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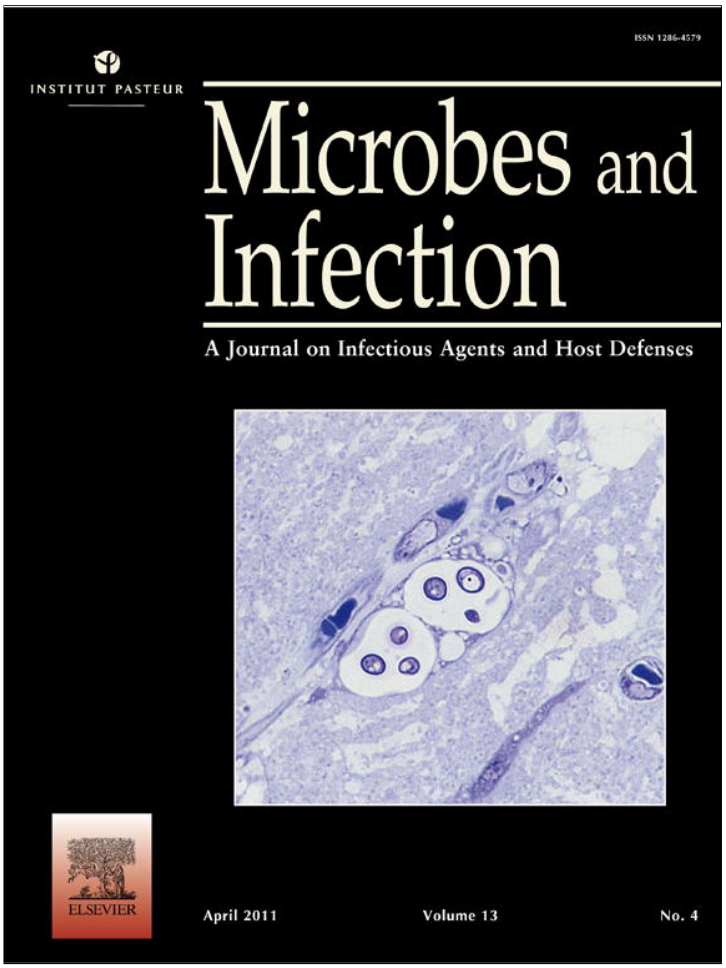

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Microbes and Infection 13 (2011) 303–311

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Review

Junín virus. A XXI century update

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Received 29 November 2010; accepted 27 December 2010
Available online 14 January 2011

Abstract

Junín virus of the *Arenaviridae* family is the etiological agent of Argentine hemorrhagic fever, a febrile syndrome causing hematological and neurological symptoms. We review historical perspectives of current knowledge on the disease, and update information related to the virion and its potential pathogenic mechanisms.
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Keywords: Arenavirus; Molecular virology; Pathogenesis; Argentine hemorrhagic fever

1. Introduction

Junín virus (JUNV), a member of the *Arenaviridae* family, is the known etiological agent of Argentine hemorrhagic fever (AHF), an endemo-epidemic disease mainly affecting agricultural workers in Argentina (reviewed in [1]). The infection is usually acquired through small abrasions in the skin or through aspiration of particles contaminated with urine, saliva or blood from carrier rodents [1]. Since the first description of the disease in the 1950s, uninterrupted annual outbreaks have been observed in a progressively expanding region in north-central Argentina, to the point that almost 5 million individuals are considered today to be at risk for AHF (reviewed in [1,2]). Several studies have confirmed persistent JUNV infection in *Calomys musculinus* and other *Calomys* rodents, including *Calomys laucha*, *Akodon azarae* y *Orizomys flavescens*, the main animal hosts and reservoirs for the disease in nature [3].

AHF incubation period ranges from 6 to 12 days, ending with the onset of fever usually associated to a flu-like syndrome which may include myalgia, arthralgia, headache, relative bradycardia, conjunctivitis, nausea, vomiting and diarrhea, with little central nervous system (CNS) or hematological involvement during the first week. Fever persists during the second week, when petechiae in the oral mucosa and the axillary region as well as gingival bleeding can be observed. Less common, more severe hemorrhagic signs may be present including hematemesis, melena, hemoptysis, epistaxis, hematomas, metrorrhagia and hematuria. CNS involvement can also be present during the second week in the form of hyporeflexia and mental confusion. When severe, it can progress to include areflexia, muscular hypotonia, ataxia, increased irritability and tremors, followed by delirium, generalized seizures and coma [4]. After the second week, over 80% of patients improve, although bacterial infection is a frequent complication. AHF diagnosis is based on clinical and laboratory data. For this reason, if platelet counts below 100,000/mm^3 in combination with white blood cell counts under 2500/mm^3 are detected when screening patients in endemic areas, these findings can be considered potentially useful to identify individuals at risk (reviewed in [1]). A reverse transcriptase PCR-based assay has also been established for rapid diagnosis [5]. Neutralizing anti-JUNV antibodies (Abs) consisting mainly of the IgG_1 subtype are usually present from day 12 (reviewed in [1]). Significant

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doi:10.1016/j.micinf.2010.12.006

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improvement in clinical AHF management has been achieved using immune plasma from convalescents (3000 units/kg body weight), with mortality rates dropping from almost 20% to less than 1%. Additional administration of ribavirin may enhance these results even further (reviewed in [1]). Approximately 10% of cases treated with immune plasma develop late neurological syndrome (LNS). After a symptom-free period, LNS onset is characterized by fever, cerebellar signs and cranial nerve palsies. LNS has never been registered among AHF patients recovering without specific treatment. Also important is the fact that live attenuated vaccine, Candid 1, has proven effective over more than a decade (reviewed in [1]). As expected, immune response to Candid 1 boosts pre-existing immunity to JUNV, but is not changed by previous exposure to Lymphocytic Choriomeningitis Virus (LCMV) [1]. Nevertheless, AHF remains a potentially lethal infection and mandatory reporting is still enforced in Argentina.

2. Current classification

To date, the International Committee for Taxonomy of Viruses recognizes the *Arenaviridae* family as containing a single genus: Arenavirus, including 22 viral species [6]. Several additional arenaviruses have been discovered recently, but taxonomic status of them remains pending (Table 1) [7]. The *Arenaviridae* family is serologically, phylogenetically and geographically divided into two major complexes, the Old World (OW) complex (Africa, Europe, and Asia) and the New World (NW) complex (North and South America). The OW complex includes the prototype arenavirus LCMV, as well as Lassa virus (LASV), Mopeia virus (MOPV), Mobala virus (MOBV), and Ippy virus (IPPYV). The NW complex is larger and JUNV is classified in Clade B together with the also human pathogens Machupo (MACV), Guanarito (GTOV) and Sabia (SABV). Tacaribe (TCRV) and Pichinde (PICV) do not cause disease in human but are extensively used in scientific studies. With the exception of LCMV, all virus species show restricted geographical distribution. This is at least partially explained by the coinciding location of habitats of the specific natural reservoir species (almost exclusively rodents) [1,7].

3. Virion characteristics

Like other members of the same family, JUNVs are pleomorphic virions, ~120 nm in diameter, with a capsid showing helicoidal symmetry and including a variable number of

Table 1
Arenaviridae family.

Virus	Acronym[b]	Evolutionary lineage	Distribution[a]	Reservoirs	Human Pathogen	Cat. A
Junín	JUNV	NW-B	Argentina	*C. musculinus*	Yes	Yes
Machupo	MACV	NW-B	Bolivia	*C. callosus, C. laucha*	Yes	Yes
Guanarito	GTOV	NW-B	Venezuela	*Zygodontomys brevicauda, Sigmodon alstoni*	Yes	Yes
Sabiá	SABV	NW-B	Brazil	Unknown	Yes	Yes
Lassa	LASV	OW	Nigeria, Ivory Coast, Guinea, Sierra Leone	*Mastomys natalensis*	Yes	Yes
Lymphocytic choriomeningitis	LCMV	OW	Worldwide	*M. musculus*	Yes	No
Mobala	MOBV	OW	Central African Republic	*Praomys* sp.	nr	No
Mopeia	MOPV	OW	Mozambique	*Mastomys natalensis*	nr	No
Ippy	IPPYV	OW	Central African Republic	*Arvicanthus* spp.	nr	No
Flexal	FLEV	NW-A	Brazil	*Oryzomys* spp.	Yes	No
Pichindé	PICV	NW-A	Colombia	*O. albigularis*	nr	No
Parana	PARV	NW-A	Paraguay	*O. buccinatus*	nr	No
Allpahuayo	ALLV	NW-A	Peru	*Oecomys bicolor, Oecomys paricola*	nr	No
Pirital	PIRV	NW-A	Venezuela	*Sigmodon alstoni*	nr	No
Tacaribe	TCRV	NW-B	Trinidad	*Artibeus* spp. *(bat)*	Yes	No
Cupixi	CPXV	NW-B	Brazil	*Oryzomys capito*	nr	No
Amapari	AMAV	NW-B	Brazil	*Oryzomys goeldi, Neacomys guianae*	nr	No
Oliveros	OLVV	NW-C	Argentina	*Bolomys obscurus*	nr	No
Latino	LATV	NW-C	Bolivia	*Calomys callosus*	nr	No
Whitewater arroyo	WWAV	NW-rec	EEUU	*Neotoma albigula, N. mexicana, N. micropus, N. cinerea*	Yes	No
Tamiami	TAMV	NW-rec	EEUU	*Sigmodon hispidus*	nr	No
Bear Canyon	BCNV	NW-rec	EEUU	*Peromyscus californicus, Neotoma macrotis*	nr	No
Catarina	na	NW-rec	EEUU	*Neotoma micropus*	nr	
Chapare	na	NW-B	Bolivia	Unknown	nr	
Dandenong	na	OW	Australia	Unknown	nr	
Kodoko	na	OW	Guinea	*Mus Nannomys minutoides*	nr	

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; na: not assigned. NW: New World; OW: Old World. Recombinant lineage as reported previously.

[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. Adapted from Charrel et al. (2010).

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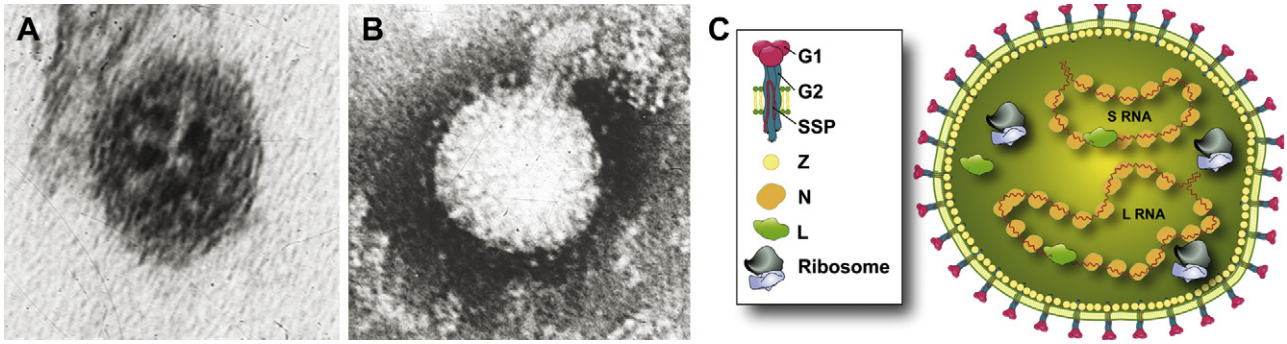

Fig. 1. A. First picture of a mature JUNV particle in the extracellular space near a plasma membrane. Notice granules and peripheral dense zone of the core surrounded by an envelope. Mouse primary fibroblast culture. 150,000X magnification. B. Negative staining of a JUNV particle with a pedicle budding from the plasma membrane. BHK cultures. 200,000X magnification. Original pictures taken by E.F. Lascano, kindly provided by A. Guevara. C. Schematic of JUNV particles. For details see text.

ribosomes inside, giving them a characteristic sand-like (arenous) appearance (Fig. 1). During release they acquire a lipid envelope from the host cell membrane. Their genome consists of two single-stranded negative RNA segments, one small in size (S) and the other large (L) of ~3,5 kb and ~7,3 kb, respectively. Each segment has two non-overlapping open reading frames of opposite sense known as *ambisense* [8], separated by a non-coding inter-gene region predicted to fold into a stable secondary structure. The L segment encodes for both the 94 amino-acid (aa) zinc-binding Z protein matrix which drives virus budding [9] [predicted molecular weight (mw) 10,459 Da], as well as for the RNA-dependent RNA polymerase L (2210 aa and 252,764 Da mw) [10]. S RNA encodes for both the glycoprotein precursor GPC as well as the nucleocapsid protein N. GPC is post-translationally cleaved to yield mature virion glycoproteins (G) G1 (192 aa, 22,154 Da mw), G2 (235 aa, 27,118 Da mw) and unusually retains its stable signal peptide SSP (58 aa, 6370 Da mw) [11]. G1/G2/SSP trimers form the spikes decorating the virus surface [12] (Figs. 1C and 2). G1 is located at the top of the spike and mediates virus interaction with host cell surface receptors [13], and G2 is similar to others class I viral fusion proteins [14]. SSP is generated by signal peptidase cleavage but unlike conventional signal peptides is stable, unusually long (58 vs. the usual 15–25 aa), myristoylated [15], contains two hydrophobic segments that each span the lipid bilayer with both N and C termini residing in the cytosol [16] (Fig. 2) and contribute to G2 fusion activity [17] through its C-terminal region [18]. The nucleocapsid protein N (537 aa, 60,003 Da mw) [19] can bind zinc and, according to a recent structural study of LASV N protein, the N domain has a structure with a cavity for binding the cap that is required for viral RNA transcription, whereas the C domain contains 3′-5′ exoribonuclease activity involved in suppressing interferon induction [20,21]. In PICV or LCMV, N is the most abundant virion protein, followed by G2, G1, Z and L protein (~1500, 650, 650, 450, and 30 molecules per virion, respectively) [22]. JUNV is believed to have the same distribution [23].

Finally, it is worth mentioning that a protein sequence database for pathogenic arenaviruses has been constructed [24]. First, monoclonal Abs (mAbs) against JUNV were generated against N and other glycosylated proteins mainly G2 [25], and more recently, new mAbs against N, identifying 3 reactive epitopes in residues 12–17 (WTQSLR), 72–79 (KEVDRLMS), and 551–558 (PPSLLFLP) were incorporated [26].

4. Replication cycle

4.1. Cell entry

Specific virus interaction with receptor molecules on the cell membrane drives subsequent entry into the host cell, making cell receptors the major determinants of viral cell-tropism, host-range and pathogenesis [27]. Until 2005, little was known about the mechanism through which JUNV entered host cells. Early in 2006, by using pseudotype viruses, Rokej et al. confirmed that JUNV, as well as other clade B NW arenaviruses, did not use α-dystroglycan, the receptor used by

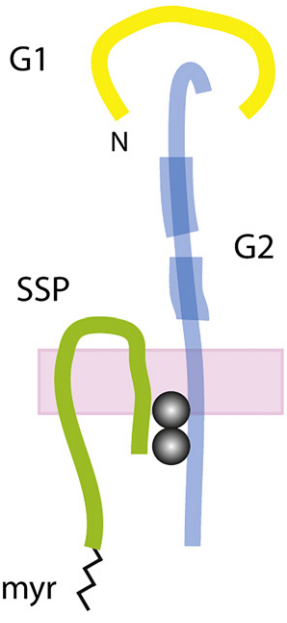

Fig. 2. Schematic of JUNV G1/G2/SSP trimers as proposed in [44]. For details see text.

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OW arenaviruses, for entry into cells [28]. The next big breakthrough came one year later when using a proteomic pull-down approach, applying a recombinant receptor-binding G1 moiety of MACV as bait, transferrin receptor 1 (TfR1) was identified as the first known JUNV, MACV, GTOV and SABV cell receptor [13]. Authors also showed that expression of human TfR1, but not TfR2, greatly enhanced susceptibility of otherwise somewhat resistant hamster cell lines to JUNV, MACV, GTOV and SABV pseudotypes, but not to LASV or LCMV. In addition, infection of human cells with JUNV, MACV, GTOV and SABV, but not with LASV, was efficiently blocked with anti-TfR1 Abs. Furthermore, iron loading of culture medium also affected infective capacity since depletion enhanced, and supplementation decreased infection efficiency of JUNV and MACV but not of LASV pseudoviruses. Together, these results provide strong evidence of TfR1 as a major cell receptor for pathogenic human clade B NW arenaviruses. Interestingly, TfR1 is also used by canine and feline parvoviruses and by the mouse mammary tumor virus (reviewed in [28]). In contrast, nonpathogenic human clade B NW arenaviruses like AMPV and TCRV attach to cells through a TfR1-independent pathway [29].

After binding to its receptor, a virus can resort to different internalization mechanisms. It has been demonstrated in Vero cells that clathrin-mediated endocytosis is the main one used by JUNV [30]. Compounds impairing clathrin-mediated endocytosis reduce virus internalization without affecting virion binding. In contrast, drugs altering lipid-raft microdomains and therefore impairing caveola-mediated endocytosis, do not block virus entry. Finally, direct evidence of JUNV cell entry was obtained using transmission electron microscopy [30]. PICV, a related virus, has also been shown to enter cells through a clathrin-dependent endocytic pathway, trafficked through the dynamin 2 endocytic pathway in which the virus travels through Rab5-mediated early and Rab7-mediated late endosomes [31]. Similar results have been observed for JUNV [32]. JUNV internalization leads to PI3K/Akt signaling pathway activation [33] which requires both intact actin and a dynamic microtubule network [34]. It is not known whether other molecules are involved in the process. Because TfR1 is recycled through the early endosome, but JUNV GP requires delivery to more acidic late endosomal compartments [18], it has been suggested that JUNV manipulates endocytic host cell machinery by modifying the oligomeric state of TfR1 ligand [28].

4.2. Transcription and replication

3′ and 5′ terminal sequences of 19-nucleotide RNA segments are extremely conserved as well as complementary in arenaviruses including JUNV, suggesting hybridization between them probably occurs. These termini are essential for replication and transcription and are believed to function as a binding site for viral polymerases (reviewed in [35]).

Genome replication and transcription takes place in the cytoplasm of infected cells and requires that viral proteins combine with viral RNA to form ribonucleoprotein (RNP) complexes. Purified RNPs are competent for RNA synthesis *in vitro* [36]. During genome replication, full-length copies of genomic S and L RNAs are synthesized generating the corresponding antigenomic S and L RNAs. Due to the *ambisense* coding strategy, both genomic and antigenomic RNAs serve as template for viral mRNA transcription (Fig. 3). Transcripts contain a cap but are not polyadenylated (reviewed in [35]). Termination at the inter-gene region [37] suggests the stem-loop structure plays a role in transcription termination. However, elements regulating termination have not yet been well defined. Genomic and antigenomic S RNAs as well as S RNA transcripts often contain non-template extensions at terminal position 5′. These are thought to be generated at the beginning of replication and transcription and may be important to maintain integrity of genomic terminal segments (reviewed in [35]). Viral RNA species packaged into virions are defined as genomic RNAs; however, smaller amounts of antigenomic RNA and Z gene mRNA are also included [38]. Ribosomal RNA is also found inside virions but its relevance is not known.

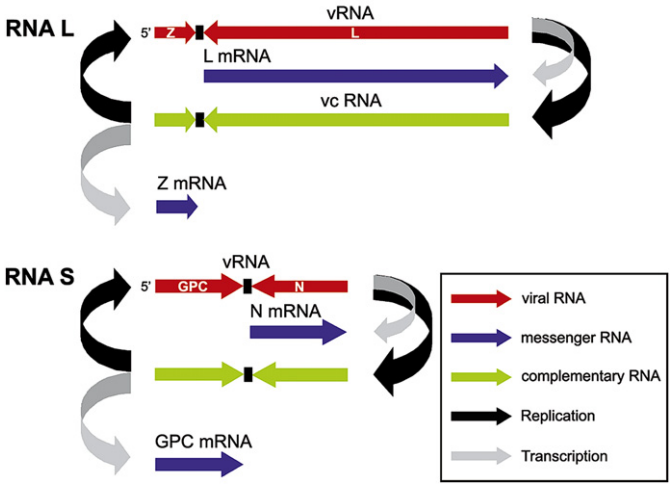

Fig. 3. Schematic of JUNV functional genome organization. For details see text.

In TCRV, N and L proteins together with virus RNA are the minimal components of RNP complexes, and are sufficient for genome replication and transcription [10]. The functional role of the Z protein in genome replication and transcription is unclear, but it has been assigned both a role in genomic RNA and N mRNA synthesis [39], as well as an inhibitory effect on transcription and replication, but not on encapsidation, [10] via interaction with the L protein [40].

4.3. Assembly, maturation and release

SSP is required for transport of the G1G2 precursor protein from the endoplasmic reticulum (ER). The interaction with SSP appears to mask endogenous dibasic ER retention/retrieval signals in the cytoplasmic domain of G2 [41] to allow transit through the Golgi compartment, where the G1G2 precursor is cleaved by the cellular SKI-1/S1P protease to form the mature

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G1 and G2 subunits [11,42]. As with other class I viral fusion proteins, proteolytic cleavage of the G1G2 precursor is required to render the GPC complex competent for membrane fusion [11,15,42]. It have been suggested that SSP is retained and positioned in GPC through interaction with a zinc-binding domain in the cytoplasmic tail of G2 [41,43], a fact that recently have found structural evidence [44].

SKI-1/S1P plays a role in regulation of cell lipid metabolism. Interestingly, cleavage by SKI-1/S1P is not necessary for GPC transport to the cell surface, where budding occurs, but it is a prerequisite for GP incorporation into viral particles. In the absence of LASV GPC cleavage, enveloped non-infectious virus-like particles containing at least N, Z protein and viral RNA, but devoid of viral GP are released. Thus, proteolytic processing of GPC is necessary for infectivity. The consensus motif at the cleavage site appears to be preserved in arenaviruses. However, SKI-1/S1P cleaves peptides representing the processing site of GTOV only to negligible levels, indicating that some arenavirus GPCs are indeed processed by a related protease [35].

By using a TCRV-JUNV hybrid system, it has been shown that interaction between Z and N is required for assembly of both nucleocapsids and glycoproteins into infectious budding particles [45]. Sequence analysis revealed the presence of so-called late domains, short proline-rich sequences, in the Z protein of LCMV (PPPY) and Lassa virus (PTAP and PPPY). Late domains were previously identified in gag proteins of a number of retroviruses as well as in matrix proteins of rhabdo- and filoviruses, and are known to interact with cellular proteins such as Tsg101 and Nedd4 promoting virus particle release; their integrity is essential for this function [35].

Z protein has been assigned a major role in virus particle budding, at least for some arenavirus including JUNV [46]. Additionally, Z function may be related to cell response to viral infection. It has been recently demonstrated that Z proteins from NW arenaviruses such as JUNV, GTOV, MAVC, and SABV, but not from OW arenavirus like LCMV or LASV, bind to RIG-I, resulting in IFNβ response down-regulation [47]. Similar findings had been reported earlier for N protein in several arenaviruses including JUNV, after observing inhibition of IFNβ and IRF3-dependent promoter activation, as well as nuclear translocation of IRF-3 [20,48]. Confirmation of other JUNV Z protein functions, such as a reported interaction between LCMV or LASV Z protein and promyelocytic leukemia protein or the ribosomal protein P0, will require future studies.

5. AHF pathogenesis

JUNV may enter the body through skin, respiratory tract or gastrointestinal mucosa. After replication, generalized dissemination occurs but gross pathology changes are non-specific. Capillary dilatation ensues with perivascular erythrocyte diapedesis and bleeding, minor edema of the vascular wall has also been observed [49]. Erythroblastopenia with morphologically abnormal erythroid and leukopoietic cell lines and normal megakaryocytes have been described in bone marrow (reviewed in [50]), as well as severe meningeal edema and hemorrhages in Virchow–Robin spaces in the CNS.

Decreased T and B lymphocyte counts and a diminished response to mitogens are expressions of immuno-supression during the acute phase of the disease. Low numbers of null, B, and T cells, as well as a lower T4/T8 ratio have been observed during the acute phase of AHF. Null and T8 cell numbers improve after immune plasma infusion, and all cell subsets return to normal in early convalescence. It has been proposed that circulating monocytes (macrophages) are targets for JUNV replication contributing to viral spread during the acute AHF [50]. However, at this stage as well as in early convalescence, patient peripheral-blood mononuclear cells may exert antibody-dependent cell cytotoxicity [50], suggesting JUNV replication in macrophages does not affect their killing capacity.

Although causes of bleeding are poorly understood, impaired hemostasis, endothelial cell dysfunction and low platelet counts are considered the major determinants of AHF.

5.1. Altered hemostasis

Prolonged kaolin partial thromboplastin time (KPTT) with low levels of factors VIII, IX and XI and elevated levels of factors V and von Willebrand (vWF) has been found in early disease stages. Fibrinogen is significantly increased in moderate and severe cases, and increased plasma levels of prothrombin fragment 1 + 2 and thrombin-anti-thrombin complexes, suggest F Xa and thrombin are also generated [50]. These results, in combination with patient clinical features and absence of fibrin deposits in microcirculation rule out disseminated intravascular coagulation (DIC) as a contributing factor in AHF pathogenesis [4].

In moderate and severe cases, marked increase in tissue plasminogen activator is found in early stages of AHF, and functional and antigenic plasminogen levels decrease slightly [50]. Fibrin split products show raised D-dimer levels in almost all patients on admission, indicating insoluble fibrin is broken down before being deposited in the microcirculation [50]. Because a plasma inhibitor of platelet aggregation was found in patients with severe Lassa fever, search for a similar abnormality was conducted in AHF patients. An inhibitor of platelet aggregation and serotonin release was found. However, it was considered to be different from the one present in Lassa fever because it was not found in immunoglobulin G fraction and because its activity was abolished by heat (reviewed in [50]).

AHF patients show reduced complement hemolytic activity with a drop in antigenic levels of Clq, C3, C5 and functional C2, but with increased C4 antigen and Factors B and C3 degradation products [51], suggesting the alternative complement pathway is the one most affected. To gain further insight into complement activation pathways in AHF patients, levels of C3 (C3b, C3bi, C3c) and C4 activation products (C4b, C4bi and C4c) were measured using enzyme-linked immunosorbent assay [50]. High levels were present in all cases regardless of AHF severity. In addition, antigenic C2 and C4 levels were

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also elevated, thus confirming both complement pathways are activated.

On most cells, TfR1 expression is subject to regulation by cellular iron, with an inverse correlation between cytosol iron concentration and TfR1surface expression levels [52]. As mentioned earlier, iron loading of culture medium affected cell infection levels by JUNV pseudotypes [13] or infectious JUNV [53]. It has been suggested that iron deficiency *in vivo* may enhance susceptibility to these pathogens [52]. However, a revision of 190 AHF patient records showed hematocrits were below 38% in less than 20% all cases, indicating that anemia, an expected condition for individuals with iron deficiency, was not associated to early or late AHF [54]. Nevertheless, the issue may merit further investigation in experimental animal models.

During the first week after symptom onset, patients with AHF show very high serum α interferon titers (IFNα) [55]. Even though these values slowly normalize during the second week of illness in survivors, they remain elevated in severe cases. Interferon levels at admission correlate with outcome, and are significantly lower in patients who survive [56]. This apparent lack of endogenous IFNα efficacy led researchers to consider that circulating IFN might be biologically inactive. This hypothesis was later ruled out, after 2′-5′oligoadenylate synthetase, a marker of IFN biological activity, was found in above normal range in peripheral-blood mononuclear cells [57].

Other cytokines described as significantly elevated in the serum of acute AHF patients, include TNFα, IL-6, IL-8 and IL-10 [58] although their individual role in disease pathogenesis has not been studied. For more details on human disease findings, readers should consult the review by Marta et al. [50].

6. Experimental infections for pathogenic studies

In an attempt to better understand disease pathogenesis and host response, many *in vivo* models (wild rodents such as *Calomys musculinus* and laboratory-bred animals including mice, rats, guinea pigs, or non-human primates), as well as *ex vivo* models of endothelial, blood-derived or CNS cell cultures have been used to study JUNV infection. Most non-human primates differ in JUNV infection outcome, only guinea pigs reproduce human hematological disorders more closely making them the model of choice for virulence studies [2,50].

6.1. Guinea pigs

Adult guinea pigs (*Cavia porcellus*) infected with virulent JUNV strains show increasing viremia from day 7 post-infection (pi) until death at around day 13 pi [2]. Virus can be isolated from bone marrow, lymph nodes, lungs, liver, kidney and adrenal glands, but not from CNS tissue. If death is delayed by treatment with immune serum, animals infected with the XJ strain of JUNV develop encephalitis [2]. Infected animals suffer the majority of AHF hemorrhagic manifestations observed in humans including leucopenia and thrombocytopenia with areas of focal necrosis in affected organs, dying without detectable Abs [2]. Blood clotting abnormalities cause progressive KPTT prolongation and reduced levels of factors VIII, IX and XI. Conversely, fibrinogen levels are initially normal increasing in the last days of illness [59]. When increased fibrin monomers last until day 9 pi and subsequently return to normal, this is a sign of thrombin generation, although no fibrin deposits have been found in microcirculation, thus ruling out DIC. In JUNV-infected guinea pigs complement is activated through the classical pathway, low levels of Clq, C4, C2 and C3 are observed, while C3 degradation products are detected from day 9. However, no immune complexes or anti-JUNV Abs have been found to explain complement activation (reviewed in [50]).

6.2. Primates

Susceptibility to JUNV infection varies among species. *Alouatta caraya, Saimiri sciureus* and *Cebus* spp show little or no susceptibility, whereas *Callitrix jacchus* develops blood disorders including anemia, leucopenia and thrombocytopenia as well as CNS involvement dying 17–24 days pi, without demonstrable anti-JUNV Abs. Coagulation abnormalities consist only of shortened KPTT with increased levels of factors VII–X and FVIII. Hemolytic complement activity and C3 levels are low on day 7 pi, increase by day 14 and return to normal by the time terminal stages develop (reviewed in [50]).

6.3. Calomys musculinus

Although intraperitoneal (ip) infection of newborn *C. musculinus* with virulent JUNV strains causes 100% mortality, inoculation with an attenuated strain decreases mortality to 50% during the first month, mainly through less CNS involvement. Animals older than 20 days are resistant when infected ip but remain susceptible to intranasal infection. Survivors present one of three possible outcomes: they can remain asymptomatic, recover after clinically manifest neurological disease or suffer persistent illness with growth arrest and infectious virus detectable in CNS tissue. Mechanisms underlying disease and viral persistence in *Calomys* remain poorly understood due in part to lack of inbred strains. For a more complete description, readers may consult the review by Weissenbacher et al. [2].

6.4. Mice and rats

Newborn mice (*Mus musculus)* are highly susceptible to JUNV, developing persistent infection without blood alterations. Rats (*Rattus norvegicus*) are also susceptible. The course of infection has been associated to the viral strain used with high lethality for virulent strains [2]. Histopathology shows relatively mild meningo-encephalitis with JUNV antigen present in the cytoplasm of neurons of the cerebral cortex, basal nuclei, cerebellum, spinal cord, and to a lesser extent, in the cytoplasm of astrocytes. Despite heavy infection of neural structures, neurons do not usually show major alterations. In contrast, a severe reaction is seen in astrocytes,

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which show enhanced expression of glial fibrillary acid protein (GFAP) [60]. In chronically infected animals no correlation between viral antigen and reactive astrocyte distribution has been observed [61]. Evidence of a protective role for induced nitric oxide (NO) is thought not to be related to reduced viral replication but rather to enhanced astrocyte activation suggesting the behavior may represent a beneficial cell response to virus-induced CNS damage [62].

Susceptibility to the infection decreases with age and 21 day-old mice are usually resistant to infection by any route. However, C3H/HeJ mice strains have been reported as susceptible until 5 months of age when infected intracerebrally (ic) with virulent XJ JUNV strains [63]. A similar situation has been reported very recently in mice with disruption of interferon signaling, in which, in addition to CNS involvement, a significant and unexpected myocarditis was observed [64]. In contrast, in congenitally athymic nu/nu mice or in thymectomized or cyclophosphamide-treated mice, CNS disease but not persistent infection can be prevented, suggesting immune-mediated disease pathogenesis [2].

6.5. Endothelial cell cultures

Clinical and experimental studies on viral hemorrhagic fevers have revealed a crucial role of the endothelium in their pathogenesis [52]. Viruses causing such as filoviruses Marburg (MARV) and Ebola, arenaviruses JUNV and LASV, or Bunyavirus causing Rift Valley fever, efficiently enter and replicate in endothelial cells, and some (e.g. MARV) may induce direct endothelial damage [52]. In contrast, JUNV replicate in endothelial cells without apparent cytopathic effects [65]. However, selective endothelial cell dysfunction has been observed, including enhanced expression of cell adhesion molecules ICAM-1 and VCAM-1, decay accelerating factor, endothelial NO synthase, as well as greater prostacyclin (PGI_2) release. In contrast, vWF production is decreased. Similarly, significant increases in NO and PGI_2 values have also been found in AHF patient sera. These studies provide the first evidence of vascular abnormalities in AHF patients [65] although their *in vivo* relevance will have to be further investigated [52].

6.6. Megakaryocytic cell cultures

With respect to thrombocytopenia, recent findings have shown that although JUNV may replicate in megakaryocytes and in their precursors, $CD34^+$ cells, they do so only in a restricted fashion. Moreover, the degree of proliferation, survival, and commitment in JUNV-infected $CD34^+$ cultures is similar to levels observed in mock-infected cultures. However, viral infection impairs thrombopoiesis by decreasing *in vitro* pro-platelet formation, platelet release, and P-selectin externalization via a bystander effect. The decrease in platelet release is also TfR1-dependent, mimicked by poly(I:C), and type I interferon (IFN α/β) has been implicated as a key paracrine mediator [53]. As mentioned earlier, high levels of circulating IFNα, correlating with virulence and prognosis have been described in clinical and experimental AHF [53]. Moreover, during systemic viral infection, bone marrow hematopoietic cells appear to be the most important source of type I IFN [53].

6.7. CNS-derived cell cultures

Brain tissue cultures have been used for pioneering ultrastructure studies on JUNV morphogenesis [66]. GFAP labeling has been subsequently applied in astroglial cell cultures to demonstrate how JUNV infection accelerates spontaneous astrocyte differentiation normally occurring in culture, as observed in animal tissue [67]. JUNV infection of astrocytes does not induce apoptosis but does enhance iNOS expression and NO synthesis. These changes occur earlier than GFAP expression increase. The fact that iNOS inhibition abolishes enhanced GFAP expression in infected monolayers suggests that NO is directly involved. In addition, iNOS inhibition enhances virus replication. These results suggest that JUNV induces iNOS expression in infected astrocytes and that the resulting NO has an important role both in reducing viral replication and in enhancing subsequent astrocyte activation [68].

7. Reverse genetics

Until recently, manipulation of genetic material from arenaviruses was mainly restricted to generating viral reassortants or to selection of variants *in vitro* and *in vivo*. In cells infected with closely related viruses, L and S RNA segments can be exchanged between viruses (so-called reassortment), and the resulting reassorted virus can subsequently be purified from plaques and characterized (reviewed in [35]). RNA segments have been reassorted between different PICV strains, LCMV strains as well as between LASV and MOPV.

Reverse genetics techniques have been used to study LCMV and TCRV for a few years now. Since their introduction, methods have been further simplified and improved. Very recently, a JUNV functional minigenome system and a reverse genetics system for production of infectious JUNV have been reported. The two-plasmid system involves transfection of cells with only two plasmids which transcribe S and L antigenomic viral RNAs. The enhanced efficiency of this system offers great promise for addressing important unanswered questions on arenavirus hemorrhagic fever as well as for future development of more precise attenuation procedures for live arenavirus vaccines [69–71].

8. Perspectives

This review presents a detailed report of the most significant findings related to JUNV since its discovery, more than 50 years ago. Of note is the fact that many were achieved with very limited resources and at the expense of great effort on behalf of the scientists involved, even incurring in health risks on the way. However, there are strong reasons to think that the best is yet to come. Whether JUNV or other NW arenaviruses,

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are involved in congenital neurological diseases such as LCMV [72], remains to be elucidated. The possibility of having new *ex vivo* models, genetically-modified susceptible adult mice, as well as incipient reverse genetics technology now opens wide the door to unveiling more of the mechanisms involved in viral replication at the molecular level, furthering our understanding of the diseases they cause.

Acknowledgments

This work was supported by grants from Agencia Nacional de Promoción Científica y Tecnológica (ANPCyT) PICT 07-00642, PICT 07-00028 (RMG) and X592 from Universidad Nacional de La Plata. The authors would like to thank Dr. A. Ure for kindly providing Figs. 1C and 3; Dr. J. Nunberg for Fig. 2 and for many helpful suggestions; Dr. F. Molinas for providing important information relative to clinical records; Ms. A. Guevara for the original ultrastructural photographs of Dr. E. Lascano; and Dr. V. Romanowski for his constructive suggestions on the manuscript. RMG and MS are researchers and CJG is a fellow of the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina.

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