Highly Pathogenic New World and Old World Human Arenaviruses Induce Distinct Interferon Responses in Human Cells

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ABSTRACT
The arenavirus family includes several important pathogens that cause severe and sometimes fatal diseases in humans. The highly pathogenic Old World (OW) arenavirus Lassa fever virus (LASV) is the causative agent of Lassa fever (LF) disease in humans. LASV infections in severe cases are generally immunosuppressive without stimulating interferon (IFN) induction, a pro-inflammatory response, or T cell activation. However, the host innate immune responses to highly pathogenic New World (NW) arenaviruses are not well understood. We have previously shown that the highly pathogenic NW arenavirus, Junin virus (JUNV), induced an IFN response in human A549 cells. Here, we report that Machupo virus (MACV), another highly pathogenic NW arenavirus, also induces an IFN response. Importantly, both pathogenic NW arenaviruses, in contrast to the OW highly pathogenic arenavirus LASV, readily elicited an IFN response in human primary dendritic cells and A549 cells. Coinfection experiments revealed that LASV could potently inhibit MACV-activated IFN responses even at 6 h after MACV infection, while the replication levels of MACV and LASV were not affected by virus coinfection. Our results clearly demonstrated that although all viruses studied herein are highly pathogenic to humans, the host IFN responses toward infections with the NW arenaviruses JUNV and MACV are quite different from responses to infections with the OW arenavirus LASV, a discovery that needs to be further investigated in relevant animal models. This finding might help us better understand various interplays between the host immune system and highly pathogenic arenaviruses as well as distinct mechanisms underlying viral pathogenesis.

IMPORTANCE
Infections of humans with the highly pathogenic OW LASV are accompanied by potent suppression of interferon or proinflammatory cytokine production. In contrast, infections with the highly pathogenic NW arenavirus JUNV are associated with high levels of IFNs and cytokines in severe and fatal cases. Arenaviruses initially target macrophages and dendritic cells, which are potent IFN/cytokine-producers. In human macrophages, JUNV reportedly does not trigger IFN responses. We here demonstrated that JUNV activated IFN responses in human dendritic cells. MACV, another highly pathogenic NW arenavirus, also activated IFN responses. LASV did not induce detectable IFN responses, in spite of higher replication levels, and blocked the MACV-triggered IFN response in a co-infection assay. Although these viruses are highly pathogenic to humans, our study highlights distinct innate immune responses to infections with the NW arenaviruses JUNV and MACV and to infection with the OW arenavirus LASV and provides important insights into the virus-host interaction and pathogenesis.

Arenaviruses are enveloped, negative-sense RNA viruses which belong to the Arenaviridae family (1). The viral genome is bi-segmented and encodes four viral proteins utilizing an ambisense coding strategy. The large segment of genomic RNA encodes the viral non-structural proteins L protein and the small zinc finger Z protein, while the small segment of genomic RNA encodes the viral nucleoprotein (NP) and the glycoprotein precursor (GPC). Based on their antigenicity, phylogeny, and geographical distribution, arenaviruses are classified into the Old World (OW; Lassa-lymphocytic choriomeningitis complex) arenaviruses and the New World (NW; Tacaribe complex) arenaviruses (2, 3). The lymphocytic choriomeningitis virus (LCMV) from the Old World arenaviruses is the prototype arenavirus. The New World arenaviruses are further classified into clade A, B, and C NW arenaviruses.

The arenavirus family includes several important human pathogens, which may cause severe or fatal diseases in patients (4–6). These viruses are usually rodent borne and may cause chronic/persistent infection in their natural rodent hosts (1). Human infections with arenaviruses likely occur through inhalation of virus-containing aerosols, ingestion of contaminated food, or direct contact of abraded skin with infectious materials (1, 7–9). Host antigen-presenting cells, namely, macrophages and dendritic cells, are proposed to be the early targets of infection. Arenaviruses may later disseminate from their initial sites of infection to establish systemic infection and may cause severe morbidity and mortality in humans or animals. Among human-pathogenic arenaviruses, the OW Lassa fever virus (LASV) is highly pathogenic and probably the most important human pathogen in the arenavirus family. LASV has been estimated to infect up to 300,000 humans and cause approximately 5,000 to 6,000 deaths annually.
annually in West Africa (10, 11). Lassa fever (LF) is of major public importance in areas of endemicity that overlap the regions currently being affected by the Ebola virus outbreak (http://www.cdc.gov/vhf/ebola/outbreaks/2014-west-africa/). Severe and fatal LASV infections are typically immunosuppressive in humans and are characterized by a lack of IFN induction, proinflammatory response, or T cell activation in vitro or in vivo (12). LASV productively infects macrophages and dendritic cells but fails to activate these cells and the CD4+ and CD8+ T cells (13, 14), which contributes to the severe disease progression of Lassa fever in fatal cases.

On the other hand, the NW clade B arenaviruses also contain several important human pathogens (6, 15), including the highly pathogenic Junin virus (JUNV), Machupo virus (MACV), Guanarito virus (GTOV), Sabia virus (SABV), and Chapare virus (CHAV). JUNV and MACV are the causative agents for Argentine hemorrhagic fever (AHF) and Bolivian hemorrhagic fever (BHF), respectively. GTOV and SABV infections cause Venezuelan hemorrhagic fever and Brazilian hemorrhagic fever, respectively. CHAV infection has been associated with human hemorrhagic fever cases in Bolivia. Because of their high morbidity and mortality in humans and the high risks to public health, the NW arenaviruses JUNV, MACV, GTOV, and CHAV, along with the OW arenavirus LASV, are classified as category A priority pathogen agents by the U.S. National Institutes of Health. Most of these highly pathogenic viruses, including JUNV, MACV and LASV, must be handled in biosafety level 4 (BSL-4) facilities.

While the pathogenesis and the host immune response to the highly pathogenic OW arenavirus LASV have been relatively well characterized in patients and in laboratories, less is understood about these highly pathogenic NW arenaviruses. Past studies suggest that the viral pathogenesis and host innate immune responses, including the interferon (IFN) response, to these highly pathogenic NW arenaviruses are likely different from those described for the pathogenic LASV from the OW (16). In contrast to the general immunosuppression associated with LASV infection, clinical studies have revealed that human infections with the NW arenavirus JUNV are often accompanied by elevated and high levels of serum IFN-α (2,000 to 64,000 IU/ml) (17, 18), which correlates with the severity and fatality of AHF disease in patients. MACV infection also induces robust IFN production in a lethal nonhuman primate (NHP) model (19). Nevertheless, it has been reported that experimental JUNV infection in macrophages and monocytes does not induce IFN and cytokine production (20).

We have recently reported that JUNV infection elicited strong IFN responses in human lung epithelial A549 cells (21). In the present study, we expand our work and demonstrate that MACV, another NW arenavirus highly pathogenic to humans, also induces IFN responses in A549 cells. More importantly, our data identified for the first time (to the best of our knowledge) that infections with JUNV and MACV could activate the IFN response in primary human dendritic cells. In contrast, LASV infection failed to induce IFN in either A549 cells or dendritic cells despite its ability to replicate to higher levels. Interestingly, LASV could effectively inhibit MACV-triggered IFN responses as late as 6 h post-MACV infection without noticeable impact on MACV replication.

**MATERIALS AND METHODS**

**Viruses and cells.** Recombinant JUNV (Romero strain), MACV (Carvallo strain), and LASV (Josiah strain) were prepared as described previously (22, 23). Briefly, virus stocks were propagated at a multiplicity of infection (MOI) of 0.001 on Vero cells (American Tissue Culture Collection, Manassas, VA). Supernatants were harvested and centrifuged, followed by filtration (0.45-μm pore size) to remove cell debris and further purification with Ultrapac 100K filters (molecular weight cutoff of 100,000; Amicon, Millipore). All infection work with pathogenic arenaviruses was performed at BSL-4 facilities in the Galveston National Laboratory at the University of Texas Medical Branch in accordance with institutional health and safety guidelines and federal regulations. Human lung epithelial A549 cells were obtained from the ATCC.

**Preparation of human primary monocyte-derived dendritic cells (MoDCs).** Human blood buffy coats containing peripheral blood mononuclear cells (PBMCs) from three healthy, anonymous donors were obtained from the University of Texas Medical Branch (UTMB) Blood Center under an IRB exemption. Phosphate-buffered saline (PBS)-diluted PBMCs were layered on Histopaque 1083 (Sigma-Aldrich, St. Louis, MO) and centrifuged at 800 x g for 30 min. PBMCs at the interface were washed twice with PBS by centrifugation at 400 to 600 x g for 10 min. Monocytes were positively selected with CD14-specific microbeads (human CD14 MicroBeads; Miltenyi Biotec, Inc., San Diego, CA) using an autoMACS Pro Separator according to the manufacturer’s instructions. More than 95% of cells of the monocyte preparations were confirmed as human IFN-α/H9251 and IFN-β/H9252 by fluorescence-activated cell sorting (FACS) analysis. CD14+ monocytes were cultured in the RPMI 1640 differentiation medium containing 10% heat-inactivated fetal bovine serum (FBS), 100 U/ml streptomycin, 100 U/ml penicillin, 2 mM glutamine, 50 ng/ml human interleukin-4 (IL-4), and 250 ng/ml human granulocyte-macrophage colony-stimulating factor (GM-CSF; R&D Systems, Inc., Minneapolis, MN). Cells were incubated at 37°C under 5% CO2 for 6 days. On days 2 and 4, half of the medium was removed and replaced with fresh differentiation medium.

**Arenavirus infection of human MoDCs.** Differentiated MoDCs (105 cells) were mock infected or infected with JUNV, MACV, or LASV at an MOI of 1 in differentiation medium containing 2% FBS for 1.5 h. Then cells were harvested by centrifugation and maintained in fresh differentiation medium. At 0, 1, 2, and 3 days postinfection (dpi), supernatants of the average results were purchased from Bio-Rad. The CFX Manager software program (Bio-Rad) was used to analyze the PCR results.

**Measurement of human IFN.** Human IFN-β and IFN-α proteins were measured by ELISA using a VeriKine Human IFN Beta ELISA kit and a Human IFN-α Multi-Subtype ELISA kit (PBL Interferon Source, NJ) according to the manufacturer’s instructions.

**RNA extraction and real-time qRT-PCR.** RNA purification was performed as described previously (24, 25). Briefly, RNA lysates were prepared from infected cells with TRIzol reagent. Samples were further purified by using an RNeasy Micro or RNeasy Mini Kit (Qiagen) according to the manufacturer’s instructions. To avoid potential host chromosome DNA contamination, RNA samples were treated with DNase I (Qiagen). cDNA synthesis was carried out using an iScript Advanced cDNA synthesis kit for real-time quantitative reverse transcription-PCR (RT-qPCR) (Bio-Rad, CA). Real-time PCR amplification was performed with SsoAdvanced Universal SYBR Green Supermix (Bio-Rad) on a CFX96 real-time PCR detection system (Bio-Rad). Threshold cycle (CT) values of targeting genes were normalized to the average CT values of the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and β-actin housekeeping genes. Validated primers for quantitative PCR (qPCR) detection of human IFN-β, IFN-stimulated gene 15 (ISG15), GAPDH, and actin genes were purchased from Bio-Rad. The CFX Manager software program (Bio-Rad) was used to analyze the PCR results.

**RNA transcription and real-time qRT-PCR.** 5′-UTR-PCR. RNA purification was performed as described previously (24, 25). Briefly, RNA lysates were prepared from infected cells with TRIzol reagent. Samples were further purified by using an RNeasy Micro or RNeasy Mini Kit (Qiagen) according to the manufacturer’s instructions. To avoid potential host chromosome DNA contamination, RNA samples were treated with DNase I (Qiagen). cDNA synthesis was carried out using an iScript Advanced cDNA synthesis kit for real-time quantitative reverse transcription-PCR (RT-qPCR) (Bio-Rad, CA). Real-time PCR amplification was performed with SsoAdvanced Universal SYBR Green Supermix (Bio-Rad) on a CFX96 real-time PCR detection system (Bio-Rad). Threshold cycle (CT) values of targeting genes were normalized to the average CT values of the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and β-actin housekeeping genes. Validated primers for quantitative PCR (qPCR) detection of human IFN-β, IFN-stimulated gene 15 (ISG15), GAPDH, and actin genes were purchased from Bio-Rad. The CFX Manager software program (Bio-Rad) was used to analyze the PCR results.

**Measurement of human IFN.** Human IFN-β and IFN-α proteins were measured by ELISA using a VeriKine Human IFN Beta ELISA kit and a Human IFN-α Multi-Subtype ELISA kit (PBL Interferon Source, NJ) according to the manufacturer’s instructions.
Laemmli secondary antibodies for incubation at room temperature. Proteins were incubated with primary antibodies overnight at 4°C and with appropriate electrophoretic transfer cell apparatus (Bio-Rad). The membranes were samples resolved on 4 to 20% SDS-PAGE gels and transferred to Mouse donkey anti-goat IgG (sc-2020; Santa Cruz). conjugated goat anti-rabbit IgG (Cell Signaling), and HRP-conjugated goat anti-mouse IgG (115-035-146; Jackson Immunology), HRP-protein expression was detected from 1 dpi to 3 dpi (Fig. 2A). IFN-β mRNA levels were upregulated by approximately 150-fold (JUNV) or 250-fold (MACV) at 1 dpi compared with the levels of mock-infected samples (Fig. 2A). Meanwhile, IFN-β mRNA expression levels in LAVS-infected samples were similar to those in mock-infected samples during the course of infection. IFN-β protein expression was detected from 1 dpi to 3 dpi (Fig. 2B). IFN-α protein was also detected in the medium of JUNV- or MACV-infected MoDCs from 1 dpi to 3 dpi, while neither IFN-β nor IFN-α could be detected in the medium from mock- or LASV-infected samples during infection (Fig. 2B and C).

In addition to IFN production, phosphorylation of STAT1 was upregulated in JUNV- and MACV-infected MoDCs but remained the same in LASV-infected samples as that in mock-infected cells during infection (Fig. 3A). Consistently, we found enhanced ISG protein expressions (ISG15 and STAT1) in JUNV- or MACV-infected MoDCs but not in LASV-infected or mock-infected samples from 1 dpi to 3 dpi. These results indicated that the IFN signaling pathway was activated and subsequently led to ISG expression in MoDCs infected with the NW arenavirus JUNV or MACV but not in MoDCs infected with the OW arenavirus LASV. The replication of LASV in MoDCs was productive, with peak virus titers at 2 × 10^6 PFU/ml at 2 dpi (Fig. 3B). However, NW JUNV and MACV infections in MoDCs were remarkably less productive: virus titers increased slightly after infection, reached peak titers of 2 × 10^3 PFU/ml for JUNV and 3 × 10^4 PFU/ml for MACV at 1 dpi, and then slightly declined (Fig. 3B). Consistently, we observed that approximately 10% of the MoDCs were positive for JUNV NP by FACS analysis (data not shown). Despite the lower infectivity of JUNV and MACV in MoDCs, infections with JUNV and MACV readily triggered IFN responses in MoDCs. In comparison, LASV productively infected human MoDCs but failed to activate an IFN response, which is in agreement with previous studies (13, 27).

The NW human-pathogenic arenaviruses JUNV and MACV, but not the OW highly pathogenic arenavirus LASV, activated IFN responses in human dendritic cells. Macrophages and dendritic cells are regarded as the initial target cells in the early stage of arenavirus infection. It remained to be established whether pathogenic NW arenavirus infection of human dendritic cells could trigger the IFN response. To address this question, we infected human monocyte-derived dendritic cells (MoDCs) with JUNV, MACV, or LASV at an MOI of 1. The NW JUNV and MACV both induced remarkably and significantly higher levels of IFN-β mRNA in MoDCs from 1 dpi to 3 dpi (Fig. 2A). IFN-β mRNA levels were upregulated by approximately 150-fold (JUNV) or 250-fold (MACV) at 1 dpi compared with the levels of mock-infected samples (Fig. 2A). Meanwhile, IFN-β mRNA expression levels in LASV-infected samples were similar to those in mock-infected samples during the course of infection. IFN-β protein expression was detected from 1 dpi to 3 dpi (Fig. 2B). IFN-α protein was also detected in the medium of JUNV- or MACV-infected MoDCs from 1 dpi to 3 dpi, while neither IFN-β nor IFN-α could be detected in the medium from mock- or LASV-infected samples during infection (Fig. 2B and C).
In contrast, the IFN-β mRNA level in LASV-infected A549 cells was almost the same as that of mock-infected samples during infection (Fig. 4B). Upregulation of ISG15 mRNA expression was also identified in JUNV- or MACV-infected cells but not in LASV-infected cells (Fig. 4C). Consistently, increasing amounts of IFN-β protein were readily detected in the medium from JUNV- or MACV-infected A549 cells but not in that from LASV-infected cells. Further characterization revealed that LASV infection did not lead to detectable changes in phosphorylation of STAT-1 (Fig. 5), indicating that the IFN signaling pathway remained inactivated during LASV infection. Consistently, LASV infection did not result in observable changes in ISG protein expression (ISG15, RIG-1, and STAT1) compared with expression in mock-infected cells (Fig. 5). These data provided evidence that the OW human-pathogenic arenavirus LASV but not the NW human-pathogenic arenavirus JUNV or MACV escaped and/or suppressed host IFN responses in A549 cells, whereas all viruses replicated at comparable levels.

The OW human-pathogenic arenavirus LASV blocked NW human-pathogenic arenavirus MACV-induced IFN response. It was evident that the host IFN responses to infections with highly pathogenic NW JUNV and MACV were essentially distinct from the response to the infection by the highly pathogenic OW arenavirus LASV in cultured human cells. To understand if LASV evades or actively suppresses host IFN responses, we performed an MACV and LASV coinfection assay in which the NW arenavirus MACV was used because it exhibited, overall, a relatively earlier and stronger IFN-inducing activity than JUNV (Fig. 1, 2, and 4). A549 cells were infected with MACV at an MOI of 1. LASV (MOI of 1) was introduced to MACV-infected cells at 1 h before (time T−1) MACV infection, at the same time (T0) as MACV infection,
or at 1 h after \((T_{+1})\) or 3 h after \((T_{+3})\) MACV infection. At 40 h post-MACV infection, high levels of IFN-β protein were measured in the medium harvested from MACV-infected cells (Fig. 6A). In contrast, no upregulation of IFN-β was detected in any of the LASV/MACV-coinfected samples (Fig. 6A). Expression of ISG proteins, such as STAT1, RIG-I, and ISG15, increased notably in response to MACV infection (Fig. 6B). However, LASV coinfection diminished the MACV-induced upregulation of ISGs in the \(T_{-1}\), \(T_{0}\), \(T_{+1}\), and \(T_{+3}\) samples. MACV replication was apparently not affected by coinfection with LASV as MACV NP levels in the coinfection samples were similar to the level in the sample infected with MACV only (Fig. 6B). MACV infection did not affect LASV replication either (Fig. 6B). These data showed that LASV could completely block the MACV-triggered IFN response as late as 3 h after MACV infection.

We further extended the coinfection experiments to investigate the ability of LASV to diminish MACV-mediated IFN induction at 6 h \((T_{+6})\) or 16 h \((T_{+16})\) after MACV infection. At 6 h post-MACV infection, LASV coinfection was able to suppress MACV-induced IFN-β protein expression by 95% (Fig. 6C), exhibiting the potent inhibitory effect of LASV infection on the IFN pathway at this time point. At 16 h post-MACV infection, IFN-β protein expression in LASV-coinfected samples was approximately 50% of that in samples infected by MACV alone. This result indicated that LASV was less efficient at blocking the MACV-activated IFN response at 16 h after MACV infection. Viral NPs were expressed at similar levels among these samples (Fig. 6D), indicating that in coinfection MACV and LASV did not interfere with each other’s replication at these time points.

**DISCUSSION**

In this study, we demonstrated that the host IFN responses to infection with highly pathogenic NW arenaviruses (JUNV and MACV) are largely distinct from the responses to infection with the OW arenavirus LASV. Among arenaviruses, the highly pathogenic OW arenavirus LASV is clinically the most important human pathogen and has been studied comprehensively in the past. Potent suppression of innate and adaptive immune responses is the hallmark of severe LASV infections in humans. LASV productively infects human macrophages and dendritic cells but fails to activate these cells or induce significant amounts of IFN/cytokine expression. In addition, LASV infection poorly induces T cell proliferation (28). In comparison, Mopeia virus, a nonpathogenic OW arenavirus closely related to LASV, induces strong IFN/cytokine responses and grows poorly in human macrophages (29). Therefore, the innate immune responses, including the IFN response, are believed to be critical to the control of nonpathogenic arenavirus infection. In contrast, the highly pathogenic OW arenavirus LASV blocks host innate and adaptive immune responses and causes severe and often fatal diseases. Among the highly pathogenic NW arenaviruses, JUNV and MACV are probably two of the most important human pathogens since they account for most of the viral hemorrhagic fever cases in South America (6, 30, 31). JUNV infections are often accompanied by very high levels of serum IFN-α in severe and fatal human cases (17, 18) as well as in NHPs (32) and guinea pigs (33). MACV infection also induces IFN production in a lethal NHP model (19). Consistent with clinical and animal studies, our previous and present studies collectively demonstrated that infection with the NW arenavirus JUNV or MACV could elicit an IFN response in human dendritic cells and A549 cells. These results highlighted the finding that the host IFN pathway reacts differently to infections with the NW arenaviruses JUNV and MACV and to infection with the OW arenavirus LASV although all of these viruses are highly pathogenic to humans.

Our studies revealed that infection with highly pathogenic NW arenaviruses (JUNV and MACV) stimulated IFN responses in human primary dendritic cells. Macrophages and dendritic cells are believed to be the early target cells of arenavirus infection. These cells are innate immune cells capable of producing IFNs and cytokines. JUNV infection does not trigger an IFN response in human macrophages (20), whereas the vaccine strain of JUNV elicits IFN and cytokine responses in mouse macrophages (34, 35). In the present study, we demonstrated that JUNV and MACV could activate IFN responses in human dendritic cells. Our results indicated that, unlike human macrophages, human dendritic cells were capable of detecting infections by JUNV and MACV and of
responding by activating the IFN pathway. This observation is also in agreement with previous studies reporting that infection of murine dendritic cells with pathogenic JUNV isolates resulted in IFN production (36). In human dendritic cells, we observed not only the upregulation of IFN-α at the protein and mRNA levels but also the upregulation of IFN-β to a greater extent (Fig. 2). IFN-β is the initial IFN produced in the early stage of the IFN response and subsequently stimulates the expression of most IFN-α subtypes. Our data demonstrated a sustained increase in the IFN-β level following IFN-α production in MACV- and JUNV-infected dendritic cells, supporting the biological significance of IFN-α expression. IFN-α expression was measured until 3 dpi (Fig. 2). It is possible that higher levels of IFN-α protein could be measured in MACV- and JUNV-infected samples later on. The biological significance of IFN upregulation was also supported by the activation of the IFN signaling pathway and the expression of ISGs (Fig. 3). We examined the IFN response at multiple time points and obtained consistent results. The IFN response to LASV infection was not detected throughout our study. Our results from dendritic cells and A549 cells are generally consistent with clinical and experimental animal studies (17–19, 32, 33), supporting the biological significance of our findings.

We used the A549 cell line as it is an IFN/cytokine-competent cell line that has been used in many studies of the host innate

FIG 5 ISG expression in MACV- and LASV-infected A549 cells. Human A549 cells were mock infected or infected with the highly pathogenic NW arenavirus MACV or the OW arenavirus LASV at an MOI of 1. At 1, 2, and 3 dpi, cellular protein lysates were prepared and subjected to Western blotting for phosphorylated STAT1 (p-STAT1), STAT1 protein, ISG15, RIG-I, MACV NP protein, LASV NP, and human β-actin. As a control, A549 cells were treated with human IFN-α for 16 h.
immune responses to virus infections, including those to arenaviruses (37–42). Considering that aerosol contact is one of the transmission routes for arenavirus infection, the lung epithelial A549 cell line as a cell culture model might potentially facilitate study of the host response to arenavirus infection. However, data from study with A549 cells needs to be further validated in dendritic cells and macrophages. Importantly our results showing differential IFN responses to MACV, JUNV, and LASV in dendritic cells were largely reproducible in A549 cells, supporting the relevance of this cell culture model. While it is known that dendritic cells and macrophages are primary target cells in the early stage of arenavirus infection, the lung epithelial A549 cell line as a cell culture model might potentially facilitate study of the host response to arenavirus infection. However, data from study with A549 cells needs to be further validated in dendritic cells and macrophages. Importantly our results showing differential IFN responses to MACV, JUNV, and LASV in dendritic cells were largely reproducible in A549 cells, supporting the relevance of this cell culture model. While it is known that dendritic cells and macrophages are primary target cells in the early stage of arenavirus infection, clinical and experimental studies also demonstrate that arenavirus infection is not exclusively restricted to these cell types: viral antigens are widely detected in various cell types other than macrophages or dendritic cells in liver, kidney, brain, and lung during JUNV infection (43, 44). Our data with A549 cells also suggested that parenchymal cells, in addition to dendritic cells, could be the source of IFN production in vivo, which needs to be investigated in future studies.

In human dendritic cells, we noticed that the viral replication of JUNV and MACV was less efficient than that of LASV (Fig. 3B). The titers of JUNV and MACV increased only moderately after infection, which was different from the rapid increase in LASV titers. The peak titers of JUNV and MACV were about 200 times lower than the peak titer of LASV (Fig. 3). We also observed that approximately 10% of the MoDCs infected by JUNV were positive for JUNV NP staining by FACS analysis (data not shown). The lower infectious virus titers for JUNV/MACV than LASV in MoDCs might be due to differences in viral entry, replication, or assembly/budding. Nevertheless, these results indicated that the host IFN pathway responded more efficiently to infections by JUNV and MACV in MoDCs despite their lower replication levels. In human A549 cells, where all viruses replicated at comparable levels (Fig. 4A), the NW pathogenic arenaviruses JUNV and MACV, but not the OW pathogenic arenavirus LASV, readily elicited IFN responses. Our experimental data obtained from different cell types supports the conclusion that the host IFN pathway is activated rapidly after infection with the NW highly pathogenic arenaviruses (JUNV and MACV) while remaining silent after infection with the OW pathogenic arenavirus LASV.

Our coinfection studies further supported the observation that the highly pathogenic NW arenaviruses and the OW arenavirus LASV had distinct interactions with the host IFN pathway, thus leading to different outcomes. The inability of the IFN pathway to
respond to LASV infection could possibly be due to either LASV active inhibition of the IFN pathway or evasion of host detection by the IFN pathway that renders LASV infection hardly visible to the host or to both (45). The coinfection data strongly suggested that it is more likely that LASV is capable of potently inhibiting the IFN pathway rather than just hiding itself from detection by the host. Interestingly, coinfection of LASV potently inhibited an MACV-mediated IFN response as late as 6 h post-MACV infection (Fig. 6). Previous studies on the cell entry of JUNV and LASV have revealed that both viruses enter cells with similar kinetics, despite the different receptors and endocytotic pathways involved (46). Also JUNV is recognized by RIG-I in A549 cells (21). Considering the similarity between JUNV and MACV, it is likely that MACV enters the cells with kinetics similar to those of JUNV and is also recognized by RIG-I. Therefore, the window for LASV inhibition as shown in Fig. 6 is unlikely due to differential entry kinetics of LASV and MACV. The data shown in Fig. 6 also suggested that host cells more likely have detected a “danger signal” associated with MACV replication rather than an immediate early signal, such as incoming viral RNA, which allowed LASV to replicate and consequently inhibit the IFN response. Otherwise, one would observe an early wave of IFN production, which is not the case. Infection of rhesus macaques by the OW LASV WE strain results in a fatal, Lassa fever-like disease, while the LCMV Arm strain does not induce disease. Interestingly, the LCMV WE strain also inhibited LCMV Arm-induced Toll-like receptor 2 (TLR2)/Mal-dependent cytokine production in similar coinfection studies (47, 48).

Notably, it has been well established that NPs of all arenaviruses, regardless of their pathogenicity potentials, as well as the Z proteins of NW arenaviruses are strong IFN antagonists (37, 40–42, 49–51). Recently, it has been reported that the Z proteins of pathogenic arenaviruses, including LASV, LCMV, JUNV, and MACV, but not the Z proteins from nonpathogenic arenaviruses block IFN-β activation in 293T cells (52). Nevertheless, NW pathogenic arenaviruses, such as MACV and JUNV, stimulate the IFN response during infection in dendritic cells and A549 cells, indicating that the arenavirus-host interaction is more complicated and dynamic and that the overall outcome of the host response to arenavirus infection ultimately needs to be further characterized in the context of virus infection. It also remains unclear why the host IFN pathway reacted so differently to the infections by the NW pathogenic arenaviruses (JUNV and MACV) and those by the OW arenavirus LASV. Future studies are required to address these interesting and important questions.

For the NW arenaviruses that are highly pathogenic to humans, the impact of IFN or cytokines on virus infection and pathogenesis is not well understood. The race between the host immune response and virus replication begins very early during the course of viral infection, when IFNs and various cytokines are produced. IFNs act to control virus infection directly by inducing the expression of a variety of interferon-stimulated genes that execute IFN-mediated antiviral activities. In addition, IFNs and cytokines also stimulate and regulate cell-mediated innate and adaptive immune responses. JUNV and MACV are relatively resistant to IFN treatment in some cell lines (21, 53), suggesting that IFNs alone might not be able to counteract JUNV and MACV infections effectively and that additional host immune responses are required. Rhesus monkeys infected with MACV developed even higher viremia in poly(I - C)-treated groups than in untreated controls in spite of the appearance of serum IFNs (19). In severe cases, it is possible that virus infection might progress unchecked and out-compete host innate and adaptive immune responses, leading to high levels of IFN and cytokine production during the late stage of the disease. High levels of IFNs and proinflammatory cytokines might be detrimental to these patients and contribute to JUNV pathogenesis. Indeed, JUNV infection has been shown to impair platelet formation and platelet release via the IFN signaling pathway, which might contribute to the thrombocytopenia observed in patients (54). On the other hand, many factors, such as the pace of virus multiplication, host health condition, genetic background, and species, etc., could also determine the outcome of infections. In some cases, it is also possible that IFNs and cytokines might actually help the host to control or limit NW JUNV or MACV infection, which might explain how certain patients recovered from infections. In agreement with this, we find that IFNs are critical to control JUNV and MACV infections in murine systems (22, 23, 55, 56). It is also worth noting that in the case of LASV infection in an NHP model, an early peak of type I IFN production is correlated with animal survival, whereas fatal infection was characterized by a lack of early type I IFN production (57).

In summary, our studies demonstrated that the host IFN responses to NW JUNV and MACV infection differ from the response to infection with the OW LASV, whereas all these viruses are highly pathogenic to humans. Future studies are required to investigate the mechanism underlying the differential IFN responses noted herein and the influence of IFN on the pathogenesis of NW pathogenic arenaviruses.

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