Coll. Antropol. **31** (2007) 4: 1159–1167
Original scientific paper

Pathogenic Hantaviruses Elicit Different Immunoreactions in THP-1 Cells and Primary Monocytes and Induce Differentiation of Human Monocytes to Dendritic-Like Cells

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ABSTRACT

Hantaviruses cause two important human illnesses, hemorrhagic fever with renal syndrome (HFRS) and hantavirus pulmonary syndrome (HPS). Both syndromes are believed to be immune-mediated diseases. Monocytes/macrophages are thought to be the main target cells for hantaviruses and important sources of and targets for cytokines/chemokines secretion. THP-1 cells have been used extensively as models for primary monocytes in biocompatibility research. The aim of our study was to determine if hantaviruses induce the same immunoreactions in THP-1 cells and primary monocytes/macrophages and might therefore be suitable for immune studies of hantaviral infections. For that purpose we compared various cytokines/chemokines and their receptors in THP-1 cell line and primary monocytes/macrophages. Infected primary monocytes/macrophages induced mostly β-chemokines and their receptors. In contrast, THP-1 cells, expressed receptors for CXC chemokines. Surprisingly, infected macrophages underwent morphological changes toward dendritic-like cells and increased expression of co-stimulatory molecules: CD40, CD80, CD83 and CD86. Our data indicate that THP-1 cells are not ideal for in vitro research of the immunopathogenesis of hantaviruses in humans. Further, our studies revealed potential roles for cytokines/chemokines in HFRS/HPS immunopathogenesis and point to intriguing possibilities for the possible differentiation of infected macrophages to dendritic-like cells.

Key words: *pathogenic hantaviruses, THP-1 cells, primary monocytes, dendritic-like cells, cytokines/chemokines*

Introduction

Hantaviruses cause two important human illnesses, hemorrhagic fever with renal syndrome (HFRS) and hantavirus pulmonary syndrome (HPS)[1]. HFRS is manifested mainly by fever, variable degrees of circulatory failure, hemorrhage and renal failure. In HPS, capillary leakage is localized exclusively in the lungs, the kidneys are largely unaffected, and death occurs from shock and cardiac complications[1]. Several studies support the view that HFRS/HPS are immune-mediated diseases[2–6].

The *in vivo* study of the pathogenesis of HFRS and HPS is limited by the lack of a suitable animal model. An exception is Andes virus, which has been found to cause HPS in humans and an HPS-like disease in hamsters[7]. Consequently, most information on the immunopathogenesis of HFRS/HPS has been derived from the analysis of patient samples[2,4–6,8]. Although such data are extremely valuable, the available samples do not always represent the optimal window for measuring organ-specific immune responses characteristic of the disease[9].

Hantaviruses replicate *in vitro* in the cytoplasm of a variety of mammalian cell lines, and in primary endothelial cells and monocytes/macrophages[10]. Monocytes/macrophages are thought to play a part in hantaviral spread from the primary site of infection[3,11–12]. Kanerva et al. suggested that mature macrophages show increased susceptibility to Puumala virus (PUUV) and that after dif-

Received for publication September 12, 2007

ferentiation to tissue macrophages they might function in the spread of the virus during the infection[12]. It is also known that human alveolar macrophages are permissive for infection with Sin Nombre virus (SNV) and react with low production of tumor necrosis factor alpha (TNF-α)[13]. We theorized that monocytes/macrophages could be important sources of and targets for cytokines/chemokines secretion[14].

THP-1 cells[15], which were derived from the peripheral blood of a 1-year-old male with acute monocytic leukaemia, have been used in numerous studies to measure immunoreactions of human macrophages during infections. THP-1 cells have Fc and C3b receptors and lack surface and cytoplasmic immunoglobulins. These cells also stain positive for alpha-naphthyl butyrate esterase, produce lysozymes, and are phagocytic. THP-1 cells can also restore the response of purified T lymphocytes to Concanavalin A, show increased CO_2 production on phagocytosis, and can be differentiated into macrophage-like cells[15]. Although for some microorganisms that THP-1 cells are suitable for such research, there is evidence that particular microorganisms may induce different immunoreactions in THP-1 cells than they do in primary human monocytes/macrophages[16–18].

The aim of our study was to find out if pathogenic hantaviruses will induce the same immunoreactions in THP-1 cells and primary monocytes/macrophages, thus would be suitable surrogates for the primary cells. Here we report a comparative evaluation of cytokines/chemokines and their receptors elicited by pathogenic hantaviruses in the two types of cells.

Material and Methods

THP-1 cells and isolation of primary monocytes

The monomyelocitic THP-1 cell line (ATCC TIB 202) and primary monocytes/macrophages were maintained in RPMI 1640 medium (Life Technologies, Grand Island NY) supplemented with 10% heat-inactivated fetal bovine serum, 0.2% fungizone, 1% penicillin -streptomycin and 1% glutamine (Life Technologies). For primary monocytes isolation, leukocytes were obtained by cytopheresis, after informed consent, from normal donors at the National Institutes of Health (NIH). These cells represent excess existing samples derived from human donors through approved NIH protocols (89-100/A-C and 99-CC-0168). Monocytes were isolated from the leukocytes by countercurrent centrifugal elutriation as described[19]. The purity (>90%) of the monocyte preparations was confirmed on cytocentrifuge slide preparations by morphology with Wright's staining. Monocytes were cultured for 2–3 days before infection. After that period of culture, >95% of the cells were adherent.

Viruses and infection

Hantaan virus (HTNV), strain 76118[20], Andes virus (ANDV) strain Chile-9717869[21], and Sin Nombre virus (SNV), strain CC107[22] propagated in Vero E6 cells (Vero C1008, ATCC CRL 1586), were used to infect cells. Cells were infected at multiplicities of infection (MOI) of 10^{-1} pfu/cell (SNV), 10^{0} pfu/cell (ANDV), or 10^{1} pfu/cell (HTNV). The different MOI were used due to our inability to achieve equivalent MOI, because of low-titer seed stocks of some hantaviruses, like SNV. As controls for the infected cultures, uninfected cells were treated identically to infected cultures; that is, control cells were incubated in the same medium that we used for diluting the viruses before adsorption. The cells were then washed and refed and maintained with appropriate medium. The cells were incubated for 7–12 days at 37 °C, 5% CO_2 and medium was not changed during the observation period. All infected and uninfected cells were tested for *Mycoplasma* contamination by ELISA-PCR (Roche Diagnostics Corporation, Roche Molecular Biochemicals, Indianapolis, IN, USA) and were found to be *Mycoplasma* free.

Plaque assay

To confirm cell infection with HFRS- and HPS-causing viruses, supernatants of infected and uninfected cells were collected at various times and were stored at –70 °C until assayed. Plaque assays were performed as previously described using Vero E6 cell monolayers[7].

Direct immunofluorescence assay for detecting hantaviruses in infected cells

Slides of infected or uninfected cells, were fixed in acetone and stained as previously described using a primary specific monoclonal antibody to the G2 protein of HTNV (8E10) or hyperimmune mouse ascites fluid (HMAF) to the specified hantavirus[23].

RNA extraction and ribonuclease protection assay (RPA)

Total cellular RNA was extracted using Trizol reagent (Life Technologies) followed by phase separation with chloroform, then precipitation with isopropyl alcohol. Measurements of cytokines, chemokines and their receptors were determined using a RiboQuant MultiProbe RNAse protection system (PharMingen, San Diego, CA, USA) and different multi-probe template sets (hCK-2, hCR-4, hCR-5, hCR-6) following instructions provided by the manufacturer. Quantitative comparisons of mRNA expression levels among samples were made using a CYCLONE™ storage phosphorimaging system (Packard Instrument Company, Meriden, CT) with molecular analysis software (Optiquant, version 3.0). The values obtained for each level of mRNA measured were normalized against the combined levels of expression obtained for mRNA from GAPDH (glyceraldehyde-3-phosphate dehydrogenase) and L32 housekeeping genes loaded within the same lane on the RPA gel.

Enzyme-linked immunosorbent assay (ELISA)

Cytokines measured were: interleukin (IL)-1β, IL-6, IL-10, IL-12p40, granulocyte-macrophage colony-stimulating factor (GM-CSF), interferon gamma (IFN-γ) and

TNF-α. Chemokines measured were: IL-8/CXCL-8, regulated upon activation, normal T-cell expressed, and secreted (RANTES/CCL5), monocyte chemo-attractant protein (MCP-1/CCL2) macrophage inflammatory protein- MIP-1α/CCL3 and MIP-1β/CCL4) levels were determined in the supernatants of infected (HTNV, SNV, ANDV) and uninfected cells by specific ELISA (Pharmingen or R&D Systems, Inc., Minneapolis, MN, USA) according to the instructions provided by the manufacturer. Samples were collected at various time points and stored at –70 °C until assayed.

Giemsa-Wright staining

Slides with infected or uninfected THP-1 cells were washed in PBS, 11 days after infection, fixed in metha-

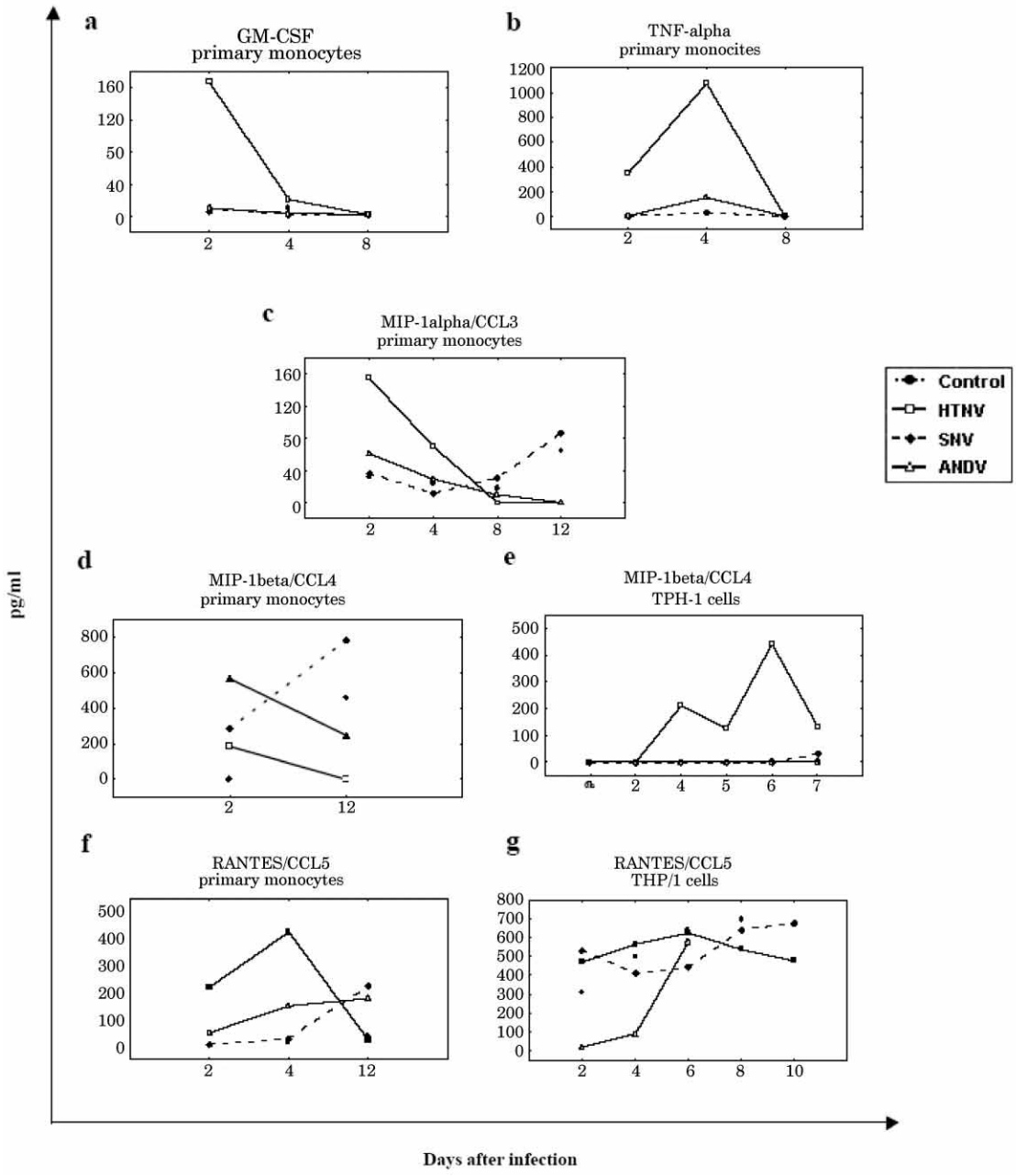


Fig. 1. Detection of cytokines and chemokines in the supernatants of primary human monocytes and THP-1 monomyelocytic cells by enzyme-linked immunosorbent assay (ELISA). Hantaan virus (HTNV) induced granulocyte-macrophage colony-stimulating factor (GM-CSF) (a) and tumor necrosis factor alpha (TNF-α) (b) in primary monocytes. All three hantaviruses, HTNV, Sin Nombre (SNV), and Andes virus (ANDV) induced β-chemokines: macrophage inflammatory protein-1 alpha (MIP-1α/CCL3), (c), MIP-1β/CCL4 (d) and regulated upon activation, normal T-cell expressed, and secreted (RANTES/CCL5) (f). In THP-1 cells only HTNV induced appreciable levels of MIP-1β/CCL4 (e) and RANTES/CCL5 (g).

nol, air dried, stained with Wright Giemsa stain for 5 min, then washed in distilled water and examined under a microscope. This stain, which contains polychrome methylene blue and eosin Y, is traditionally used for staining blood smears, parasites and viral inclusions. We used it for our studies to provide better resolution of cellular details.

Flow cytometry

Infection of THP-1 cells with hantaviruses produced an apparent alteration in THP-1 cell type morphology. Cells became adherent and appeared to develop dendritic cell-like characteristics. To assess if infection of THP-1 cells altered the expression of cellular markers, infected cells were stained for expression of various cluster of differentiation (CD) markers and examined by flow cytometry as previously described[4]. Briefly, THP-1 cells were infected with hantaviruses or were untreated and were cultured for 9 days. At days 3, 5, 6, 7, 8 and 9 postinfection, 1 x 10^6 cells were harvested and stained with monoclonal antibodies for CD14, CD40, CD80, CD83 or CD86 (Pharmingen), or double stained for CD14 and CD40, CD80, CD83 or CD86. Monoclonal antibodies were directly conjugated to fluorochromes (FITC and PE). Control cells were prepared using appropriate labeled isotype controls. In addition uninfected cells were also processed in a similar manner and left unstained. The same was repeated with primary human monocytes 12 days after infection with hantaviruses.

Direct immunofluorescence (IFA) assay

Infection of primary monocytes with hantaviruses resulted in changing of the cells' morphology toward dendritic cell-like characteristics. To assess if infection of primary monocytes altered the expression of cellular markers, the slides of infected or uninfected cells were fixed in acetone and stained for expression of various CD markers and examined by direct IFA. Briefly, primary monocytes were infected with hantaviruses or were untreated and were cultured for 11 days. Cells (infected and uninfected) were stained with monoclonal antibodies for either CD14, CD40, CD80, CD83 or CD86 (Pharmingen), or double stained for CD14 and CD80. Monoclonal antibodies were directly conjugated to fluorochromes (FITC and PE).

Results

Detection of THP-1 cells and primary monocytes/macrophages infection with hantaviruses

Several human cell lines are susceptible to infection with hantaviruses[10]. In this study, we infected primary human monocytes/macrophages and THP-1 cells with hantaviruses and confirmed the infection by plaque assay of supernatants and/or immunofluorescence antibody assays. Low viral titers ranging from 9,5 x 10^4 pfu for SNV to 1,2 x 10^4 pfu for HTNV were detected in the supernatants of human monocytes by plaque assay (data not shown).

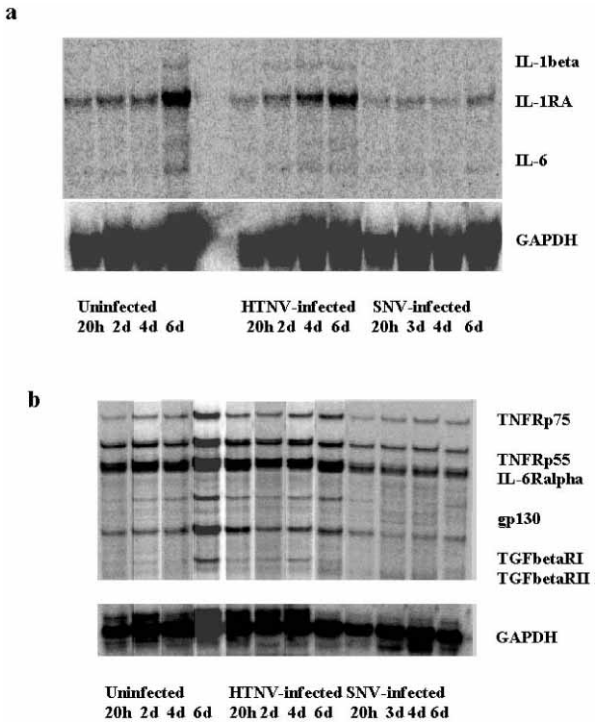


Fig. 2. Detection of cytokines mRNA expression in THP-1 cells infected with hantaviruses from 20 h to 6 days postinfection. Ribonuclease protection assays showed that Sin Nombre virus (SNV) in comparison to Hantaan virus (HTNV) or uninfected THP-1 cells, down-regulated mRNA for interleukine-1 receptor antagonist (IL-1RA) (a) and also slightly down-regulated the gene-expression for tumor necrosis factor alpha (TNF) and tumor growth factor (TGF) receptors (b).

Hantaviruses induce different cytokines, chemokines and their receptors in primary human monocytes than they do in THP-1 monomyelocytic cells

In primary monocytes, HTNV induced GM-CSF (Figure 1*a*) and TNF-α (Figure 1b). ANDV also induced low levels of TNF-α (Figure 1b) and all three viruses induced MIP-1α/CCL3 (Figure 1c), MIP-1β/CCL4 (Figure 1d) and RANTES/CCL5 (Figure 1f). In contrast, in THP-1 cells, only HTNV induced MIP-1α/CCL3 (Figure 1e) and low levels of RANTES/CCL5 (Figure 1g). When we measured mRNA for cytokines and their receptors in the infected THP-1 cells, we found that SNV suppressed gene expression of IL-1RA (Figure 2a) and slightly suppressed expression of TNF-α, IL-6 and TGF receptors (Figure 2b). Further we found that all three hantaviruses that we tested increased gene expression for receptors of CC chemokines in infected primary monocytes: including the CCR1 receptor for MIP-1α/CCL3, and RANTES/CCL5 and CCR5 receptors for MIP-1α/CCL3, MIP-1β/CCL4 and RANTES/CCL5 (Figure 3*a*). Only HTNV induced higher CCR1 and CCR5 mRNA expression in comparison with control cells on day 2 after infection, while

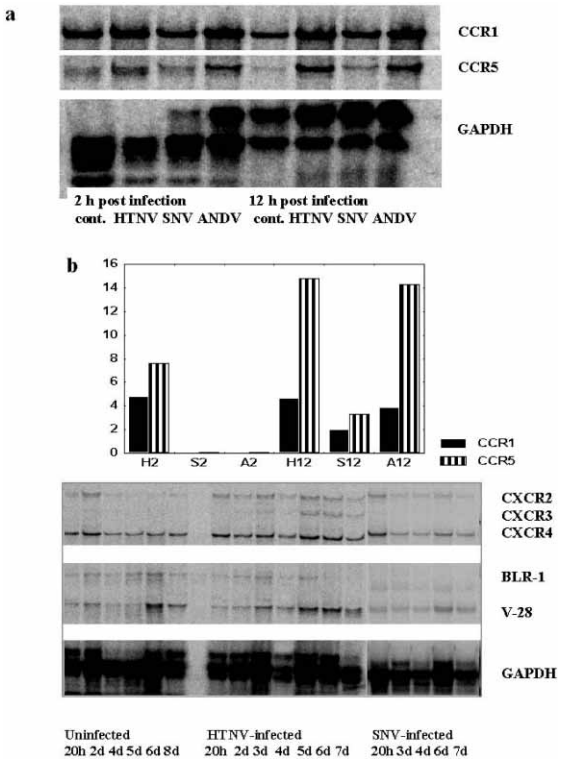


Fig. 3. Detection of chemokine receptor's mRNA expression in primary human monocytes and THP-1 cells infected with hantaviruses. Ribonuclease protection assays performed at 2 or 12 days postinfection showed that Hantaan virus (HTNV), Sin Nombre virus (SNV) and Andes virus (ANDV) increased expression of chemokine receptors: CCR1 and CCR5 in infected as compared to uninfected primary human monocytes (a). The highest expression was induced by HTNV, 12 days after infection (b). In contrast, in THP-1 cells all three hantaviruses induced expression of CXCR2, while HTNV induced expression of CXCR3 (c).

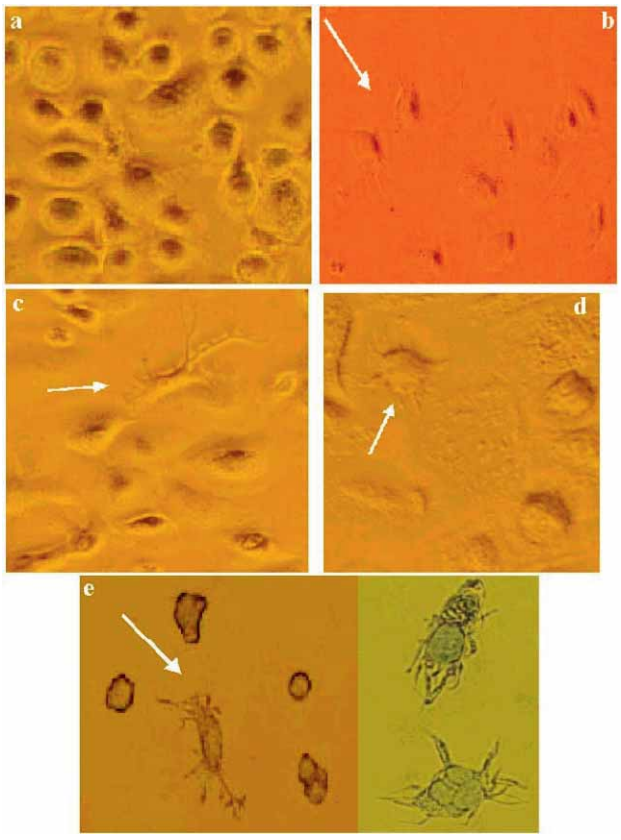


Fig. 4. Morphological changes in primary human monocytes and THP-1 cells infected with hantaviruses. In comparison to uninfected primary human monocytes (a), Hantaan virus (HTNV) (b), Sin Nombre virus (SNV) (c), and Andes virus (ANDV) (d) induced morphological differentiation to dendritic-like cells. In THP-1 cells, HTNV (e) ANDV, and SNV (data not shown) also induced morphological changes.

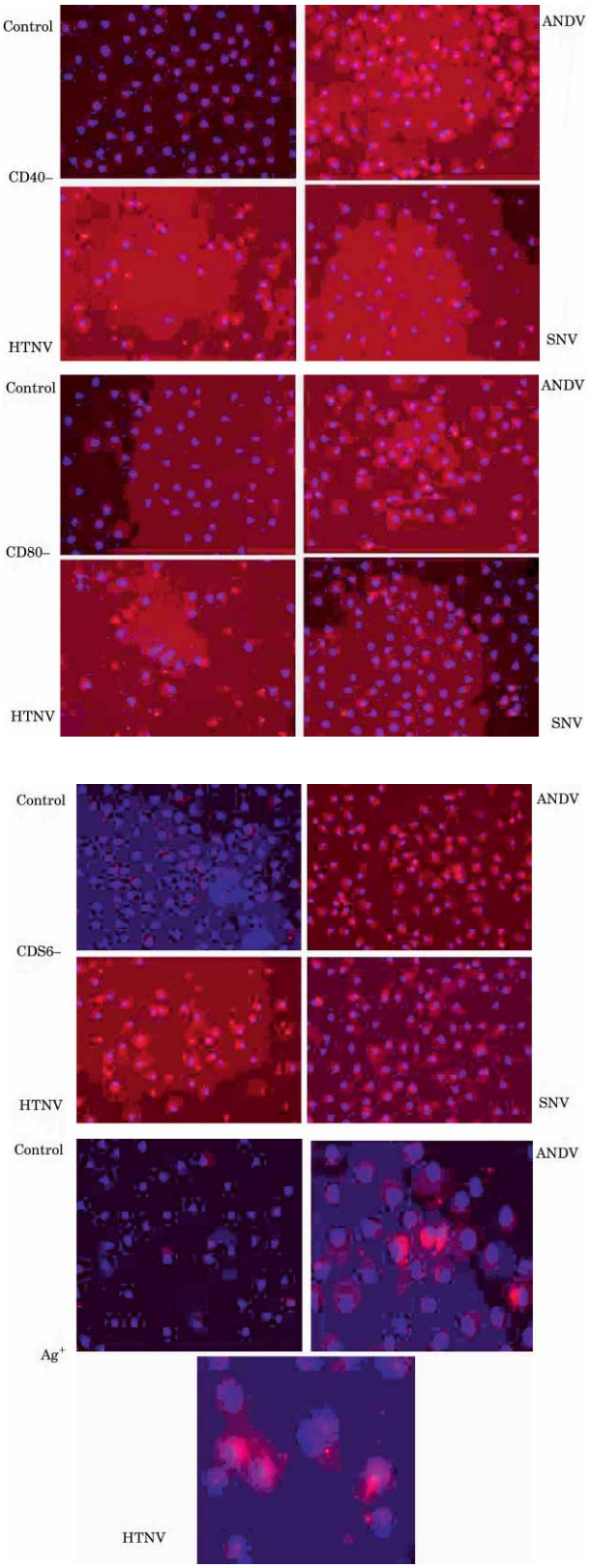
three viruses induced higher levels 12 days after the infection (Figure 3 a,b). The expression of CCR5 was about 3–14 times higher in infected than in control cells and CCR1 expression was about 2-5-fold higher than in control cells 12 days after infection (Figure 3b). Surprisingly, in THP-1 cells, hantaviruses induced expression of CXC chemokine receptors. Both, HTNV and SNV induced CXCR2, which is the receptor for CXCL8, and HTNV induced CXCR3, which is the receptor for IP-10 and other chemokines (Figure 3c).

Hantaviruses induce morphological changes of primary human monocytes and THP-1 cells toward dendritic-like cells

Unexpectedly, during our experiments, we found that monocytes infected with HFRS- and HPS-causing hantaviruses underwent morphological changes toward dendritic-like cells. Although we found specific differences in the profiles of cytokines, chemokines and their receptors between primary monocytes and THP-1 cells infected with hantaviruses, both types of cells underwent morphological changes (Figure 4). The most prominent morphological changes in primary monocytes were observed 10–12 days after infection (Figure 4a–d). In infected THP-1 cells, morphological changes were observed 48–72 h after infection. The most prominent changes toward dendritic-like cell shapes in THP-1 cells attached to the plastic were observed 5 to 7 days after infection with HTNV (Figure 4*e*), SNV or ANDV (data are not shown). Eight to 10 days after infection, both infected and control THP-1 cells began to die.

Hantaviruses induce expression of CD40, CD80, CD83 and CD86 in infected primary human monocytes

To evaluate if the morphological changes that we observed in the primary monocytes infected with hantaviruses coincided with the induction of co-stimulatory molecules (CD40, CD80, CD86) or markers for the maturation of dendritic cells (CD83), we performed two-color flow cytometry with HTNV-infected THP-1 cells (5 days after infection) or primary monocytes (11 days after infection) with monoclonal antibodies to CD14, CD40, CD80, CD83 and CD86. We did not observe the increased



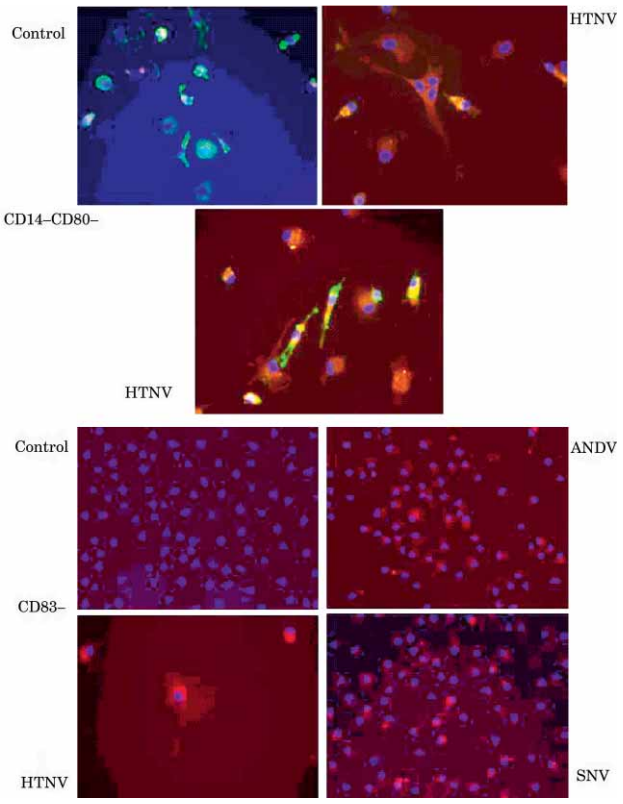


Fig. 5. Expression of co-stimulatory molecules on primary human monocytes infected with hantaviruses. In comparison to uninfected primary monocytes, monocytes infected with Hantaan (HTNV), Sin Nombre (SNV) or Andes ANDV viruses increased expression of CD40, CD80, CD83 and CD86 by direct immunofluorescence (IFA). In double IFA staining with CD14 and CD80 antibodies, uninfected monocytes expressed only CD14, while monocytes infected with HTNV co-expressed CD14 and CD80 or lost CD14 and expressed only CD80. We also confirmed by IFA that primary monocytes were infected with hantaviruses. Blue-dapi (nuclear stain); green-FITC (CD14); red-PE (CD40, CD80, CD83 or CD86).

expression of any of the tested molecules in infected compared to uninfected cells. In only one experiment, with THP-1 cells infected with HTNV, did we observe a slightly higher signal for CD40 than was seen in control cells (data not shown). Because we observed morphological changes in a low percentage of infected monocytes, we suspected that flow cytometry might not be sensitive enough to detect the changes in a large pool of cells. Consequently, we fixed primary monocytes infected with HTNV, SNV or ANDV, and performed IFA with specific antibodies. We found that all three of the hantaviruses induced higher expression of CD40, CD80, CD83 and CD86 molecules than were seen in uninfected control cells (Figure 5). When we double-stained primary monocytes infected with HTNV with anti-CD14 and anti-CD80 antibodies we found that uninfected cells expressed only CD14, while monocytes infected with HTNV co-expressed CD14 and CD80 or lost CD14 and expressed only

CD80. It is possible that some of the infected cells, which lost CD14 and had only CD80, had already acquired the dendritic cell-like phenotype, whereas the cells that co-expressed both CD14 and CD80 might have been in a transient phase between macrophage and dendritic cell-like morphologies.

Discussion

Hemorrhagic fever with renal syndrome and HPS are both thought to be mediated by immunopathological mechanisms[1,3,6]. The selective induction of cytokine and chemokine synthesis has been implicated as a common contributing factor in the pathology of many diseases, including acute infectious diseases[4–6]. The myelomonocytic THP-1 cell line has been used extensively as a model for primary monocytes in biocompatibility research. The lipopolysaccharide (LPS)-induced transcriptional response in the THP-1 cell line is very similar to primary peripheral blood mononuclear cell (PBMC)-derived macrophages. Therefore, THP-1 cells represent a good model system for studying the mechanisms of LPS and NF-$\varkappa$B-dependent gene expression[16]. In contrast, there are some reports that show differences in immunoreactions induced by certain microorganisms in these two cell types. For example, *Mycoplasma fermentans*-derived high-molecular-weight material-stimulated induction of cytokine mRNA expression is accompanied by different proto-oncogene responses in primary monocytes/macrophages and THP-1 cells, which may represent different regulatory pathways of the two cell systems[17]. Likewise, in a study in which the responses of monocytes and THP-1 cells to some dental materials were compared by measuring TNF-? secretion, it was found that the monocytes were 5–10 times less sensitive than the THP-1 cells to these materials[18]. To date, however, there has been no information reported with regard to the use of THP-1 cells as models for primary monocytes in hantavirus research.

In this study we sought to measure differences in cytokine/chemokine profiles elicited by HFRS or HPS-causing hantaviruses in THP-1 cell line and primary monocytes/macrophages. When we infected human monocytes with various hantaviruses, we did not find any noticeable differences in the cytokine/chemokine produced. However, a striking finding was that all of the hantaviruses showed similar profiles in primary monocytes and also in THP-1 cells, but the profiles displayed differed between the cell types. That is, in primary monocytes, the hantaviruses elicited proinflammatory cytokines and β-chemokines, but in infected monomyelocytic THP-1 cells they produced only low amounts of β-chemokines.

Interestingly, when we measured gene expression of mRNA in the THP-1 cells, we found that cells infected with SNV had decreased levels of mRNA expression of IL-1Ra, which is reported to serve an important role in regulation of the potentially injurious effects of IL-1. In other studies, there are examples in which increases in circulating IL-1Ra have been detected during viral or bacterial infections[27]. For example, the rapid induction of IL-1Ra by LPS, IL-1 or IL-6 suggests that IL-1Ra acts as an acute phase protein[27]. In our studies, we did not detect IL-1β or IL-6 or their mRNAs in infected THP-1 cells. However, in THP-1 cells infected with the HFRS-causing virus, HTNV, the mRNA level of IL-1Ra was not down-regulated as it was for the HPS-causing virus, SNV. In a recent clinical study it was found that non-carriage of the IL-1Ra allele 2 and IL-1β allele 2 might contribute to susceptibility to HFRS caused by PUUV[28]. Whether or not the down-regulation of IL-1Ra in THP-1 cells induced by SNV is similarly related to an increased susceptibility to infection with SNV has yet to be determined.

Another intriguing finding appeared when we measured chemokine receptors. We were not surprised to find increased expression of CCR1 and CCR5 in infected primary monocytes, as CCR1 and CCR5 are receptors for β-chemokines. We assumed that β-chemokines produced by primary monocytes infected with hantaviruses would have autocrine as well as paracrine effects. Surprisingly, however, we found that hantavirus-infected THP-1 cells displayed receptors for CXC chemokines. Our results showing differences in the biological responses of primary human monocytes and THP-1 cells to β-chemokines, suggest that there is a lack of subsequent responses in THP-1 cells, which indicates some missing link in the downstream signaling transduction pathways[29]. Our data suggest that THP-1 cells are not ideal for *in vitro* research of the immunopathogenesis of hantaviruses in humans, at least for cytokine/chemokine research. However, we believe that THP-1 cells may be useful for studying more general immune mechanisms of hantaviral infections. Further parallel analysis of downstream signaling pathways in primary monocytes and THP-1 cells infected with hantaviruses may yield interesting results.

Unexpectedly, in our study, we observed that monocytes/macrophages infected with hantaviruses differentiated toward dendritic-like cells. It is known that hantaviruses may infect dendritic cells[30], but the differentiation of monocytes/macrophages infected with hantaviruses to dendritic-like cells was not previously described. Although we found more striking differences in cytokine/chemokine production in primary human monocytes than in THP-1 cells, both types of monocytes differentiated upon infection with hantaviruses. To substantiate our morphological observation of dendritic-like cells, we looked for expression of co-stimulatory molecules indicating dendritic cells by FACS analysis. Although such molecules were not detected with this method, we were able to detect their expression in infected primary human monocytes by IFA staining of fixed cells. Thus, we suspect that there were too few cells undergoing morphological changes to be measured by FACS analysis of the molecules.

Viruses alone are not known to induce monocyte differentiation toward dendritic cells. Earlier studies demonstrated that HTNV can infect and activate immature

dendritic cells, resulting in up-regulation of major histocompatibility complex (MHC), co-stimulatory and adhesion molecules[30]. Although we do not have definitive proof, our results suggest that hantaviruses may induce monocyte differentiation toward dendritic cell-like morphology; however, the low number of dendritic-like cells that we observed may indicate that only certain monocyte subsets may acquire dendritic cell-like characteristics after infection with hantaviruses. Further study is needed to confirm this hypothesis.

Our results suggest many possible roles for cytokines/chemokines in HFRS/HPS immunopathogenesis and point to several intriguing possibilities for the interaction of hantaviruses and macrophages and their possible differentiation to dendritic cell-like cells. However, the generation of immunological data in cell culture or even animal models, might not be an accurate reflection of immune responses that occur in a targeted organ of a patient. Therefore, our *in vitro* studies will need to be confirmed in future clinical studies as well as additional *in vitro* studies.

Acknowledgements

These studies were performed while Dr. A. Markotić held a National Research Council fellowship at USAMRIID. We thank to Dr Ljiljana Cebalo on useful suggestions and comments. The opinions, interpretations, conclusions, and recommendations contained herein are those of the authors and are not necessarily endorsed by the U.S. Army.

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PATOGENI HANTAVIRUSI UZROKUJU RAZLIČITE IMUNOREAKCIJE U THP-1 STANICAMA I PRIMARNIM HUMANIM MONOCITIMA I INDUCIRAJU DIFERENCIJACIJU MONOCITA U STANICE SLIČNE DENDRITIČKIM STANICAMA

S A Ž E T A K

Hantavirusi uzrokuju dvije važne bolesti u ljudi, hemoragijsku vrućicu s bubrežnim sindromom (HVBS) i hantavirusni plućni sindrom (HPS). Za oba sindroma se pretpostavlja da su imunološki posredovani. Monociti/makrofagi su vjerojatno izvor i ciljne stanice za citokine/kemokine. THP-1 stanice se često koriste kao model za primarne monocite u biokompatibilnim istraživanjima. Cilj naše studije je istražiti mogu li hantavirusi inducirati iste imunoreakcije u THP-1 stanicama i primarnim monocitima/makrofagima i mogu li se koristiti za istraživanje imunoreakcija na hantaviruse. U tom cilju smo komparirali indukciju različitih citokina/kemokina i njihovih receptora u THP-1 stanicama i primarnim monocitima/makrofagima. Inficirani primarni monociti/makrofagi induciraju većinom β-kemokine i njihove receptore. Naprotiv, u THP-1 stanicama nalazimo ekspresiju CXC kemokina. Iznenađujuća je bila promjena u morfologiji inficiranih stanica koje su poprimile oblik stanica sličnih dendritičkim stanicama, te povisile ekspresiju kostimulatornih molekula: CD40, CD80, CD83 and CD86. Naša istraživanja pokazuju da THP-1 stanice nisu idealne za *in vitro* itraživanja imunopatogeneze hantavirusa u ljudi. Nadalje, naše studije potvrđuju važnu ulogu citokina i kemokina u imunopatogeneze HVBS/HPS i upućuju na intrigantnu mogućnost diferencijacije inficiranih makrofaga prema stanicama sličnim dendritičkim stanicama.