are species of rodents. LCMV is found in *Mus sp* and the African viruses mainly in the rodents *Mastomys* and *Praomys* (82, 111) in the sub-family *Murinae*. The New World viruses are mostly found in the Sigmodontine rodents *Calomys*, *Neacomys*, *Neotoma*, *Oryzomys* and *Sigmodon* (79, 87,156). The majority of the rodents associated with arenaviruses are commensals or semi-commensals, living within human dwellings or in cultivated fields. Exceptionally, Tacaribe virus was isolated from fruit-eating bats (*Artibeus spp*.) (49), but subsequent attempts to recover it from bats or from other potential hosts have been unsuccessful. It is notable that the geographic range of an arenavirus is generally much more restricted than that of its cognate rodent host. Most of the viruses induce a persistent, frequently asymptomatic infection in their reservoir hosts, in which chronic viremia and viruria occur (139).

Strain, dose, route of exposure and passage history of arenaviruses inoculated into experimental animals have a marked effect on lethality, tissue tropism and development of persistent infection (50). Age of the host can also have a critical effects on the type of infection that results (164).

Most arenaviruses do not normally infect other mammals or humans, however, Lassa virus is the cause of widespread human infection (Lassa fever) in West Africa (Nigeria, Sierra Leone, Liberia, Guinea) (102), and Junin virus causes Argentine hemorrhagic fever in agricultural workers in an increasingly large area of that country (167). Machupo virus has caused isolated outbreaks of similar disease in Bolivia (78), and Guanarito virusis associated with human disease in Venezuela (155). Sabia virus was isolated from a fatal human case in Brazil (36). LCMV acquired from mice has also caused a highly fatal hepatitis in captive Callitrichid primates (8, 151, 152). Severe laboratory-acquired infections have occurred with LCMV, Lassa, Junin, Machupo, Sabia and Flexal viruses and asymptomatic infections with Pichinde virus have also been reported (19, 51, 159).

Vertical transmission in uterus is the major mechanism of LCMV maintenance in *Mus musculus*. Horizontal transmission also occurs in the natural hosts by milk-, saliva- or urine-

borne routes. Horizontal transmission within and between species occurs by aerosol routes and gastrointestinal tract (113, 114). No arthropod vectors are thought to be involved in the normal transmission process.

Rodent-to-human infections are thought to probably occur primarily through aerosols or wound-contact with rodent blood, droplets and fomites (35, 91, 153). In the past LCMV has been implicated in about 8% of the patients diagnosed with viral meningitis, and serological studies have suggested an incidence of LCMV infection of up to 10-15% in the general population. Most of these infections are probably mild or sub-clinical (124). The aerosol stability of arenaviruses seems to be high. Studies with Lassa virus indicate a biological half-life of 55 minutes at 25°C and 30% relative humidity or 18 minutes at 25°C and 80% relative humidity. Arenaviruses are susceptible though to heat and desiccation (153).

Some relatively recent studies support the feasibility of mucosal infection by intragastric (ig) inoculation of mice (130, 131) guinea pigs (Lukashevich et al, unpublished data) and of monkeys (95). Several pathogens, such as *Vibrio cholera* and HIV, depend on mucosal transmission for the majority of natural infections, even though the pathogens are highly unstable in the gastrointestinal tract (5, 32). Acid-treated LCMV and Lassa virus clearly lose infectivity (43, 44, 70), but they are still able to infect by the ig route. Acidic pH triggers membrane fusion activity and the glycoprotein spike complex undergoes irreversible changes hat result in the release of GP-1 from LCM virions, in this way previously concealed GP-2 epitopes are exposed (43, 44). Nevertheless, it is likely that infectious particles are protected within aggregates of virus after exposure to stomach acid or the loss of GP-1 could expose regions of GP-2 (fusion peptide of arenaviruses) capable of mediating virus entry (70, 72). In this sense, previous biochemical results showed that proteolytic removal of all viral glycoproteins (to an undetectable level) did not affect dramatically the infectivity of LCMV particles (26). This suggested that only a few molecules of GP-2 could be enough to initiate fusion and entry into cells. In addition it is also likely that virus uptake occurs so rapidly that infection might happen before complete viral inactivation, this theory is supported by previous studies in mice, where 10^4 reduction of infectivity may take 30 minutes in acid yet uptake may occur within the first 5 minutes after inoculation (130, 131).

Human-to-human spread has been reported for Lassa fever in the community and in hospital settings (104), whereas only a few cases of nosocomial transmission have been reported for Bolivian hemorrhagic fever (127) and none for Argentine hemorrhagic fever virus. There have been reports suggestive of sexual transmission of Lassa and Machupo viruses from convalescing patients (48). Aerosol spread and direct contact are the most likely routes of infection between humans. Neonates are at risk of infection through their mother's milk.

So far there are no reports of experimentally controlled transmission studies in the monkey model as a way to test the likelihood of mucosal human infection by this route. It has been proven that ingestion of food contaminated with rodent urine or the direct consumption of rodents, as it is customary in regions of Africa where Lassa fever is endemic; represents a clear opportunity for human infection with the Lassa virus (154, 162). The experiments performed during the PhD program of the first author attempted to fill out the experimental gap in our knowledge of mucosal infection with hemorrhagic fever viruses.

Clinical manifestations of viral infection

All arenaviruses establish persistent infection in the natural rodent host after virus infection *in uterus* or within a few days of birth. Adult mice inoculated intracerebrally with LCMV develop tremor with characteristic extensor spasm of the legs and they finally go into convulsions and die (120). A diversity of clinical manifestations, most of which are mediated by the immune system, has been described in the mouse

model (123). A summary of the role played by the LCMV murine model in the development of immunology as a science can be found somewhere else (141, 169).

LCMV infection of human beings may be asymptomatic, mild or moderately severe with CNS manifestation. Lymphocytic choriomeningitis begins with fever, malaise, weakness, myalgia and headache associated with photophobia. Anorexia, nausea and dizziness are common (55). It has also been reported that LCMV has teratogenic capacity (14, 18).

In man, the arenavirus hemorrhagic fevers are often severe, generalized febrile diseases with multi-organ involvement. Tissue and pulmonary edema with prominent hypovolemic shock and acute respiratory distress syndrome are associated with fatality rates of about 16–30% in untreated hospitalized patients (58, 60). The onset of Lassa fever is characterized by generalized symptoms such as high fever, joint and back pain and severe headache, leading to dry cough and exudative pharyngitis (63, 81, 104). Edema and bleeding may occur together or independently. Acute neurological manifestations such as unilateral or bilateral deafness and moderate or severe diffuse encephalopathy with or without seizures are also common in Lassa fever. Lassa fever is severe in pregnant women especially during the third trimester when fetal/ neonatal loss is 87% (129).

Several of the South American arenaviruses have been found by screening rodent populations but are not associated with human disease; for example, Oliveros virus is carried by 20–30% of *Bolomys obscurus* in the Argentine hemorrhagic fever areas (109).

South American Hemorrhagic Fevers are clinically very similar (36, 78, 96, 98, 127, 166). Symptoms include malaise, high fever, severe myalgia, arthralgia, anorexia, relative bradycardia, lumbar pain, epigastric pain, abdominal tenderness, conjunctivitis and retro-orbital pain, with photophobia. In severe cases there is nausea, vomiting, diarrhea, tremor and convulsions (158). Fatal cases show hemorrhagic disorders due to vascular collapse with hypotensive shock, hypothermia and pulmonary edema. In contrast to Lassa fever, bleeding with severe thrombocytopenia is more common in Argentine and Bolivian hemorrhagic fevers (58).

Arenavirus hemorrhagic fever (HF) in man is unlike the classical LCMV diseases of rodents, neither the acute T-cell mediated disease nor the chronic immune-complex disease are seen. In fact there is no mouse model for HF and hence the reliance on less thoroughly characterized and more expensive species such as guinea pigs and non-human primates. Guinea pig studies showed that animals infected with Lassa virus developed homologus neutralizing antibodies, but that other Old world arenaviruses induced little or no serum neutralizing response to Lassa virus. Nevertheless, guinea pigs infected with other Old World arenavirus were often protected against virulent Lassa challenge (94, 95, 126). In the Lassa infection of guinea pigs the mortality rate is very high and is marked by respiratory symptoms and pathologic evidence of myocarditis, pulmonary edema and hepatocellular damage. Survivors apparently harbor no virus but develop high antibody titers. In addition, pathogenicity of LCMV or Lassa virus for guinea pigs varies widely with strain. Whereas the Josiah strain of Lassa has an LD_{50} of 0.3 plaque forming units (pfu) for strain 13 guinea pigs, the same virus only kills about 30% of outbred Hartley animals with doses varying from 2 to 200.000 pfu. For LCMV the lethal dose is less than 1 pfu for the WE strain and more than 10^6 pfu for the Armstrong strain (99). Reassortants of WE and Arm strain have been used to map the virulence determinants of LCMV to the large RNA segment (45, 93, 134, 135).

Infected primates as a model for hemorrhagic fever

Lassa virus infection of rhesus and cynomolgus monkeys results in fever by day 5, accompanied by significant anorexia and progressive wasting. The infections end almost invariably with death after 10-15 days with vascular collapse, shock, and moderate hemorrhage affecting primarily mucosal

surfaces. Focal hepatic and adrenal necrosis and interstitial pneumonitis are consistent pathological findings (107, 161). In agreement with the symptoms described in humans, high levels of virus in blood and hepatic liver enzymes, particularly ALT, were regularly high in all the animals tested. It must be borne in mind, however, that the rhesus model differs from humans with Lassa fever in their higher mortality rate and the prominence of meningo-encephalomyelitis, pulmonary vascular lesions (e.g. thrombocytopenia) and systemic arteritis. Although Lassa virus is pantropic, the most consistent findings are mild hepatic focal necrosis without significant inflammatory response, some evidence of interstitial edema and focal adrenal cortical necrosis. In addition even though the liver is the most affected organ, biochemical measures of liver function and the extent of tissue necrosis are inadequate to account for death due to hepatic failure (107). Likewise, the absence of significant disturbances in coagulation makes disseminated intravascular coagulation (DIC) unlikely as a primary pathologic process. Trombocytopenia is rare, but platelet function is markedly depressed. The existence of a plasma inhibitor of platelet aggregation has been suggested in patients with Lassa fever (38, 39, 56, 57). There is also evidence for disturbance of endothelial function related to depression in production of prostacyclin in postmortem vascular samples from Lassa infected animals compared to uninfected controls (62). Presumably these changes in vascular function could be enough to account for the failure of integrity in the intravascular compartment leading to edema, shock and effusions observed at the autopsies (58). The viral glycoprotein G2 has been found associated with circulating neutrophils and although the significance of this finding is uncertain since the platelet inhibitor may also affect neutrophil function some consideration should be given to the role of neutrophils in the pathogenesis of severe Lassa fever (136).

Since the 1960's it was known that LCMV-WE produces a rapidly fatal infection in rhesus and cynomolgus monkeys after inoculation by peripheral, intravenous (iv) or aerosol routes. In contrast monkeys inoculated with LCMV Arm had an uneventful course (94, 126). Previous work has exploited the remarkable similarity of LCMV WE infection of macaques to human Lassa fever (95). The use of a BSL-3 virus, LCMV-WE, instead of Lassa, has allowed us to do monkey research at a lower biosafety level, and it is much easier to find facilities that will support this research. In rhesus and cynomolgus monkeys the infection with the WE strain of LCMV is fatal within two weeks and the disease course resembles the severest form of Lassa fever. Viremia reaches 10^7 to 10^8 pfu/ml by the time of death (75, 126). Initial leukopenia is followed by a leukocytosis, and primarily neutrophilia as it has been previously observed in Lassa fever infected primates and humans. All monkeys show intradermal hemorrhage (petechia and ecchymoses) and epistaxis. At the autopsy large effusions have been found and high titers of virus have been detected in all tissues. Serum aspartate aminotransferase (AST) levels are elevated in LCMV-WE infection (95) as in Lassa fever (102, 103). Junin and Machupo virus infections of non-human primates also simulate the disease in humans, with fever, anorexia, weight loss and gastrointestinal symptoms. The animals die with cachexia and severe dehydration (11, 52, 84, 165).

Most infected monkeys seroconvert with antibodies being detectable by immune-fluorescence or complement fixation. Early antibody responses are neither associated with reduced viremia nor with recovery from disease. In contrast the neutralizing antibody response was undetectable before 45 days, suggesting that neutralizing antibody was not critical to viremia clearance (126). *In vitro* lymphocyte proliferation tests during the acute phase of the disease show impaired responses to non-specific mitogens suggesting inhibition of lymphocyte function that could explain the lack of inflammatory infiltration observed in tissues from Lassa infected humans and monkeys (102, 161-163).

Past vaccine studies have suggested an important role the cell-mediated immunity in protection against a lethal challenge with Lassa fever virus (LFV) in monkeys (59, 61). These suggestions stem from weak humoral responses and highlight the abysmal

absence of cell-mediated immunity (CMI) data in the literature. A recent study in our laboratory provides the first measure of cytotoxic T lymphocytes (CTL) responses in monkey surviving lethal exposure to an Old World arenavirus (137). According to this, cytotoxic T lymphocytes would be playing a crucial role in recovery after infection and protection against the otherwise lethal iv challenge with the WE strain of LCMV. This study shows also the ability of the avirulent Arm strain given by iv or intragastric (ig) routes to cross-protect against lethal challenge with the WE strain. By performing several immunological measurements of infected monkeys our lab addresses a large gap in the literature.

Arenavirus vaccines and treatments

The development of a safe and effective vaccine for arenavirus infections of humans has proved difficult. Several killed and live attenuated vaccines have been tested for Lassa, Junin and Machupo viruses, none of which has shown to be suitable for widespread human use. Many of these vaccines are still in the stage of animal trials. A live attenuated Junin virus vaccine, Candid1, has been shown to be safe and immunogenic in non-human primates (16, 37, 108). Laboratory animals infected with various avirulent viruses serologically related to Lassa virus, including LCMV, Mopeia and Mobala viruses, survived a subsequent challenge with virulent Lassa virus (77, 161). Similar strategies for protection against Junin virus with heterologous live vaccines have repeatedly demonstrated protection against Junin virus in guinea pigs and hamsters (16). Other vaccine trials with inactivated Lassa and Machupo viruses have given mixed results, although they are immunogenic. Immunization with inactivated Lassa virus protects *Papio hamadryas* monkeys from a subsequent challenge with Lassa virus (86), but fails to protect rhesus monkeys even though there is a secondary, high titer antibody response to the major structural proteins of Lassa virus in these vaccinated monkeys (106).

Killed antigen vaccine has proved ineffective for Lassa virus (106) and an attenuated virus vaccine is not available; so a live recombinant vaccine provides a very attractive alternative. Recombinant vaccinia virus vaccines, which express either the Lassa virus nucleoprotein or the glycoprotein gene, successfully protect guinea pigs from a lethal Lassa virus infection, but offer incomplete protection in primates (9, 10, 115). Fisher-Hoch et al have tested a variety of LAS vaccines delivered to non-human primates via the NYBH vaccinia vector. In one study the authors described the outcome after the vaccination of 44 monkeys with Mopeia or vaccinia expressing Lassa S segments genes (G1, G2, N, G1+G2 or combinations of them). A third of the monkeys were Cynomolgus macaques and the remainder monkeys were Rhesus and all of them were challenged with a lethal dose (10^5 pfu) of Lassa virus (Josiah strain). The data indicate that vaccines delivering all genes of the Lassa S RNA (both N and G) are more protective than vaccines with only the glycoprotein genes and these later are more protective than vaccines with only the N gene (61). In murine experiments with the recombinant Lassa vectors, a weak but measurable cross-protection against LCMV intracranial challenge can be mediated by Lassa Gp-specific CD4+ T cells or by Salmonella or Vaccinia recombinants expressing Lassa Np (46, 47, 89). Vaccine trials so far have suggested but not proved that cell-mediated immune response must be activated to protect against challenge with arenaviruses.

Immunization with recombinant vaccinia virus that expresses the LCMV glycoprotein (VV GP) or nucleoprotein (VV NP) protects mice from LCM disease by induction of a protective CTL response in an H-2 haplotype-dependent manner (73, 85, 119). Mice can be specifically protected by subcutaneous inoculation of recombinant LCMV proteins (GP or NP) or just the T cell epitope of the LCMV nucleoprotein as an unmodified free synthetic peptide in incomplete Freund's adjuvant (12, 146, 147). Vaccination with DNA encoding the LCMV nucleoprotein or the glycoprotein also confers protection against lethal LCMV challenge and against persistent LCMV infection in an MHC-dependent manner by priming CD8+ cytotoxic lymphocytes (100, 168). In certain circumstances, however, immunization with VV GP or VV NP aggravates disease. For example, Balb/C mice infected with a high dose of the LCMV - Docile usually survive, unless they are pre-injected with

VV NP or VV GP (119). In this case, low-level immunization may accelerate development of immunopathology. High dose immune-supression, often mentioned by the Zinkernagel lab, is caused by high viral antigen being inappropriately presented thereby inactivating the cell response (20).This example illustrates the potential value of CTL vaccines and highlights at the same time the limitations of subunit vaccines. To protect an outbred population in an MHC-restricted fashion, it will be necessary to make a vaccine that consists of a cocktail of relevant peptides and to ensure that none of its components aggravates the disease in a subsequent virus challenge.

LCMV-induced persistent infection in mice is a classic example of viral persistence and serves as a model to study basic principles of immune clearance in persistent and disseminated infections in general. This model system makes it possible to test the potential of specific immune therapy to clear virus from a chronically infected host and to study the effector mechanisms responsible for clearing such infections. Volkert was the first to show that the adoptive transfer of spleen cells from LCMV-challenged immune adult mice results in reduction of infectious virus in carrier mice (160). This has been confirmed by a number of workers (1, 3, 13, 69). Nevertheless there is no evidence for persistent arenavirus infection of man and our monkey model is more applicable for the acute human disease.

Clearance of viral materials (infectious virus, viral nucleic acid and proteins) from several organs of persistently infected mice probably occurs by reconstitution of LCMV-specific CTL that had been deleted during viral infection. By using mice that are recombinant in the H-2 region and by selective depletion of lymphocyte subpopulations, it has been shown that viral clearance is mediated by co-operation between virus-specific CD8+ T cells and non-specific bone marrow-derived mononuclear cells from the carrier host (2). The effector mechanisms responsible for eliminating the persistent and disseminated LCMV infection of mice are dependent on the lytic ability of CTL, because perforin-negative transgenic mice are unable to clear infection (83). Likewise, the important role played in protection by the CMI has also been observed through cross-protection experiments carried out using spleen cells from guinea pigs inoculated with Lassa, Mopeia or LCMV Armstrong and syngeneic guinea pig kidney target cells. Spleen cells from guinea pigs infected with Lassa virus lyse infected kidney cell targets. In contrast Mopeia-immunized spleen cells lysed Lassa or Mopeia-infected targets but not LCMV-infected ones and LCMV-immune spleen cells recognized Lassa and LCMV-infected targets but not Mopeia-infected ones (126). Unfortunately, adoptive transfer of effector cells has been a failure in human trials (66) and is not really a viable approach for treatment of outbred populations (e.g. human beings).

Early success of Lassa virus immune plasma in the treatment of Lassa fever (92) and immunotherapy of Machupo virus infections in primates (53) showed promise for the treatment of arenavirus infections in humans. Convalescent phase plasma from Junin virus patients reduced mortality from 16% to 1% in those who were treated in the first 8 days of illness (97), and the efficacy of the plasma seemed to be directly related to the concentration of neutralizing antibodies of the plasma. However, better understandings of the limitations of this approach and reduced success in subsequent cases have restricted its use. A late neurological syndrome developed 4–6 weeks after the onset of acute illness in about 10% of the cases treated with Junin virus immune plasma (4). Passive antibody therapy depends on collection of plasma from people known to have been infected with the virus, testing the plasma or screening the donor for antibodies to blood-borne agents such as hepatitis and proper storage of plasma until it is used.

In summary, research is needed in primate models of acute arenavirus infection to further define vaccines, treatments and immune correlates of protection. The antiviral drug ribavirin has proved effective in the treatment of Lassa fever in laboratory animals (75, 76) and in humans (105), especially when administered during the first 6 days after the onset of illness. Later the pathogenesis of the infection is less reversible. Ribavirin is perhaps more effective if given intravenously than orally (101, 105). It is the drug of choice for treatment and

for prophylaxis in cases of possible exposure to Lassa virus, in laboratory or hospitals. Studies with Junin virus infections indicate that ribavirin may also have beneficial effect in Argentine hemorrhagic fever (54). A single case of laboratory-acquired Sabia virus infection was successfully treated with intravenous ribavirin (17) at a dosage recommended by the Centers for Disease Control and Prevention (CDC, USA) for other arenavirus infections (a loading dose of 30 mg/kg body weight, followed by a dose of 15 mg/kg every 6 hours for 4 days and then by a dose of 7.5 mg/kg three times daily for 6 days). A study for combined antibody and Ribavirin therapy has also been performed in the mouse model (148) and shows that the two treatments are complementary.

Resumen

Cuentos de ratones y de hombres: Historia Natural de los Arenavirus

En la actualidad, los arenavirus son considerados uno de los grupos de virus más temidos debido a su potencial uso como armas para el bio-terrorismo, debido a su diversidad creciente y a que son portados por roedores, cuyo éxito para sobrevivir, y adaptarse, solo puede compararse con el de los mosquitos y los seres humanos. El prototipo de esta familia viral, el virus de la coriomeningitis linfocítica, ha servido como herramienta para una gran cantidad de descubrimientos sobre la respuesta inmune. Los arenavirus han sido conocidos por más de 70 años, pero la familia aún sigue creciendo, gracias a su subsistencia insidiosa en los países del tercer mundo, y a su naturaleza genética, que les permite comportarse como máquinas "habilidosas" para la evolución. Esta revisión, recoge algunos de los resultados de los autores sobre los rasgos mejor conocidos, entre los muchos que aún no han sido descubiertos en grupo de virus zoonóticos, intrigante y muy difíciles de estudiar.

Palabras clave: *Fiebre hemorrágica, LCMV, virus trasmitidos por roedores.*

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