CHAPTER 2

Recent Advances in the Development of Antiviral Approaches against Hemorrhagic-Fever-Causing Arenaviruses

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Abstract: Arenaviruses, enveloped viruses containing a bisegmented single-stranded RNA genome with ambisense coding strategy, include important hemorrhagic-feve--causing viruses representing a public health threat in endemic areas of Africa and South America. In spite of the danger of pathogenic arenaviruses and their increased emergence in recent years, no specific and safe chemotherapy for these viruses is currently available. This chapter covers recent advances in the development of antiviral strategies to face arenavirus infections. New insights in molecular aspects of virus replication and virus-host interactions have allowed the identification of viral and cellular factors as potential target for antiviral therapy. We will revise the main features of arenavirus biology and the mechanism of antiviral action of different molecules derived from natural sources, chemical synthesis and rational structure-based antiviral drug design. The advantage of targeting viral and cell host factors as complementary approaches for therapy intervention will be discussed. We will particularly discuss the use of novel inhibitory strategies and the main advances in the development of innovative screening platforms.

Keywords: Attachment, Antiviral activity, Arenavirus, Argentine hemorrhagic fever, Budding, Candid #1, Emerging viruses, Entry, Favipiravir, Guanarito virus, High-throughput screening, Interferon, Junin virus, Lassa fever, Lassa virus, Lujo virus, Machupo virus, Membrane fusion, Monoclonal antibody, Pathogenesis, Peptide, Replication, Reverse genetic, Ribavirin, Sabia virus, Small interfering RNA, Small molecule, Therapy, Uncoating, Hemorrhagic fevers.

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Atta-ur-Rahman (Ed)

INTRODUCTION

Viral hemorrhagic fevers (VHFs) are a set of human diseases caused by viruses belonging to four distinct families: *Arenaviridae* (Junin, Machupo, Guanarito, Sabia, Lassa, Lujo and Chapare viruses), *Bunyaviridae* (Crimean-Congo, Rift Valley, Hantaan, Puumala, Seoul, Dobrava, Sin Nombre, Andes, Choclo and severe fever viruses), *Filoviridae* (Marburg and Ebola viruses), and *Flaviviridae* (yellow fever, dengue, Omsk, Kyasanur Forest and Alkhumbra viruses). These families include enveloped viruses with RNA genomes that are maintained in nature by infection of a mammal or insect host, which is usually referred as the reservoir. Reservoirs include nonhuman primates, bats, rodents, domestic ruminants, humans, mosquitoes, and ticks. This characteristic leads to a geographically restricted distribution of the viruses around the world that is coincident with that of the corresponding reservoir and matches the area of incidence of the disease. Up to the present, VHFs cannot be cured or controlled by any specific antiviral treatment being supportive therapy the main therapy option. Although hemorrhages are prone to occur in VHFs, especially in cases of patients with low platelet counts or dysfunction in blood clotting, these hemorrhages use to be mild and do not represent a serious threat to health. However, in severe cases, extensive damage of the vascular system leads to capillary leakage and, eventually, shock, both characteristic features of the terminal phase of VHFs [1]. Despite clinical aspects differ among VHFs, perturbation of vascular endothelium integrity and coagulation abnormalities appear as two common features underlying VHFs pathology. Both aspects are associated not only to a direct cytopathic effect of virus replication in endothelial cells but to the contribution of the immune response mediated by proinflammatory cytokines as well. In many cases, an initial phase of the disease characterized by virus suppression of the innate immune response is established, thus allowing systemic infection by virus replication in monocytes, macrophages and dendritic cells, which in turn induce an exacerbated cytokine production that triggers severe disease [2]. Most detailed data on pathogenesis of VHFs come from studies carried on nonhuman primate (NHP) models of infection with Ebola virus (EBOV). EBOV disease is characterized by systemic virus replication at high titers, cytokine storm, liver damage due to hepatocyte infection, coagulopathy due to endothelial cells infection, perturbation of blood pressure due to adrenal cortical cells infection, and lymphopenia as reviewed in [3].

Junin virus (JUNV) pathogenesis is characterized by a marked tropism for lymphatic tissue, being macrophages, dendritic cells and lymphocytes the main cell targets for virus replication. Infection of cells from the immune system may

underlie a mechanism of immune system evasion together with a concomitant reduction in the count of these cells leading to the leucopenia observed in the majority of patients. The initial immune evasion induced by the virus may lead to a later phase of infection when a cytokine storm is produced by the infected cells that is, by far, more harmful than the scarce cytolitic potential of the virus. Eventually, permeability of endothelial cells in response to cytokine production may lead to hemorrhage and shock [4]. In line with this, the presence of high levels of interferon (IFN) may affect thrombopoiesis *via* alteration of the coagulation capability of platelets. Moreover, infection of megakaryocytes by JUNV leads to a diminished proplatelet formation and release, contributing to patient bleeding [5].

In the case of OW arenavirus Lassa (LASV), although macrophages and dendritic cells are also the main target cell types, they are not activated as in the case of infection with JUNV and a generalized immunosuppression is maintained throughout the course of infection [6, 7].

The fact that isolated outbreaks of VHFs occur sporadically all over the world, contributes to the lack of detailed information about pathogenesis. On this line, the conspicuous symptomatology of VHFs favors epidemiological studies towards the identification of the factors involved in the emergence and maintenance of viruses in nature. One important point to consider is that human infections with viruses that cause VHFs do not contribute to the evolutionary success and survival of the agent which is rather linked to the chronic infection of the host, vertebrate or arthropod, than to the acute and sequelae free infections developed in humans [8].

Despite almost two thirds of human population reside in areas of VHFs incidence limited effort is put on the development of vaccines against these diseases. Basic research is constantly performed on this subject, in fact, many experimental vaccine platforms have been evaluated in several animal models but only few of them are being considered for clinical trials, such as a recombinant vesicular stomatitis virus-based vaccine for EBOV expressing the Zaire EBOV glycoprotein and a live attenuated Rift Valley vaccine [9, 10] and recently a live attenuated DENV vaccine based on a yellow fever 17D vaccine backbone has been approved in some endemic countries [11]. A live attenuated JUNV vaccine, called Candid#1 is currently available in Argentina but it is not recommended for children and pregnant women. Although partial cross protection with Candid#1 for the arenavirus Machupo (MACV) has been reported, employment of Candid#1 has not been approved for use in other countries [12].

THE *ARENAVIRIDAE* FAMILY

The *Arenaviridae* family comprises two genera *Mammarenavirus* and *Reptarenavirus*, isolated from mammals and snakes, respectively. *Mammarenavirus* comprises at least 25 members classified into two groups: Old World (OW) and New World (NW) arenaviruses, based on serological cross-reactivity and sequence-based phylogenetic studies. Reservoirs for OW arenaviruses are rodents from *Muridae* family, *Murinae* subfamily whereas NW arenaviruses reservoirs belong to *Muridae* family, *Sigmodontinae* subfamily. Furthermore, while OW viruses form a single lineage, NW arenaviruses can be differentiated into clades A, B, A/B, and C [13, 14]. The OW arenavirus LASV is endemic of West Africa and infects several hundred thousand individuals yearly causing an elevated number of Lassa fever (LF) cases, a VHF of high morbidity and mortality [15]. The recent isolation of Lujo arenavirus (LUJV), identified as the causative agent of an outbreak of VHF in Southern Africa, settles a new territory for VHFs caused by OW arenaviruses outside the existing endemic region [16]. The NW JUNV, endemic to the central region of Argentina, a highly populated farmland area, causes Argentine Hemorrhagic Fever (AHF) with a fatality rate ranging 15% to 30% [17]. Other NW arenaviruses causative of VHFs are: MACV and Chapare (CHPV) viruses in Bolivia [18], Sabia virus (SABV) in Brazil [19] and Guanarito virus (GTOV) in Venezuela [20].

Arenavirus virions are enveloped pleomorphic particles, 40-80 nm in diameter, containing single stranded RNA bisegmented genomes harboring four ORFs arranged in an ambisense coding strategy.

The small RNA segment of 3.4 kb approx. codes for the glycoprotein precursor (GPC) and the nucleoprotein (NP). GPC and NP genes are separated by an intergenic non-coding intergenic region (IGR) that is rich in secondary structure [21]. Processing of GPC by a signal peptidase and the cellular Site 1 Protease (SKI-1/S1P) yields three polypeptides: the signal peptide (SSP) and the two mature glycoproteins: the surface glycoprotein (GP1), which elicits neutralizing antibody response and is responsible for the interaction with the cellular receptor, and the fusion glycoprotein (GP2) that mediates fusion of viral and cellular membranes during viral internalization into cells. Processing of GPC induces a series of conformational changes that triggers the formation of a tripartite structure, the envelope glycoprotein complex (GP), which is constituted by SSP, GP2 and GP1. GP in turn associates as homotrimers conforming the arenavirus spikes [22]. The spike is plausible of undergoing a re-arrangement that facilitates membrane fusion when exposed to low pH during viral entry. Although GP2 may

be classified within class I viral fusion proteins, arenavirus are unique among viruses because glycoprotein spikes keep the cleaved SSP as part of each GP complex [23].

NP protein encapsidates with RNA molecules to form viral genomes and is able to associate with the viral RNA dependent RNA polymerase (L) and the matrix protein (Z) to carry on transcription and replication of the RNA genome. In addition, NP is involved in cell innate immune response suppression, associates with Z protein driving budding of virus and exhibits 3´exonuclease and ligation of nucleotides activities [24 - 31].

The large RNA segment of 7.2 kb approx. codes for the zinc binding protein (Z) which acts as a matrix protein and interacts with NP and L modulating transcription and replication, drives budding of virions through plasmatic cell membrane, interferes with the interferon (IFN) response and promotes apoptosis of infected cells [25, 30, 32 - 34]. The large segment also codes for the L protein, the viral RNA dependent RNA polymerase that together with NP protein constitute the minimal factors necessary for transcription and replication of viral RNA. L and Z genes are also separated by an IGR as described for NP and GPC genes [24, 35, 36].

Arenavirus Replication Cycle

Entrance of Arenavirus into Cells

Cell susceptibility of infection for arenaviruses is established through successful interaction of the GP complex with a protein located in the cell surface that acts as a receptor for attachment. This interaction not only mediates adsorption of virions to cell surface but sets the beginning of a series of coordinated steps leading to endocytosis, membrane fusion in response to acidic pH of endosome and, finally, release of viral nucleocapsids into cell cytoplasm (reviewed in [37]).

Binding of GP1 to a protein receptor located in the cell surface of susceptible cells initiates the process of infection of arenaviruses. Entry by the OW and NW clade C species of arenaviruses depends on the binding of GP1 to α-dystroglycan (αDG), a ubiquitous and highly conserved glycoprotein of the cell surface that mediates adhesion to the extracellular matrix [38, 39].

NW clade B species that include pathogenic NW arenaviruses attach to cells by interacting with the human transferrin receptor-1 (TfR1) [40]. Characterization of this interaction has been described from the point of view of the host specificity

observed for these viruses and TfR1 residues involved in this interaction have been already identified [41]. Genetic studies performed with MACV GP1 pointed out several residues of this glycoprotein as critical for the interaction with the receptor. Many of these residues are conserved among other NW viruses' glycoproteins suggesting a common basis of receptor interaction and opening avenues for the rational development of viral entry inhibitors [42].

Even though all NW clade B viruses are able to interact with their specific rodent-host TfR1, only those causative of VHFs can also recognize human TfR1. Also, it has been reported that a single amino-acid change in human TfR1 is enough to permit entry by Tacaribe virus (TCRV), a non-pathogenic clade B species [41]. This finding suggests that through subtle changes in GP1 nonpathogenic arenaviruses could use human TfR1, emerging thus as human pathogens. The reciprocal has been also addressed in early studies with a host range mutant of JUNV attenuated for mice. This mutant, derived from XJCl3 strain of JUNV by induced mutagenesis with 5-fluoruracil, showed an altered GP1 protein that conferred the mutant the inability to adsorb to murine cells while keeping intact its capacity to replicate in monkey/human cells [43].

Adsorbed viral particles are internalized *via* an endocytic mechanism and subsequently transport to late endosomes where fusion of the viral and endosomal membranes takes place at low pH (Fig. **1**). In the case of the NW arenavirus JUNV, a clathrin-dependent endocytosis that requires low pH in endosomes to achieve viral and endosomal membrane fusion has been reported [44, 45]. By contrast, experimental evidence supports a clathrin-independent internalization for the prototypic OW lymphocytic choriomeningitis virus (LCMV) [46]. Recent data revealed that LASV binding to αDG links the virus to an uncommon pathway of macropinocytosis with minimal perturbation to the host cell. The hepatocyte growth factor receptor (HGFR), a tyrosine kinase receptor (TKR), has been pointed out as a probable candidate to mediate this process [47].

As mentioned above, low pH within the endocytic vesicles involved in virus uptake triggers fusion between vesicle and viral membranes. Acidic pH promotes the transition from a metastable perfusion GP complex to an activated fusion competent GP form, characterized by the reorganization of GP2 ectodomain that finally drives membrane fusion. Interestingly, amino acid changes in SSP affect GP mediated membrane fusion indicating that SSP and GP2 interaction is required for the formation of the fusion active GP complex [48] (Fig. **2**).

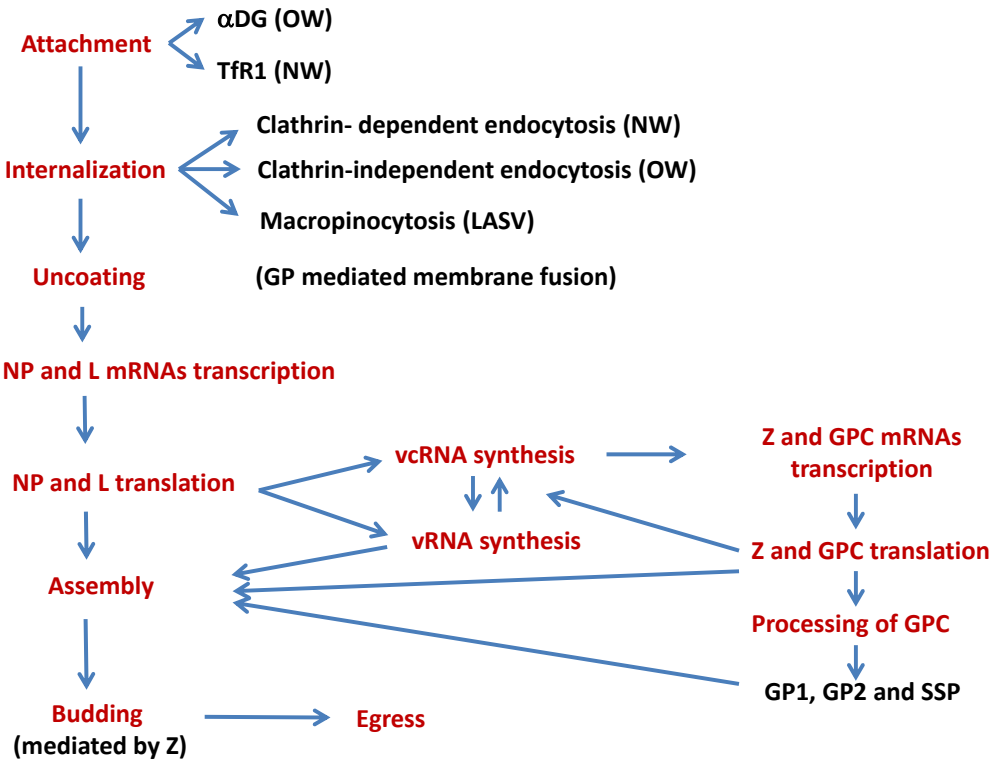

Fig. (1). Scheme of arenavirus replication steps. The sequence of the main events of arenavirus replication are shown. Arenavirus cellular receptors αDG: α-dystroglycan and TfR1: human transferrin receptor-1. OW: Old world arenaviruses; NW: New world arenaviruses; LASV: Lassa virus; NP: nucleoprotein; L: RNA polymerase; Z: matrix protein; GP: glycoprotein complex; GPC: glycoprotein precursor; GP1, GP2 and SSP: mature products of GPC processing; vRNA: viral RNA and vcRNA: viral complementary RNA.

Synthesis of Macromolecules

Once nucleocapsids are released in the cytoplasm, transcription of genes proceeds commanded by the promoters located in the 3´untranslated regions (UTRs) of the viral RNA (vRNA). Hence, NP and L proteins are the first proteins to be synthesized upon infection taking into account that NP and L genes are coded in the 3´ends of the small and large genome segment, respectively (Fig. **1**). Transcription of NP and L mRNAs is terminated by the secondary structure of the IGR in the form of a stem loop. Anti-termination activity of NP allows the replication complex formed by NP, L and Z to make a full copy of a viral complementary RNA (vcRNA) from which the mRNAs corresponding to Z and GPC genes are transcribed. At the same time, vcRNA serves as template for the synthesis of vRNA of progeny virus [13].

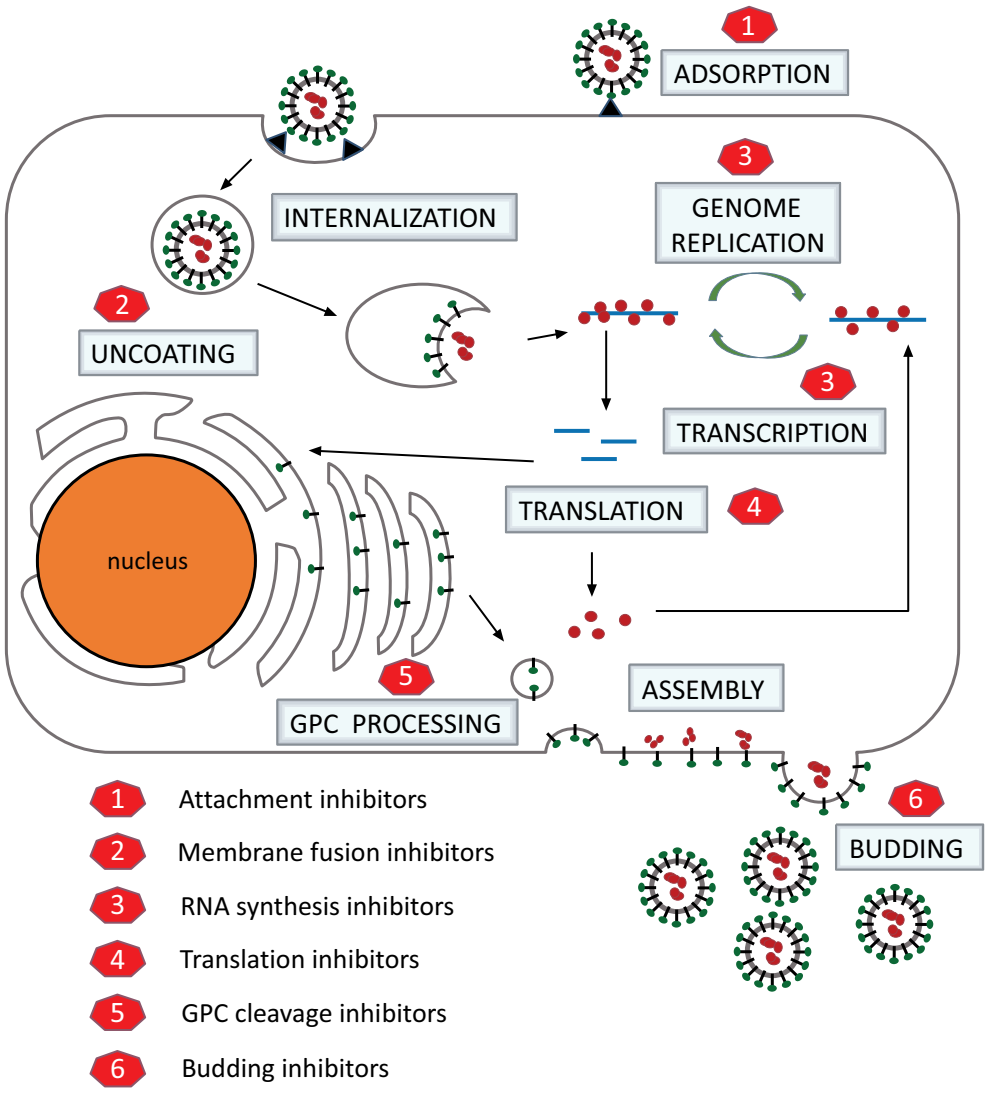

Fig. (2). Arenavirus replication cycle. The main targets of antiviral strategies against arenaviruses are indicated. GPC: precursor of viral glycoproteins.

Eukaryotic initiation factor of translation 4E (eIF4e) is dispensable for the synthesis of JUNV proteins suggesting a non-canonical initiation process of translation [49]. In the case of LCMV and LASV, it has been reported that Z protein is able to bind eIF4E and suppress translation of cellular proteins [33, 50].

Assembly and Budding

Newly synthesized vRNA is encapsidated by NP and, at a lesser extent, by L and Z, to form the nucleocapsids, which in turn interact with the Z protein underlying the plasma membrane where glycoprotein spikes are inserted [51]. Subsequent to this interaction, progeny viruses bud at the plasma membrane in a process mainly directed by Z protein (Fig. **1** and **2**) [52]. This process involves a PTAP late (L) domain motif of Z that mediates virus budding by binding cellular protein Tsg101 and other proteins of the endosomal sorting complexes required for transport (ESCRT) to induce the release of virions from the cell membrane [53]. This mechanism of egress is counteracted by the cellular protein named tetherin (also known as BST-2, CD317 or HM1.24), which is upregulated by treatment with IFN of different cell types. Tetherin locates as a homodimer in the plasma membrane of cells and its antiviral mechanism seems to be related to its ability to bind progeny viruses blocking viral release [54]. Up-regulation of Tsg101 has been also described as an antiviral factor in BHK-21 cells persistently infected with JUNV [55].

INNATE IMMUNITY MEDIATED ANTIVIRAL STATE IN ARENAVIRUS INFECTIONS

Interferon Response

IFNs are a family of cytokines that modulate diverse signaling pathways in order to allow the host to mount the most appropriate response against microbial infections, particularly, viral infections. There are three types of IFNs. The type I IFNs (IFN-I) comprise multiple subtypes of IFNα (13 in humans), IFNβ (1 in humans) and other less described IFNs such as IFNω, IFNε, IFNκ, IFNδ and IFNτ. The type II IFNs (IFN-II) include only one member, IFNγ, which has antiviral activity but is mainly considered as a strong immunomodulatory cytokine. The type III IFNs (IFN-III) or IFN-λs comprise three members: interleukin (IL)-29, IL-28A, and IL-28B [56]. To note, IFN-I and IFN-III are produced by many different cell types, while IFN-II is mainly produced by activated natural killer (NK) cells and T lymphocytes. Plasmacytoid dendritic cells (pDCs) produce the highest levels of IFN-I and other proinflammatory cytokines in response to viral infections [57]. When different cell types are infected, the interaction of viral components with cellular pattern recognition receptors (PRRs) activates signaling pathways leading to IFN-I production [58]. The IFN-I secreted by infected cells interacts with its own receptor allowing an additional increased of IFN-I production and promotes control of virus replication

in the IFN-producing cell (autocrine manner). IFN-I also acts in a paracrine manner to facilitate the establishment of an antiviral state in neighboring cells and to activate other cells types involved in the innate and adaptive response. The induction of IFN-I is controlled by three different classes of PRRs: Toll-like receptors (TLRs), retinoic acid inducible gene-I like receptors (RLRs) and nucleotide oligomerization domain-like receptors (NOD) [59]. RLRs are comprised of retinoic acid inducible gene-I (RIG-I), melanoma differentiation-associated gene 5 (MDA5) and laboratory of genetics and physiology 2 (LGP2), and together form the RLH family [59, 60]. RIG-I was identified as a detector of cytoplasmic viral RNA responsible for the production of IFN-I [61]. RIG-I recognizes 5′-triphosphate from ssRNAs as well as short (<2 kbp) dsRNAs in most cell types, and MDA5 detects 5′-triphosphate from long (>2 kbp) dsRNAs as well as a synthetic dsRNA as poly (I:C). This causes an exposure of the N-terminal domain of RIG-1, which is involved in protein-protein interactions and activates downstream signaling pathways that ultimately lead to the activation of IRF3 and IRF7 kinases [62]. Once IFN-I is produced, it binds to specific cell receptors and induces the expression of different interferon stimulated genes (ISGs) *via* the activation the Janus kinases (JAKs) signaling cascade, a family of tyrosine kinases comprised of Janus kinase 1 (JAK1) and tyrosine kinase 2 (Tyk2), which in turn activate signal transducer and activator of transcription (STAT) factors STAT1 and STAT2 by phosphorylation [59].

Inhibition of Interferon Response by Arenavirus Proteins

It has been shown that viral proteins of both OW and NW arenaviruses are capable of inhibiting the IFN-I response (Fig. **3**). For example, it has been reported that the NP proteins of OW LASV and LCMV as well as NW JUNV, PICV, MACV, White Water Arroyo virus (WWAV), TCRV and Latino virus (LATV) have the ability to inhibit the translocation to the nucleus of IRF3, and in consequence, they inhibit the induction of IFN-I [26, 28, 63, 64]. On the other hand, the Z protein is another arenavirus protein that contributes to the virus suppression of the IFN-I response. It has been reported that Z proteins of NW JUNV, GTOV, MACV and SABV are able to bind RIG-I, causing downregulation of the IFN-I response. Z seems to impede the binding of IPS-1 to RIG-I, which inhibits the trigger of signaling cascades that result in induction of IFN-I [65]. Interestingly, Z proteins of OW LASV and LCMV do not have the ability to bind RIG-I. However, it has been demonstrated that LCMV NP directly interact with RIG-I and MDA-5 [64]. Additionally, the short double-stranded RNAs with the overhanging 5′ppp-G residue present at the 5′-end of arenavirus genomic and antigenomic RNA species are bad substrates for RIG-I binding. This

probably contribute to arenavirus evasion from RIG-I recognition [66, 67]. Despite the initial IFN suppression mediated by NP and Z proteins a strong induction of IFN and proinflammatory cytokines is observed in AHF patients at a later state of infection.

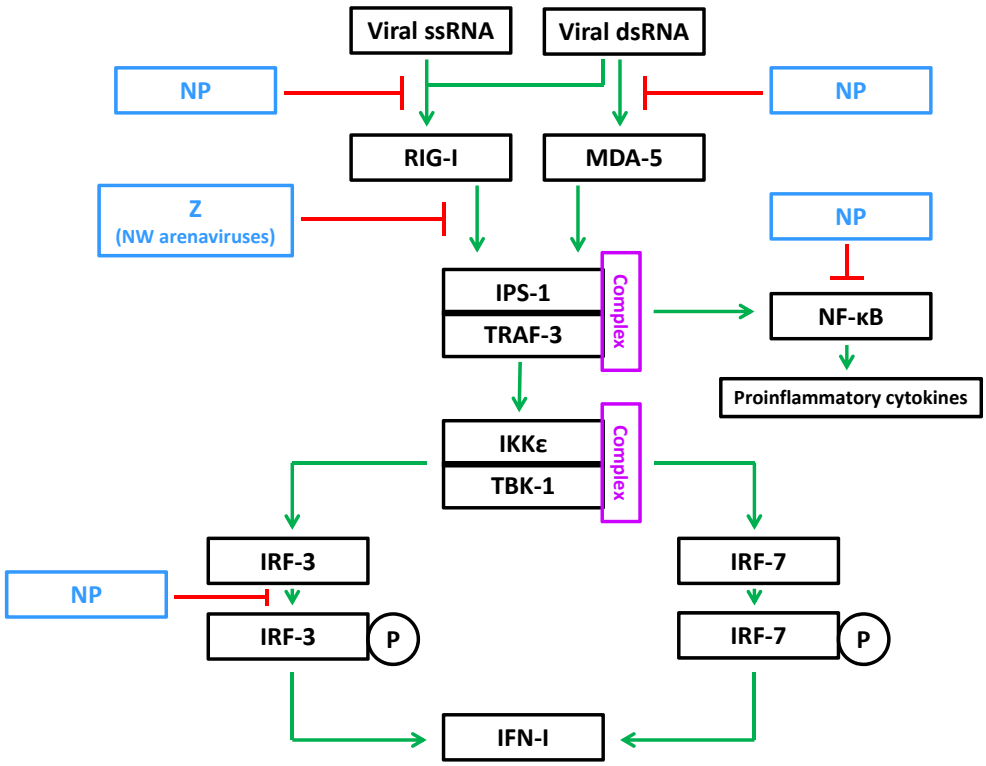

Fig. (3). Inhibition of interferon production by viral proteins. Green arrows indicate the sequence of events along the interferon (IFN) induction pathway whereas red connectors indicate inhibition mediated by viral proteins. NP: arenavirus nucleoprotein; Z: arenavirus matrix protein;ssRNA: single stranded RNA; ds: double stranded RNA; NW: New world; RIG-1: retinoic acid inducible gene-I; MDA-5: melanoma differentiation-associated gene 5; IPS-1: interferon-beta promoter stimulator 1; TRAF-3: tumor necrosis factor receptor-associated factor 3; NF-κB: nuclear factor κB; IKKε: inhibitor-κB kinase epsilon ; TBK-1: TANK-binding kinase 1; IRF-3: interferon regulatory factor 3; IRF-7: interferon regulatory factor 7; P: phosphate; IFN-I: type I interferon.

It is known that during the IFN-I response, protein kinase R (PKR) expression is augmented. PKR phosphorylates the alpha subunit of eukaryotic translation initiation factor 2 (eIF2α), blocking cap dependent translation. Thus, cellular protein synthesis is impaired in the context of viral infection [68]. Phosphorylation of eIF2α also leads to the formation of stress granules, cytoplasmic aggregates that contain pre-initiation complexes that accumulate as a consequence of translation blockage. It has been demonstrated that in JUNV-infected cells stressed with sodium arsenite, which induces phosphorylation of

eIF2α, the virus is able to inhibit stress granules formation by impairing eIF2α phosphorylation. Both viral NP as GPC are able to maintain eIF2α phosphorylation levels similar to those observed in uninfected cells, thus neutralizing the suppression of protein synthesis exerted by the infected cell [69].

Innate Immunity to Old World Hemorrhagic Arenaviruses

Patients with LASV infection suffer an immunosuppression, and this is evidenced both *in vitro* and *in vivo* through the lack of IFN-I induction, proinflammatory response or T-cell activation [70, 71]. It has been reported that in human macrophages and DCs infected with LASV *in vitro* there are low levels of IFN-I and other proinflammatory cytokines such as tumor necrosis factor (TNF- α) and IL-1β. Also, in LASV-infected DCs a lack of induction of co-stimulatory molecules such as CD86 and a failure in the activation of virus-specific CD4+ T cells and CD8+ T cells was demonstrated [70 - 72]. It is likely that the insufficient immune response both in macrophages and DCs promotes disease progression of LF, especially in lethal cases. An effective T-cell-mediated response is probably critical for recovery from infection. Furthermore, NHPs infected with LASV present uncontrolled viral replication and poor innate and cellular immune responses, similar to those observed in infected patients when a fatal outcome of disease is considered. On the other hand, the antibody response seems to be not effective in controlling virus replication because high IgG and IgM titers are not associated with a mild outcome of the disease [73, 74].

It is known that antigen-presenting cells (APCs), like DCs and macrophages, are early targets of arenavirus infection. But in the case of LASV, DCs seem to be a more important target, as this cell type produces much more virus than macrophages [6]. Infected DCs fail to mature, as they do not present increased levels of phagocytic activity and this is consistent with the generalized immunosuppression that causes LASV. Interestingly, it has been demonstrated that LASV subverts classical routes of endosomal trafficking and bypasses the early endosome, which contains the TLRs able to recognize RNA viruses. Thus, LASV may escape detection by TLRs. This can partly explain the failure of the innate immune system to detect LASV infection, causing an uncontrolled virus infection [15].

Innate Immunity to New World Hemorrhagic Arenaviruses

While the hallmark of LASV infection is a generalized immunosuppression, in the case of JUNV-infected patients the cytokine levels are correlated with disease severity. In the acute stage of disease high levels of IFNα and other cytokines

such as IL-6, IL-8, IL-10 and TNF-α in serum are detected [75]. Animal models of AHF also present induction of IFN [76]. The induction of IFN probably contributes to the AHF pathology as IFNα levels are connected to the severity of symptoms like fever, chills and backache, and to low platelet count and platelet abnormality [77]. As previously mentioned, hematopoietic progenitor CD34+ cells and megakaryocyte cells infected with JUNV show impairment of platelet formation and function, an effect that involves IFN-I signaling pathway [61]. On the other hand, JUNV and TCRV-infected mice lacking IFNα/β/γ receptor develop a disease with some histopathological changes similar to those observed in AHF patients, suggesting an important role of IFN pathway to combat JUNV and TCRV infection [78, 79]. In contrast, LASV did not generate fatal infection in IFNα/β/γR -/- mice [80]. In addition, STAT-1 knockout mice infected with MACV presented high levels of proinflammatory cytokines like TNF-α, IFNγ, IL-6 and granulocyte colony-stimulating factor (G-CSF) in serum [81]. These findings suggest that IFNs play a critical role in controlling infections of some NW arenaviruses and that proinflammatory cytokines promote pathogenesis in the murine model. Moreover, human lung epithelial A549 cells infected with both pathogenic and vaccine strains of JUNV presented RIG-I mediated IFN generation and ISG expression [82], which suggests that parenchymal cells could be a cellular source of IFN *in vivo*. Although the antibody response seems to be not effective in controlling LASV infection, in JUNV-infected patients the mortality can be reduced when immune plasma from previously infected patients is administered early within the first week of illness. The effectiveness of this treatment seems to be due the neutralizing activity of the antibodies, since the levels of virus in serum are reduced after transfusion with immune plasma [83].

ANTIVIRAL STRATEGIES AGAINST ARENAVIRUS INFECTION

Despite their importance as pathogens ribavirin is the only licensed antiviral compound available for treatment of arenavirus infections, but because of its limited efficacy in advanced cases and its undesirable side effects the development of more effective and safer antiviral options is required.

Recent advances in the design of novel animal models, viral pseudotypes and reverse genetic systems have been very important, not only to progress in the knowledge of molecular aspects of viral pathogenesis, but also to improve the methods for evaluation of new molecules with antiviral properties. Furthermore, the identification of potential viral and host proteins that might be targeted for the treatment of arenavirus infections constitutes a key factor in the design of therapeutic strategies.

In this section we focus on recent developments of diverse molecules that have been found to be active against arenaviruses, in available cell and animal model systems, within the last years. Fig. (**2**) shows steps of viral replication targeted by antiviral agents and Table **1** summarizes the main antiviral strategies.

Viral Entry as Antiviral Target

Targeting early events of virus replication cycle merits significant attention as a powerful means of preventing arenavirus infection. An efficient viral adsorption and internalization are critical for rapid virus dissemination; therefore the blockade of viral entry steps gives the host immune system the opportunity for establishing an effective antiviral immune response. Furthermore, the more detailed knowledge now available about the tripartite structure of GP complex and the requirement of SSP-GP2 interaction for pH-dependent membrane fusion have provided novel targets for antiviral intervention.

Synthetic Compounds

Since biosafety level 4 (BSL-4) or 3 (BSL-3) is required to handle hemorrhagic arenaviruses, lentiviral pseudotypes expressing arenavirus GP constitute a safe option to be used as a high-throughput screening (HTS) platform to identify inhibitors of GP mediated entry. The screening of a random library of small molecules, using lentiviral-based pseudotypes, and further structural modifications of the active compounds, led to the synthesis of a benzimidazole derivative, compound ST-193, with submicromolar anti-LASV activity *in vitro*. ST-193 was also effective against South American hemorrhagic fever viruses JUNV, MACV, GTOV and SABV [84]. Determinants of ST-193 sensitivity were mapped within GP2 [84] and the inhibitory effect of ST-193 on pH-dependent cell-cell fusion mediated by LASV and JUNV GPs [48] suggested that ST-193 stabilizes the perfusion GP complex against acidic pH, impairing membrane fusion during virus entry. The ability of compound ST-193 to deal with *in vivo* LASV infection was tested in guinea pigs, a useful small animal model for LF. Guinea pigs infected with LASV developed a severe disease resulting in 100% mortality between days 14 and 17 post-inoculation. Treatment with ST-193 reduced signs of disease, caused a significant reduction in viremia and resulted in enhanced animal survival in comparison to ribavirin or vehicle treated animals [85]. Given the antiviral activity exhibited against other hemorrhagic arenaviruses this compound is a good candidate for further development.

Another HTS study allowed the identification of different small molecule inhibitors of GP mediated membrane fusion, compounds 16G8, 17C8, and 17C9,

which exhibited broad activity against the major human pathogenic arenaviruses [86]. On the other hand, photoaffinity derivatives of the 4-acyl-1,6-dialkyl-piperazin-2-one, which were designed against LASV GP, also displayed anti-fusion property [87].

TCRV is a non-pathogen clade B arenavirus, closely related to JUNV, which is useful in early stage of drug discovery research since it can be handled in BSL-2 laboratories. A HTS campaign that analyzed the effect of approximately 400,000 small molecules on TCRV cytopathic effect was performed and compounds with inhibitory action were identified. One of the active compounds, called ST-294, also exhibited protective efficacy in a TCRV-mouse challenge model and the isolation of viral resistant mutants indicated that ST-294 targeted GP2 protein [88].

Minigenome (MG) systems are powerful tools not only to study biology and pathogenesis of many hazardous viruses but also to find new antiviral agents as part of HTS platforms. A JUNV based reverse-genetic system comprising a MG, which contains a reporter genome from the S RNA segment that recapitulates all steps of the virus replicative cycle, was adapted for HTS. After T7 RNA polymerase mediated transcription, the generated MG is recognized by NP and L proteins provided in *trans*, allowing MG replication and transcription. Packaging of newly generated MGs by the action of Z protein and the expression of GPC leads to the formation and release of virus like particles (VLPs). Moreover, these VLPs can infect new NP and L expressing cells resulting in the amplification of the MG reporter signal [89]. The HTS resulted in the identification of four compounds that exhibited anti-JUNV antiviral activity and prevented arenavirus GP mediated cell-cell fusion. Furthermore, mutations in GP2 transmembrane domain or in SSP conferred resistance to these active compounds indicating that viral entry would be the antiviral target [89].

A novel recombinant LCMV, which expresses GFP and viral NP proteins from the same bicistronic mRNA, was recently used in the screening of 30,000 compounds in the context of a cell-based HTS study. In this analysis, compound F3406, another inhibitor of LCMV GP mediated membrane fusion, was uncovered [90].

A fusion inhibitor, compound ZCL278, which targets a host factor, has been recently described [91]. This molecule, which is an inhibitor of a small GTPase (Cdc42) known to regulate actin polymerization, was proved to inhibit JUNV and LCMV replication. ZCL278 prevented pH-dependent JUNV GP mediated fusion

and perturbed intracellular trafficking leading to the redistribution of viral particles from endosomal to lysosomal compartments [91].

GP Derived Peptides

Another approach to develop a fusion inhibitor consists in the design of GP derived peptides that impair glycoprotein conformational changes required to trigger membrane fusion. A PICV GP2 derived peptide, named AVP-p, exhibited antiviral activity against pseudoviruses bearing OW and NW arenavirus GP showing no acute cytotoxicity. The interaction of this peptide with viral spike would induce a premature fusogenic rearrangement of viral glycoproteins reducing virus binding to cellular receptor and also impeding endosomal fusion [92].

DNA Polymers

Amphipathic DNA polymers were found to inhibit LCMV *in vitro* replication, in the low nanomolar range, affecting the interaction between LCMV GP and cell receptor. Structure-function studies using retroviral pseudotypes of LCMV demonstrated that the antiviral effect of the DNA polymers was sequence-independent whereas size and hydrophobicity were critical for their inhibitory action [93]. Unfortunately, evaluation of this antiviral strategy against hemorrhagic arenaviruses has not been reported.

Antibodies

As mentioned above, current treatment of AHF patients consists in the administration of immune plasma from recovered patients. This treatment provides 100% protection to guinea pigs, the most commonly used JUNV animal model, when delivered as late as 6 days after infection. Passive immunotherapy based on polyclonal immune sera has several drawbacks such as the presence of virus specific non-neutralizing antibodies, batch to batch variation, difficulties in obtaining immune donors and risks associated with the use of blood products. A mouse-human chimeric JUNV neutralizing monoclonal antibody (MAb) was proved to provide 100% protection against lethal challenge when administered at 6 days after JUNV infection in the guinea pig model of AHF. In addition, viral antigen was undetectable by immunohistochemistry in the brains of MAb inoculated animals suggesting that this treatment could be an efficacious replacement for immune plasma in AHF therapy [94]. Although the neutralizing activity of different tested MAbs correlates with protection in guinea pigs, neutralization of free virus would not be the only mechanism by which MAbs

function *in vivo*, since other studies indicate that optimal protection would also require Fc-mediated immune functions [95]. The demonstration of MAb effectiveness against clinical viral isolates and the analysis of escape mutant selection are further required to assess the potential of this antiviral strategy against JUNV.

In an another attempt to block virus-cell receptor interaction it was demonstrated that a MAb directed to the apical region of human TfR1 was able to inhibit binding and subsequent GP mediated entry of NW pathogen arenaviruses [96]. Since the capacity of NW arenaviruses to cause human disease correlates with their ability to bind human TfR1 the therapeutic use of this MAb appears as a promissory antiviral approach.

Exposure of GP2 fusion peptide provides a target for fusion inhibition by GP2 directed antibodies and early studies had described the ability of sera from AHF patients to prevent JUNV GP-mediated cell-cell fusion [97]. In line with these findings, a MAb directed toward GP2, F100G5, which recognizes a pH-induced intermediate of JUNV GP, was proved to impair GP mediated membrane fusion [98]. Even though this MAb is unable to inhibit viral replication in cell cultures, York *et al*. [98] proposed that linking of F100G5 to neutralizing MAbs, by using bifunctional reagents, would be an interesting antiviral research line to explore.

The administration of ribavirin or convalescent sera in LF patients has shown limited success depending on the time of treatment and the donor source in the case of immune sera. LASV GP specific human mABs, which display *in vitro* neutralizing activity, also prevented, individually or in combination, the development of disease in a guinea pig model. This experimental evidence supports the *in vivo* potential of these mABs in future studies using non-human primates, the gold standard animal model for LF [99].

Inhibition of RNA Replication

The purine nucleoside analogue ribavirin, which exhibits antiviral activity against diverse RNA viruses, is the only licensed antiviral compound currently available for arenavirus infections, however, even though the effectiveness of ribavirin has been proved in cell cultures and in several animal models [81, 100] this compound is not equally effective against all human arenaviruses. Ribavirin treatment early after the onset of LF reduces mortality but is ineffective in preventing neurological sequelae [101], whereas convalescent immune globulin administration is still the recommended treatment for AHF [17].

Even though ribavirin, as well as mycophenolic acid (MPA), another inosine monophosphate dehydrogenase (IMPDH) inhibitor, adversely affected LASV and JUNV infection in cell cultures, addition of guanosine reversed the inhibitory effects of MPA but did not affect the antiviral action of ribavirin. These results indicate that depletion of the intracellular GTP pool *via* inhibition of IMPDH would not account for ribavirin inhibitory effect [102, 103]. Although it has been proposed that ribavirin inhibits viral RNA synthesis by targeting the L polymerase [104] other studies suggest that ribavirin-induced lethal mutagenesis might contribute to its antiviral activity against LCMV [105].

Despite its usefulness in treating some arenavirus infections it is also known that ribavirin causes several adverse effects such as teratogenic and/or embryocidal effects and haemolytic anaemia [106]. In the search of other RNA inhibitors with antiviral activity several *in vitro* and *in vivo* assays revealed that the compound favipiravir (T-705), a nucleoside analog recently approved in Japan and in Phase 3 clinical trials in US for treatment of influenzavirus infections, displays a broad-spectrum antiviral activity against RNA viruses [107]. Favipiravir exhibited *in vitro* inhibitory action against JUNV, PICV, TCRV, GTOV and MACV rendering higher selectivity indexes than ribavirin [108, 109]. Favipiravir inhibition was reversed by the addition of purine bases and nucleosides [109]. In addition, favipiravir administration prevented the death of PICV- infected hamsters [108] and oral therapy with this drug was also effective against a PICV strain adapted to produce lethal infections in guinea pigs, protecting even those animals that were treated after the onset of signs of illness [110]. Intraperitoneal favipiravir administration in JUNV-infected guinea pigs resulted in a high level of protection (78% survival) and undetectable levels of viral titers in tissues and serum, whereas protection in ribavirin injected animals was in the range of 33-40% [111]. Potent anti-LASV activity was also demonstrated for favipiravir in the guinea pig model [112]. Interestingly, the addition of low dose of ribavirin synergistically potentiates the protective efficacy of favipiravir in JUNV-infected guinea pigs [106].

Like favipiravir, compound A3, an inhibitor of pyrimidine biosynthesis, was found to exhibit a broad antiviral activity against RNA viruses. A significant antiviral effect of A3 against LCMV and JUNV in different cell lines was demonstrated and A3 inhibitory effect would be in part due to its ability to interfere with viral RNA replication and transcription as was shown by Northern blot assays [113]. In addition, A3 would affect the dihydroorotate dehydrogenase (DHODH) enzyme activity since treatment with orotic acid, an intermediate in the *de novo* pyrimidine pathway produced by DHODH, restored normal production of

infectious progeny. Consistent with the fact that ribavirin and A3 target different metabolic pathways within the cell, an additive anti-arenavirus effect of A3 and ribavirin was demonstrated [113].

Other studies revealed that N-substituted acridones selectively inhibited JUNV, TCRV and LCMV replication in cell cultures [114] and the most active compound, designated 3f, inhibited viral RNA synthesis. The addition of exogenous guanosine partially rescued JUNV infectivity and RNA synthesis indicating that reduction of GTP pool is not the main 3f inhibitory mechanism [103].

Gene Silencing

Post-transcriptional gene silencing by RNA interference has been explored as a strategy to block arenavirus replication. Small interfering RNAs (siRNAs) targeting the conserved RNA termini of NP and L genes inhibited LASV and LCMV replication in cell cultures [115]. On the other hand, siRNAs directed against Z gene also reduced *in vitro* JUNV replication but were non-effective against the related NW arenavirus TCRV [116]. Neuman *et al*. [117] designed antisense phosphorodiamidate morpholino oligomers (PMOs) to interfere with viral mRNA translation. Unlike siRNAs, PMOs are uncharged and nuclease-resistant, and conjugation of PMOs with an arginine-rich peptide (PPMOs) greatly enhanced cellular uptake of the antisense molecules. PPMOs complementary to sequences highly conserved at the 5' termini of both arenavirus genomic segments were effective against New and Old World viruses in cell cultures and during acute LCMV infection in mice [117].

Targeting Glycoprotein Maturation

GPC proteolytic processing by the cellular subtilisin kexin isozyme 1 (SKI-1)/site1 protease (S1P) is required for the incorporation of GP1 and GP2 mature glycoproteins into viral particles, thus being a potential effective antiviral target. Engineered antitrypsins, which are derived from a1-antitrypsin, can be used as inhibitors of proprotein convertases and consistent with this fact, expression of S1P-adapted a1-antitrypsins blocked the proteolytic maturation of LASV GPC and hindered viral replication and spread [118]. On the other hand, a peptide-based S1P inhibitor, decanoyl (dec)-RRLL-chloromethylketone (CMK), blocked LCMV GPC cleavage impairing infectious virus production [119].

In vitro anti-arenavirus effect against LCMV, LASV, JUNV, MACV, GTOV, AMPV and TCRV was also demonstrated for the amino-pyrrolidine amide

compound PF-429242, a small-molecule inhibitor of S1P [120, 121]. In combination therapy, synergism between PF-429242 and ribavirin action was demonstrated. In addition, PF-429242 efficiently cleared persistent LCMV from infected cells and the toxicity profile and pharmacokinetic properties make PF-429242 a promising novel anti-arenavirus agent [121].

Table 1. Antiviral strategies against arenavirus infections.

Antiviral target	Antiviral strategy	References
Virus-receptor interaction	GP derived peptides	[92]
	DNA polymers	[93]
	Anti-cell receptor antibodies	[96]
	Anti-virus antibodies	[94, 99]
Membrane fusion	Synthetic compounds	[48, 84-87, 89-91]
	GP derived peptides	[92]
	Anti-virus antibodies	[98]
RNA synthesis	Nucleoside analogues	[81, 100-112, 114]
	Purine and pyrimidine biosynthesis inhibitors	[103, 109, 113, 114]
RNA expression	Viral mRNA specific siRNAs	[115, 116]
	Genome 5‘ termini specific antisense PPMOs	[117]
GPC cleavage	Modified antitrypsins	[118]
	Peptides	[119]
	Small molecules	[120, 121]
Virus assembly/ budding	Fatty acids	[122]
	Inhibitors of Tsg-101-Z PTAP interaction	[123]

GP: glycoprotein complex; siRNAs: small interfering RNAs; PPMOS: peptide conjugated phosphorodiamidate morpholino oligomers; Tsg-101: tumor susceptibility gene 101; Z PTAP: late assembly domain in Z protein.

Blocking Viral Assembly and Budding

Valproic acid (VPA), a short chain fatty acid, exhibited anti-LCMV activity in cell cultures and the analysis of its mechanism of action showed that VPA affected the release of viral particles from infected cells and the specific infectivity of released virions [122]. Alteration of cellular lipid metabolism induced by VPA might induce changes in the lipid composition of the plasma membrane from which LCMV acquires its envelope leading to the reduction of the infectivity of released particles. VPA is currently being used for epilepsy treatment and it displays a high *in vitro* selectivity index against LCMV, which is a good predictor of its potential as antiviral agent.

As mentioned above, arenavirus Z protein promotes virus egress and is able to induce budding from mammalian cells in the form of VLPs. Separation of arenavirus Z VLPs from the plasma membrane is promoted by a PTAP type L domain within Z protein which interacts with host Tsg101, a component of the ESCRT machinery. Based on the knowledge of the structure of the Tsg101-PTAP interaction site, an *in silico* screen for competitive binding inhibitors was performed and several compounds that blocked Z VLPs formation were identified. One of these compounds, called 0013, not only reduced Z VLP production but also inhibited *in vitro* JUNV replication at nanomolar concentrations [123]. Bearing in mind that PTAP L domain mediated Tsg101 recruitment is utilized by other RNA virus pathogens, PTAP inhibitors would represent a potent broad-spectrum host-oriented antiviral drug.

An interesting cell-based budding assay for the identification of small inhibitors of Z-mediated budding and adaptable to HTS studies has been developed [124]. In this assay, a chimera of LASV Z fused at its C-terminus to *Gaussia* luciferase (Z-Gluc) was used, thus the amount of released VLPs can be directly measured by detecting luciferase activity in the supernatant of Z-Gluc-transfected cells.

Other Anti-arenavirus Compounds

The *in vivo* efficacy of a phenolic dibenzylsulfide called D746, which showed inhibitory action against TCRV and JUNV in cell cultures, was studied in type I and II IFN receptor knockout mice infected with TCRV. It was proved that D746 is active as a pre-exposure prophylaxis but not as a post-exposure intervention. However, pre-treatment did not reduce viral titers and induced the accumulation of ascites fluid suggesting that stimulation of host immune response would account for D746 protective effect [125].

Several other natural and synthetic compounds were proved to impair *in vitro* arenavirus replication [126 - 130]. For instance, the zinc finger- reactive disulfide compound NSC20625, which induced unfolding and oligomerization of Z protein, blocking the interaction of this protein and cellular promyelocytic leukemia protein, exhibited anti-arenavirus activity [128]. On the other hand, natural and synthetic steroids also displayed anti- JUNV activity in cell cultures [126]. The efficacy of these agents in animal models should be further analyzed to estimate their potential in the anti-arenavirus therapy.

Kinase Signaling Cascades as Antiviral Targets

Cell signaling networks control different cellular processes such as differentiation,

proliferation, cell cycle, apoptosis and the assembly of immune response against pathogens. They consist of a series of proteins that activate or inhibit each other changing their 3D structure through post-translational modifications, such as phosphorylations, and these changes can determine the features as well as the success of viral infections. Several reports describe that the mitogen-activated protein kinase (MAPK) pathways and the phosphatidylinositol-3-kinase (PI3K/Akt) pathway are important modulators of virus replication. Since it has been demonstrated that numerous viruses hijack cell signaling pathways to achieve a productive infection, the modulation of these processes appears to be an interesting strategy for the development of new antiviral drugs [131 - 135].

MAPK Pathways

In mammalians there are three main MAPK cascades: extracellular-regulated kinase 1/2 (ERK1/2), c-Jun N-terminal kinase (JNK) and p38 pathways. Several DNA and RNA viruses take advantage of ERK pathway [131, 134, 136 - 139] probably by affecting the activation of certain genes. Non-hemorrhagic arenaviruses LCMV, PICV and TCRV as well as JUNV are able to activate ERK pathway and inhibition of this cell cascade reduced JUNV, TCRV and PICV replication (Fig. **4**) [140, 141]. JUNV induces a biphasic activation of ERK signaling in Vero cells: an early activation occurs at 15-30 min post-infection and a second phase of activation takes place after 7-9 h post-infection. Although it is still not clear which is the role of ERK activation in virus infection, early activation might contribute to suppress the antiviral IFN-dependent immune response [142].

By contrast, there is evidence that binding of the OW arenavirus LASV to the cellular receptor αDG prevents ERK activation [143], however, future studies will be needed to determine the role of this inhibition in LASV replication cycle.

Numerous inhibitors for the ERK pathway have been described and drugs directed towards different components of this cell signaling cascade have been approved for cancer treatment while other compounds are in different stages of clinical trials or approval (Fig. **4**) (recently revised in [144]).

On the other hand, it has been described that PICV promotes p38 activation in cell cultures [145], however the involvement of p38 and JNK pathway in hemorrhagic arenaviruses has not been studied yet.

A novel approach for the inhibition of kinases is the use of peptides as signaling inhibitors. The design and synthesis of cell permeable peptides that block MAPK

signaling would result in the development of highly specific MAPK inhibitors with antiviral action [144, 146].

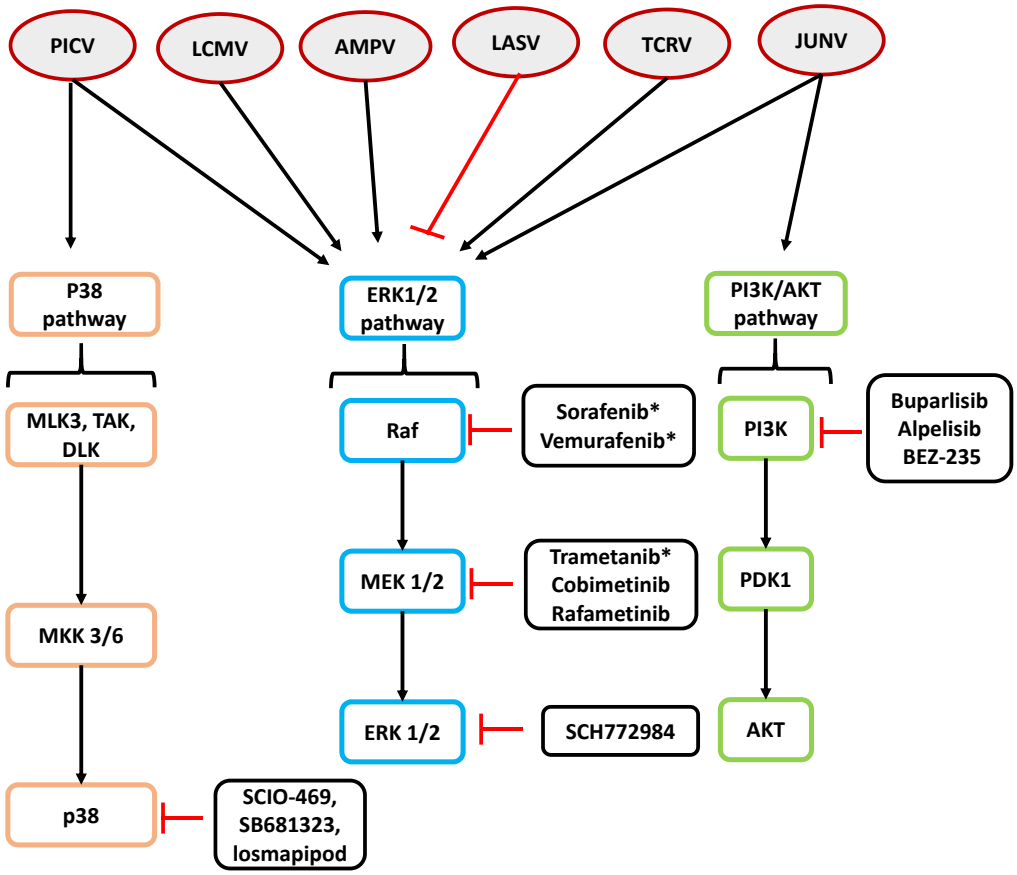

Fig. (4). Arenavirus modulation of cell signaling pathways. The main components of p38, ERK 1/2 and PI3K/AKT pathways are represented. Black arrows indicate virus activation whereas red connectors indicate virus inhibition of the indicated pathway. Chemical inhibitors acting at different levels of the corresponding pathway are displayed and the asterisk indicates that the inhibitor is approved for treatment of other human diseases. PICV: Pichinde virus; LCMV: lymphocytic choriomeningitis virus; AMPV: Amapari virus; LASV: Lassa virus; TCRV: Tacaribe virus; JUNV: Junin virus; MLK3: mixed-lineage kinase 3; TAK:transforming growth factor β activated kinase; DLK: dual leucine bearing zipper kinase; MKK: mitogen activated protein kinase kinase; ERK: extracellular signal-regulated kinase; Raf: rapidly accelerated fibrosarcoma, MEK: mitogen activated protein kinase kinase; PI3K: phosphoinositide 3-kinase; PDK1: pyruvate dehydrogenase lipoamide kinase isozyme 1; AKT: protein kinase B.

PI3K/Akt Pathway

Several DNA and RNA viruses modulate the PI3K/Akt pathway in order to regulate apoptosis and immune response and benefit their replication [132, 147]. Early PI3K/Akt signaling activation was observed in different cell lines infected

with JUNV and it was demonstrated that clathrin mediated endocytosis of viral particles is necessary to induce AKT phosphorylation (Fig. **4**) [148]. Moreover, treatment with the PI3K inhibitor Ly294002 caused the decrease of virus production, which is consistent with a reduction in the synthesis of NP protein suggesting that the early activation of the PI3K/Akt pathway is necessary for the outcome of a productive infection. Nevertheless, treatment of cell cultures with Ly294002 did not prevent establishment or maintenance of JUNV persistent infection in cell cultures [149].

It has been described that PI3K is essential for LCMV and LASV entry in macrophages [150]. By contrast, another report showed that treatment with Ly294002 or BEZ-235, a PI3K inhibitor which is currently being tested in cancer clinical trials, inhibited LCMV and LASV infection by hindering virus budding without affecting viral uptake [151]. Despite the discrepancies between these two reports, which might be due to the employment of PI3K inhibitors with different mechanisms of action, PI3K signaling also seems to be an attractive antiviral target.

CONCLUDING REMARKS

No highly effective anti-arenavirus therapeutic is approved for use in humans, hence treatment of these viral infections is limited to the use of ribavirin, which has limited prophylactic efficacy, or immune convalescent plasma, with the drawbacks associated to the use of blood derivatives.

Recent advances in the understanding of arenavirus biology and improvements in animal and cell culture models have greatly contributed towards the discovery of different antiviral targets and a great variety of novel molecules with anti-arenavirus activity. Furthermore, reverse genetic approaches allowed the development of surrogate systems that facilitate the evaluation of potential antiviral agents in the context of HTS platforms under low stringent BSL-2 conditions. Different small molecules screens have identified promising compounds with antiviral properties using these surrogate systems.

Antiviral strategies targeting different aspects of arenavirus replication have been described. Most of the highly effective antiviral agents correspond to viral entry inhibitors, being GP interaction with the cellular receptor and GP mediated membrane fusion the main targets of the inhibitory action. Antibody based interventions have proved to provide protective efficacious even when administered late in animal models for arenavirus disease. In addition, novel manufacturing systems turn mAB therapy technically and economically feasible

and mixtures of different mABs would offer both high specificity and broad spectrum activity. Small molecules identified through HTS platforms and DNA polymers were also effective viral entry inhibitors.

Antiviral strategies based on both viral and cellular targets have proved to be effective against arenavirus infections. Antiviral agents directed to block a virus encoded function are usually more selective and specific than antiviral strategies towards cellular factors. However, targeting cellular components may represent an alternative with low risk of appearance of viral resistance and effective against different viruses, even unrelated ones, which require the same cellular factors. Moreover, cellular targets offer the possibility of the employment of licensed compounds, approved for other human diseases, with known safety-data profile and, although toxic effects can be an important disadvantage of this type of therapy, adverse side effects might be kept to a minimum in short term treatments of acute arenavirus infections. Furthermore, combined administration of cellular and viral targeted inhibitors would enhance the effectiveness of anti-arenavirus treatment.

Arenaviruses exhibit the ability of controlling cell signaling pathways regulating cellular fate and their own replication. We propose that the inhibition of cellular signaling represents an attractive therapeutic approach considering the availability of approved inhibitors or compounds that are currently being tested in clinical trials, which are promising candidates as anti-arenavirus agents.

New advances in siRNA screens as well as in the analysis of proteomic, transcriptomic and kinomic profiles of arenavirus infected cells will provide key information about virus-cell interactions that will further support the rationale design of novel cell-factor based antiviral strategies.

CONFLICT OF INTEREST

The authors confirm that they have no conflict of interest to declare for this publication.

ACKNOWLEDGEMENTS

This work was supported by Universidad de Buenos Aires (UBACyT 20020150100148BA) and Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET, PIP: 0338). LAS is member of research career of CONICET. JEB and MNA are fellows from CONICET.

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