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Human Plasmacytoid Dendritic Cells Elicited Different Responses after Infection with Pathogenic and Nonpathogenic Junin Virus Strains

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The arenavirus Junin virus (JUNV) is the etiologic agent of Argentine hemorrhagic fever. We characterized the JUNV infection of human peripheral blood-derived plasmacytoid dendritic cells (hpDC), demonstrating that hpDC are susceptible to infection with the C#1 strain (attenuated) and even more susceptible to infection with the P (virulent) JUNV strain. However, hpDC elicited different responses in terms of viability, activation, maturation, and cytokine expression after infection with both JUNV strains.

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The arenavirus Junin virus (JUNV) is the etiologic agent responsible for Argentine hemorrhagic fever (AHF) (1). The pathogenesis of AHF is not completely understood (2, 3), although immune cells are among the main targets (2–4).

Circulating human peripheral blood-derived plasmacytoid dendritic cells (hpDC) sense viral infections, responding with massive type I interferon (IFN-I) production (5, 6). They also have an assigned role in determining the outcome of some viral infections (7, 8). We hypothesized that JUNV targets hpDC in order to modulate the host's response.

hpDC were isolated from the buffy coats of 14 donors by immunomagnetic negative selection (catalog number 130-092-207; Miltenyi, Germany). According to flow cytometry analysis, the percentage of $CD123^+$ (specific marker) (9) cells ranged from 95 to 99%, with a cell viability of ≥97% (FACScan flow cytometer and FCS Express V3 software).

Viral stocks of the attenuated Candid#1 (C#1) and virulent P3441 (P) strains of JUNV (10, 11) were made and quantified as described previously (12). Briefly, monolayers of BHK21 cells were infected with each strain and clarified supernatants ($5.000 \times g$) were collected 2 to 3 days postinfection (dpi). hpDC (1×10^4) were infected with the JUNV strains at a multiplicity of infection (MOI) of 0.1 or 1. The negative controls (mock infected) were supernatants from uninfected Vero cells. Human recombinant interleukin-3 (IL-3, 20 ng/ml; Peprotech, Mexico) was added on day 1 (13). All cell cultures were free of mycoplasma.

Results are expressed as means ± the standard errors of the means. Each experiment was performed with a different donor, and its number is indicated in the corresponding legend as $n =$ x. P values of ≤0.05 after Student's paired t test or one-way analysis of variance were considered to be statistically significant.

The level of infective virus in the supernatants increased in a time- and MOI-dependent manner, as confirmed by infectivity assays (Fig. 1A) in concordance with the viral RNA levels detected by quantitative PCR (qPCR) (Fig. 1B). C#1 and P nucleoprotein (NP) was detected in 70 to 95% of the cells, respectively (Fig. 1B) with a monoclonal antibody (clone AB06-B610) (14), followed by Alexa 488-conjugated anti-mouse IgG and a phycoerythrin (PE)-conjugated rabbit anti-human CD123 (BD Biosciences, NJ). Analysis was conducted by confocal microscopy (11). Negative and positive controls were uninfected hpDC and infected Vero cells, respectively. The results demonstrated that hpDC are susceptible to both JUNV strains, and in agreement with previous studies, the P strain elicited higher values than did C#1 (15, 16).

JUNV failed to induce cell apoptosis or necrosis at any time point or MOI (Fig. 2A), as established by nuclear morphology after acridine orange and ethidium bromide staining (11). The percentages of apoptotic cells were similar in mock- and C#1-infected hpDC, while the P strain inhibited spontaneous apoptosis over time and in an MOI-dependent manner, even preventing apoptosis induced by fetal bovine serum (FBS) deprivation (data not shown). Flow cytometry analysis of $JUNV^+$ cells indicated that P, but not C#1, reduced caspase-3 activation and phosphatidylserine exposure (detected by annexin V binding) (Fig. 2B and C). In this regard, a recent study demonstrated retinoic acid-inducible gene 1 (RIG-I)-mediated IFN-I-independent apoptosis in a cell- and viral strain-dependent manner (17). Moreover, the NP of JUNV interfered with the activity of caspase-3, inhibiting apoptosis (18). Finally, the Z protein of pathogenic, but not nonpathogenic, arenaviruses interacts with the RIG-I-like receptor, resulting in inhibition of IFN-I production (19) and associated apoptosis (20). It re-

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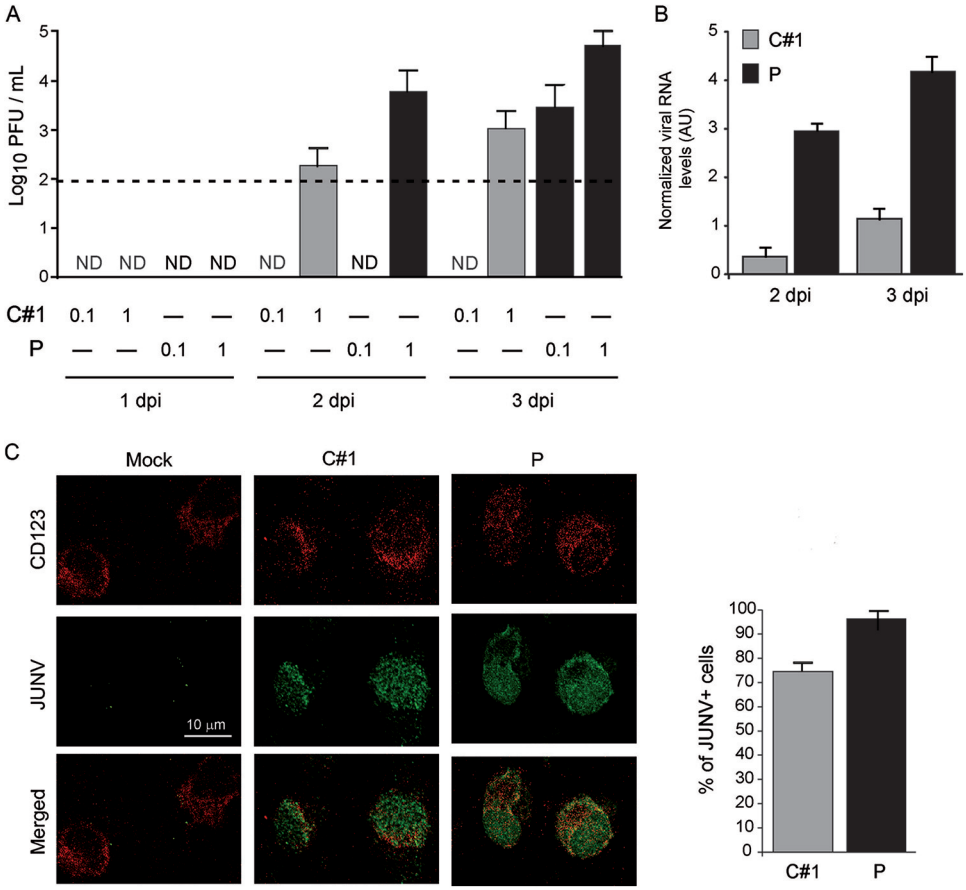

FIG 1 hpDC are susceptible to JUNV infection and replication. (A) PFU counts in supernatants at the time points indicated were quantified with infectivity titration assays on Vero cells ($n = 5$). ND, not detected. (B) Viral mRNA levels were detected by reverse transcription (RT)-qPCR in hpDC infected with JUNV strain C#1 or P at the time points indicated ($n = 3$). AU, arbitrary units. (C) Mock-, C#1-, or P-infected hpDC were costained with the specific marker CD123 (red) and anti-JUNV protein antibody (green) at 2 dpi. Original magnification, ×600. The bar graph shows the percentages of JUNV$^+$ cells in the CD123$^+$ population as determined by manual scoring of at least 200 cells ($n = 4$).

mains necessary to clarify if any of these mechanisms are involved with both of the JUNV strains studied here.

While the expression of HLA-ABC or HLA-DR at 1 (data not shown) and 2 dpi was moderately and strongly increased after C#1 or P infection, respectively, the expression of CD86 was upregulated only by the P strain (Fig. 3A). In addition, CD83 expression levels were higher than in mock-treated cells (Fig. 3B). Similar results were obtained with JUNV$^+$ cells only (data not shown). These results suggest that JUNV infection, by inducing activation and maturation of hpDC, may contribute to different adaptive immune responses according to the viral strain, as reported for other viral infections (21, 22).

IFN-I levels in cell supernatants were studied with a stably transfected WISH cell line with the enhanced green fluorescent protein gene under the control of the IFN-I-inducible Mx2 promoter (23). The percentage of positive cells was evaluated by flow cytometry with recombinant IFN-β (InvivoGen, San Diego, CA) as a positive control. Both JUNV strains were able to induce IFN-I expression, with P showing the highest values (Fig. 4A). These results were confirmed at the transcriptional level (Fig. 4B). By using imiquimod as a positive control (data not shown) (24), we determined that C#1 moderately and strongly enhanced the transcription of IL-6 and tumor necrosis factor alpha (TNF-α), respectively. Meanwhile, the P variant failed to modify IL-6 levels, even reducing the expression of TNF-α below the control values (Fig. 4C).

Several viruses have been reported to induce (8, 25–28) or inhibit (29, 30) IFN-I after hpDC infection. The high levels of IFN-I induced by P infection may explain the elevated levels of circulating IFN-α in patients with AHF (31). However, it is unclear if these enhanced levels simply reflect a greater early viral load or if they are linked to disease development (32). The reduced IFN-I production of hpDC induced by the clone 13 strain of the arenavirus lymphocytic choriomeningitis virus (LCMV^{clon13}) contributed to viral persistence (33). Moreover, hpDC-deficient mice fail to clear LCMV, associated with the reduced number and functionality of LCMV-specific T-cell responses (7), protection from NK cell cytotoxicity (34, 35), or viral replicative capacity (36). In mice intravenously infected with a high dose (2×10^6 PFU) of LCMV^{clon13}, virus persistence is controlled by blocking IFN-I signaling (37, 38). These studies suggest that the levels of IFN-I are critical for the immune host response to arenavirus. The enhanced production of IL-6 and TNF-α triggered by C#1 may also be important for the infection outcome, since these cytokines prime the CD4$^+$ T cells to differentiate into IL-10-producing regulatory T cells

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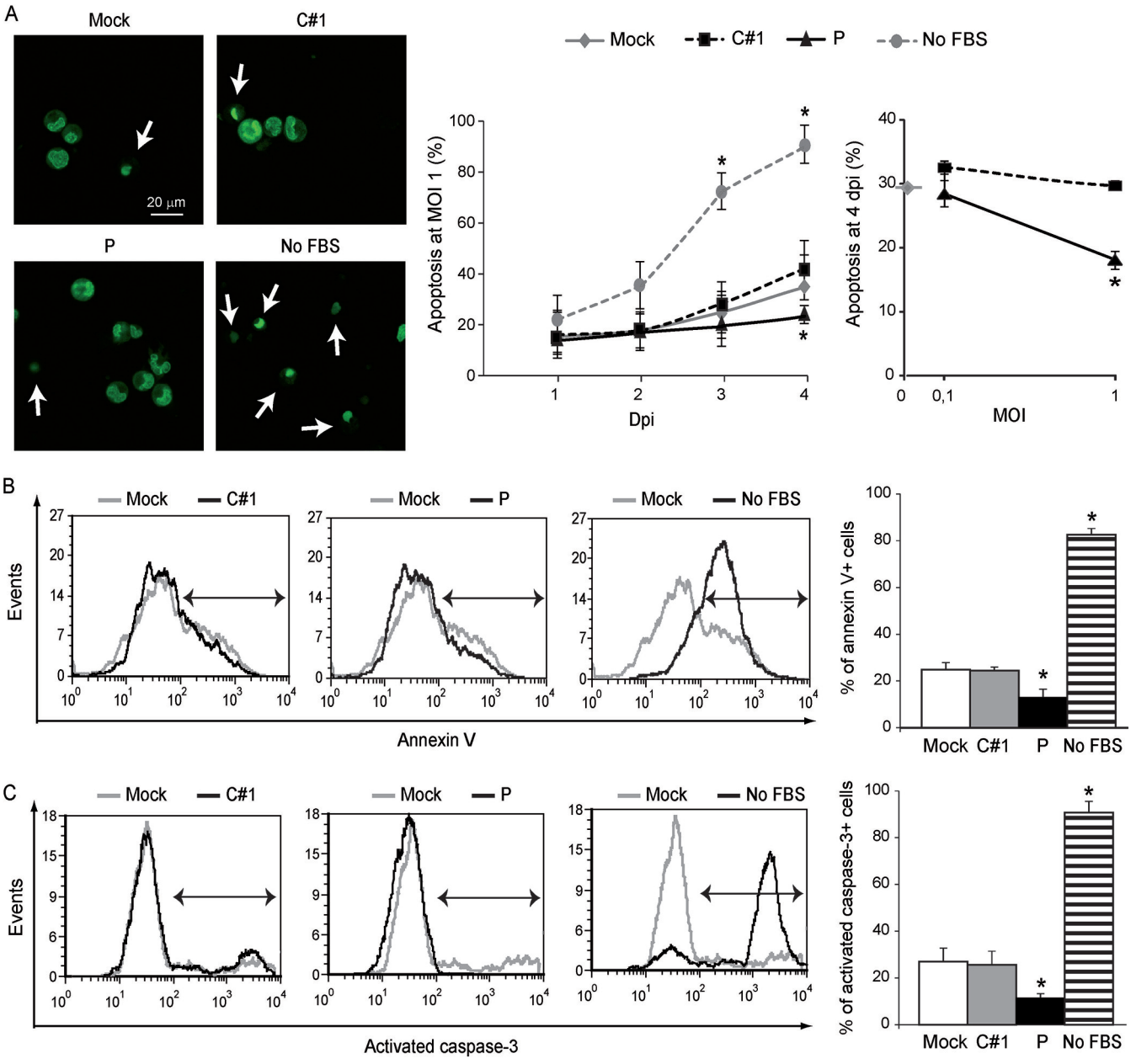

FIG 2 JUNV failed to cause cytopathic effects, and strain P suppressed spontaneous hpDC apoptosis. (A) Cells were double stained with acridine orange and ethidium bromide, and the percentage of apoptotic cells in noninfected (mock-infected) hpDC or those infected with C#1 or P was analyzed by fluorescence microscopy. Serum-deprived hpDC (no FBS) were used as a positive control ($n = 5$). Original magnification, ×200. Arrows indicate apoptotic cells. (B and C) Cells were stained with fluorescein isothiocyanate (FITC)-annexin V-conjugated (B) or FITC-conjugated (C) anti-human activated caspase-3 antibodies, and the percentage of positive cells was analyzed by flow cytometry at 4 dpi. Histograms represent one of three independent experiments. *, $P < 0.05$ versus mock infection.

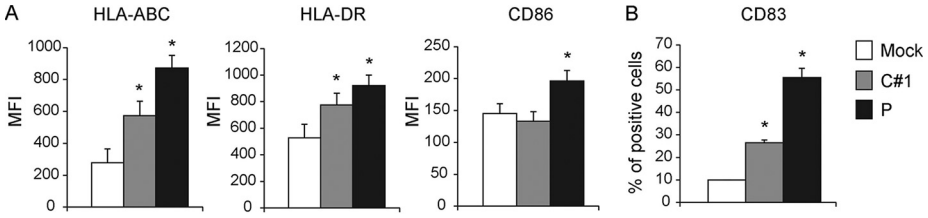

FIG 3 JUNV infection upregulated the expression levels of activation and maturation markers in hpDC. hpDC were incubated with the vehicle (mock infection), C#1, or P, and hpDC activation and maturation markers were analyzed at 2 dpi. (A) Cells were stained with anti-human FITC-HLA-ABC, PE-HLA-DR, or PE-Cy5-CD86 antibodies, and the mean fluorescence intensity (MFI) was measured by flow cytometry ($n = 5$). (B) Cells were stained with anti-human FITC-CD83, and the percentage of positive cells was determined by flow cytometry. *, $P < 0.05$ versus mock infection.

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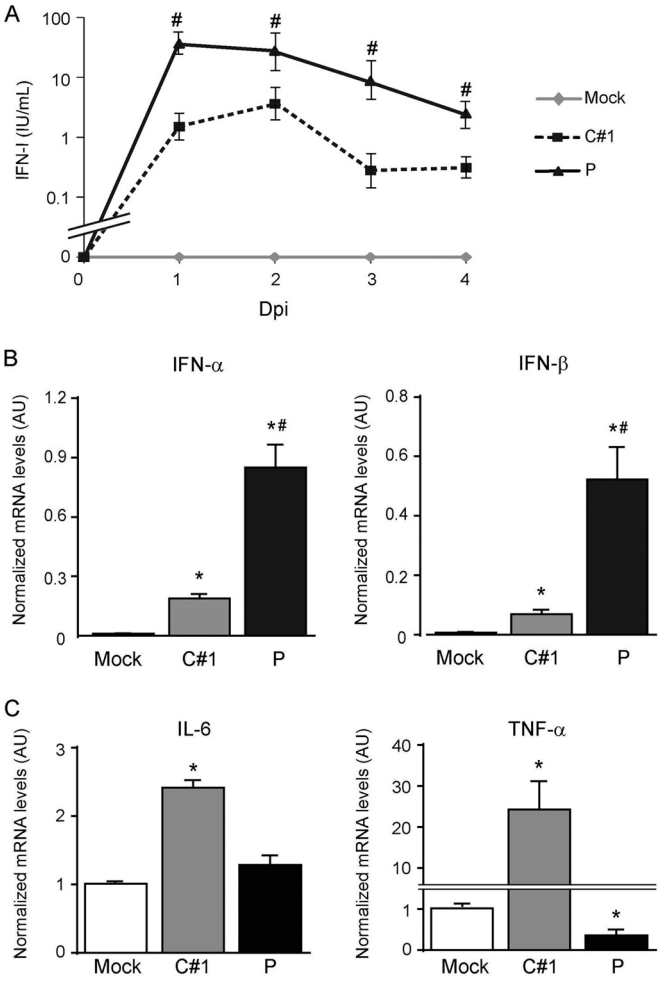

FIG 4 Cytokine profile of hpDC induced by JUNV infection. (A) hpDC were incubated with the vehicle (mock infection), C#1, or P, and supernatants were collected at the time points indicated. IFN-I levels in hpDC supernatants ($n = 4$) were measured with a reporter gene assay. (B) IFN-I mRNA expression levels in hpDC infected with C#1 or P were determined by RT-qPCR ($n = 3$) at 2 dpi. (C) IL-6 and TNF-α mRNA expression levels were determined by RT-qPCR ($n = 3$) at 2 dpi. *, $P < 0.05$ versus mock infection. #, $P < 0.05$ versus C#1. AU, arbitrary units.

(39) and late IL-6 escalates helper T cell responses to control viral infection (40).

Taken together, our results suggest that JUNV infection of hpDC may be important in pathogenesis, encouraging further studies of roles for hpDC in arenavirus-induced hemorrhagic fevers.

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