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Impact of equine herpesvirus type 1 (EHV-1) infection on the migration of monocytic cells through equine nasal mucosa

Hossein Bannazadeh Baghi, Hans J. Nauwynck*

Laboratory of Virology, Department of Virology, Parasitology and Immunology, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, B-9820 Merelbeke, Belgium

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ABSTRACT

The mucosal surfaces are important sites of entry for a majority of pathogens, and viruses in particular. The migration of antigen presenting cells (APCs) from the apical side of the mucosal epithelium to the lymph node is a key event in the development of mucosal immunity during viral infections. However, the mechanism by which viruses utilize the transmigration of these cells to invade the mucosa is largely unexplored. Here, we establish an *ex vivo* explant model of monocytic cell transmigration across the nasal mucosal epithelium and lamina propria. Equine nasal mucosal $CD172a^{+}$ cells (nm$CD172a^{+}$ cells), blood-derived monocytes and monocyte-derived DCs (moDCs) were labeled with a fluorescent dye and transferred to the apical part of a polarized mucosal explant. Confocal imaging was used to monitor the migration patterns of monocytic cells and the effect of equine herpesvirus type 1 (EHV-1) on their transmigration. We observed that 16–26% of mock-inoculated nm$CD172a^{+}$ cells and moDCs moved into the nasal epithelia, and 1–7% moved further in the lamina propria. The migration of EHV-1 inoculated monocytic cells was not increased in these tissues compared to the mock-inoculated monocytic cells. Immediate early protein positive (IEP^{+}) cells were observed beneath the basement membrane (BM) 48 hours post addition (hpa) of moDCs and nm$CD172a^{+}$ cells, but not blood-derived monocytes. Together, our finding demonstrate that monocytic cells may become infected with EHV-1 in the respiratory mucosa and transport the virus from the apical side of the epithelium to the lamina propria en route to the lymph and blood circulation.

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1. Introduction

Nasal mucosal tissue is heavily populated with immune cells, which are continuously bombarded with a flow of foreign antigens and environmental microorganisms. Professional antigen presenting cells (APCs), such as dendritic cells (DCs), monocytes/macrophages and B lymphocytes, play a crucial role in the recognition of invading pathogens and the initiation of mucosal immune responses [1,2]. To induce an appropriate immune response, these immune cells must migrate from the apical surface of epithelia through the mucosa to the blood and lymph circulation. Important elements affecting immune cell distribution, migration and function are cell-cell and cell-fiber interactions via adhesion molecules and chemokine receptors and their response to chemokine gradients [3–5]. The migration of APCs in response to viral infection in mucosal tissue and the navigation of infected cells to lymph nodes or blood vessels are driven by a complex network of cell signals and interactions. After the virus is exposed to the mucosal epithelium of the upper respiratory tract (URT), epithelial cells immediately recognize viruses via pattern

* Corresponding author.
E-mail address: hans.nauwynck@ugent.be (H.J. Nauwynck).

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recognition receptors (PRRs) such as toll-like receptors (TLRs) and intracellular viral sensors [6–8]. Cytokines and chemokines, which orchestrate the migration of blood mononuclear cells or mucosal APCs (m-APCs), are also produced during viral infection in the airways [9–11].

Alphaherpesviruses use m-APCs to invade their host [12]. Equine herpesvirus type 1 (EHV-1) is an example of an invasive virus of the airway mucosa. The mucosa of the URT is the primary replication site for EHV-1, similar to other alphaherpesviruses [13–16]. After initial replication in the epithelial cells, EHV-1 can disseminate through the basement membrane (BM) barrier via a single infected mononuclear immune cell, which then progresses to the blood vessels of the lamina propria and the draining lymph nodes. The virus is detectable in infected m-APCs in equine respiratory explants around submucosal venules at 12 hours post inoculation (hpi) [13,15,16]. These data suggest that infection with the virus may direct the migration of the m-APCs toward the lymph nodes or blood vessels. However, it is not clear whether EHV-1 stimulates the migration of these mucosal monocytic cells upon infection or just follows the normal migration of the cells.

In the present study, different types of monocytic cells (blood-derived monocytes, moDCs and nmCD172a+ cells) were placed on top of a polarized nasal mucosal explant and their migration through the mucosa was followed over time. The impact of EHV-1 infection on this transmigration was examined.

2. Materials and methods

2.1. Collection of tissues and cells

Six horses were used in this study, and each horse constituted an experimental unit. For each experiment blood and nasal samples were collected from the same horse. The horses were of both genders and different ages (3–7 years old). The samples were collected at the slaughterhouse and processed as previously described, with minor modification [17]. In brief, mucosal tissue from the deep intranasal region of the septum was collected and cut into two parts: one was transported to the laboratory in phosphate-buffered saline (PBS), supplemented with 1 μg/ml gentamicin (Gibco), 1 mg/ml streptomycin (Certa), 1000 U/ml penicillin (Continental Pharma), 5 μg/ml fungizone (Bristol-Myers Squibb) and 10% fetal calf serum and the other was transported in the same medium, but without fetal calf serum. To avoid spontaneous migration of DCs, the tissues were kept on ice during transport. The tissue that was transported without serum was cut into small pieces (approximately 50 mm^2) for nasal explant culture. The other sample was used to isolate nmCD172a+ cells.

2.2. Isolation and purification of nmCD172a+ cells

The nasal tissue that was transported in medium containing 10% fetal calf serum was chopped into 3 mm^2 pieces and transferred into medium supplemented with 10 mM EDTA (VWR BDH Prolabo). The sample was incubated at 37 °C for 30 min with shaking at 250 rpm. Afterwards, the nasal tissue was transferred into medium containing 30 μg/ml DNase I (Sigma-Aldrich) and collagenase type IV (220 U/ml, Gibco, USA) and incubated for 2 h at 37 °C on a shaking platform (Unimax 2010, Heidolph Brinkmann, Germany). The digested cell population was layered over a Ficoll-Paque™ PLUS gradient (density 1.077; GE Healthcare, Life Sciences), and the interface cell layer was collected. Next, single cell populations were stained with mouse monoclonal anti-CD172a (VMRD, clone DH59B, 1:100, IgG1), and the positive cells were selected by performing MACS with anti-mouse IgG magnetic beads (Miltenyi Biotech Ltd). Immunophenotyping of equine nasal mucosal CD172a+ cells (nmCD172a+ cells) was performed as previously described [17]. The sections were incubated with mouse monoclonal anti-CD172a (VMRD, clone DH59B, 1:100, IgG1), followed by FITC-labeled goat anti-mouse IgG1 (Immunotech) secondary antibody. The cells were analyzed using a FACS Canto flow cytometer (Becton Dickinson Immunocytometry systems). This methodology yielded >90% pure nmCD172a+ cells with >85% viability. Purified nmCD172a+ cells were cultured in 6-well plates.

2.3. Monocyte isolation and DC generation

Equine blood-derived monocytes and monocyte-derived dendritic cells were prepared as previously described, with minor modifications [18]. Briefly, monocytes were isolated from 50 ml heparinized blood (100 μl heparin). The blood was diluted 1:1 with cold calcium/magnesium-free phosphate–buffered saline (CMF-PBS), layered onto a Ficoll-Paque™ PLUS gradient (density 1.077; GE Healthcare, Life Sciences) and centrifuged at room temperature for 30 min at 2100 rpm. The PBMC interface was washed twice with phosphate–buffered saline and resuspended in RPMI-1640 containing 10% fetal calf serum (FCS), 1 μg/ml gentamycin, 0.1 mg/ml streptomycin and 100 U/ml penicillin. Cells were cultured at 37 °C in 5% CO_2 for 1–2 h. Non-adherent cells were removed from the plate, and adherent cells were maintained in RPMI-1640 medium containing 10% FCS, 1 μg/ml gentamycin, 0.1 mg/ml streptomycin and 100 U/ml penicillin for 24 h. The moDCs were obtained by adding recombinant equine GM-CSF (Kingfisher Biotech, USA) and IL-4 (R&D Systems, UK) at concentrations of 20 ng/ml and 10 ng/ml, respectively. All cells were collected using accutase detachment solution (Sigma-Aldrich) and counted, and cell viability was assessed by flow cytometry using propidium iodide.

2.4. Monocyte, moDC and nmCD172a+ cell labeling (cell tracker™ CFSE)

All prepared cells were resuspended in 1 ml Dulbecco's phosphate-buffered saline (D-PBS) with 0.1% FCS, and the final cell concentration was brought to 4×10^5 cells/ml and incubated at 37 °C. Carboxyfluorescein diacetate succinimidyl ester (CFSE; Molecular Probes, Eugene, OR) was reconstituted in dimethylsulfoxide (DMSO; BDH, Toronto) just prior to use. Two microliters of 5 mM CFSE solution per milliliter of cells was added to obtain a final working concentration of 10 μM. Cells were incubated for 10 min at 37 °C, followed by an additional 5 min at 4 °C.

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