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Mumps virus inhibits migration of primary human macrophages toward a chemokine gradient through a TNF-alpha dependent mechanism

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ABSTRACT

Macrophages are an important cell type for regulation of immunity, and can play key roles in virus pathogenesis. Here we address the effect of infection of primary human macrophages with the related paramyxoviruses Parainfluenza virus 5 (PIV5) and Mumps virus (MuV). Monocyte-derived macrophages infected with PIV5 or MuV showed very little cytopathic effect, but were found to be defective in migration toward a gradient of chemokines such as macrophage colony stimulating factor (MCSF) and vascular endothelial growth factor (VEGF). For MuV infection, the inhibition of migration required live virus infection, but was not caused by a loss of chemokine receptors on the surface of infected cells. MuV-mediated inhibition of macrophage chemotaxis was through a soluble factor released from infected cells. MuV infection enhanced secretion of TNF-α, but not macrophage inhibitory factor (MIF). Antibody inhibition and add-back experiments demonstrated that TNF-α was both necessary and sufficient for MuV-mediate chemotaxis inhibition.

Introduction

Macrophages are an important cell type that plays a crucial role in modulating both innate and adaptive immune responses to viral infection (reviewed in Mosser and Edwards, 2008; Murray and Wynn, 2011). Upon detection of a virus, macrophages are recruited to the site of infection through the release of chemokines by epithelial cells and innate immune cells. Once localized to the site of infection, macrophages are able to phagocytose viruses or infected cell debris, process and present antigen, and migrate to lymph nodes to activate T cells and stimulate the adaptive immune response (Lehtonen et al., 2007). However, a fine regulation of macrophage trafficking is needed to avoid chronic inflammation, impaired tissue healing, and recurrent infection in a host (Frascaroli et al., 2009). Therefore, it is important that macrophages are readily able to move to the site of an infection, be retained in those tissues to combat a pathogen, and then egress out of the tissues to avoid overt damaging the host. In this study, we address the question of the impact of two related paramyxoviruses, Mumps Virus (MuV) and Parainfluenza virus 5 (PIV5) on macrophage migration.

Macrophages are specialized lymphocytes whose functions include a number of important aspects in innate and adaptive responses to virus infection (Lehtonen et al., 2007). In some cases such as with influenza virus, the ability of macrophages to respond to virus has been shown to be crucial in determining the outcome of respiratory tract infection (Kim et al., 2008; Wijburg et al., 1997). Macrophages have been shown to express high levels of the co-stimulatory molecules CD80 and CD86 on their cell surface, a property which allows these cells to present antigen to lymphocytes (Mosser and Edwards, 2008; Murray and Wynn, 2011; Wijburg et al., 1997). In addition, macrophages respond to pathogens by secreting pro-inflammatory cytokines such as IL-6, IFN-β, RANTES, and TNF-α (Assuncao-Miranda et al., 2010; Mosser and Edwards, 2008), and these cytokines are able to act in both an autocrine and paracrine fashion to stimulate additional innate and adaptive immune responses. Here, we demonstrate that MuV infection of primary human macrophages induces the production of TNF-α, and this MuV-mediated production of TNF-α has a profound effect on macrophage function.

PIV5 is a prototype virus which has served as a model parainfluenza for the study of paramyxoviruses in general and the Rubulavirus family in particular (reviewed in Lamb and Parks, 2007). PIV5 is closely related to two other Rubulaviruses: MuV and Human Parainfluenza virus type 2. MuV is the causative agent of mumps in humans, a viral infection of children and adolescents characterized by swelling of the parotid glands (Hviid et al., 2008). In addition, MuV infection often results in secondary complications, including aseptic meningitis, deafness, sterility in males, and can be highly neurotropic (Rubin and Afzal, 2011). Although the introduction of the MuV vaccine has been successful

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in reducing infection in the general population, there have been increasing numbers of MuV infections reported in vaccinated populations. Available data support waning immunity as the mechanism behind these outbreaks, as opposed to immune escape (Rubin et al., 2011).

Given that macrophages rely heavily on chemotaxis to perform their functions for both innate and adaptive immune responses, we have tested the effects of PIV5 and MuV infection on macrophage motility. Using primary human macrophages, we demonstrate that infections with both PIV5 and MuV show minimal cytopathic effect (CPE), but infected macrophages have a significantly reduced ability to migrate toward chemoattractants. For MuV, TNF-α secreted during viral infection was found to be both necessary and sufficient to account for the defect in macrophage migration. Our results have implications for potential treatment of viral infections that are capable of exploiting normal host cell responses for their own benefit.

Results

PIV5 infection of human macrophages restricts migration toward chemokines

To determine the outcome of infection of primary human macrophages with PIV5, $CD14^+$ monocytes were isolated from human PBMCs and cultured for six days in the presence of MCSF. At that time, >90% of cells were found to be $CD14^+$, $CD11b^+$, and $CD64^+$, a characteristic marker profile of monocyte-derived macrophages (MDMs, Briggs et al., 2011). Macrophages were mock infected or infected with WT PIV5 that encoded GFP at an moi of 10 and then examined by microscopy at 24 h pi. As shown in Fig. 1A, PIV5 was capable of initiating a productive infection of human macrophages as evidenced by new gene expression. However, no progeny virus was detected (data not shown). Importantly, PIV5 infection did not show overt CPE compared to mock infected macrophages. To confirm the lack of CPE, macrophages from three donors were infected with WT PIV5-GFP at an moi of 10. At 24 h pi, an MTT assay was done to evaluate cell viability. As shown in Fig. 1B, infected macrophages from all three donors showed a slight reduction in cell proliferation, similar to that seen during infections of human epithelial cells in culture (Manuse and Parks, 2010; Wansley and Parks, 2002) and consistent with the finding that PIV5 slows the cell cycle (Lin and Lamb, 2000). Thus, in contrast to the dramatic cell death seen with WT PIV5 infection of human DC (Arimilli et al., 2006), infected primary human macrophages display little CPE.

We have previously shown that WT PIV5 is a poor inducer of proinflammatory cytokines in human DC and epithelial cells. To determine if cytokines were induced in infected macrophages, human MDMs were mock infected or infected with WT PIV5 at an moi of 10, and levels of IL-6 were assayed by ELISA at days 1, 3, and 5 pi. As shown in Fig. 1C, PIV5-infected macrophages produced only low levels of IL-6, in contrast to cells treated with the positive

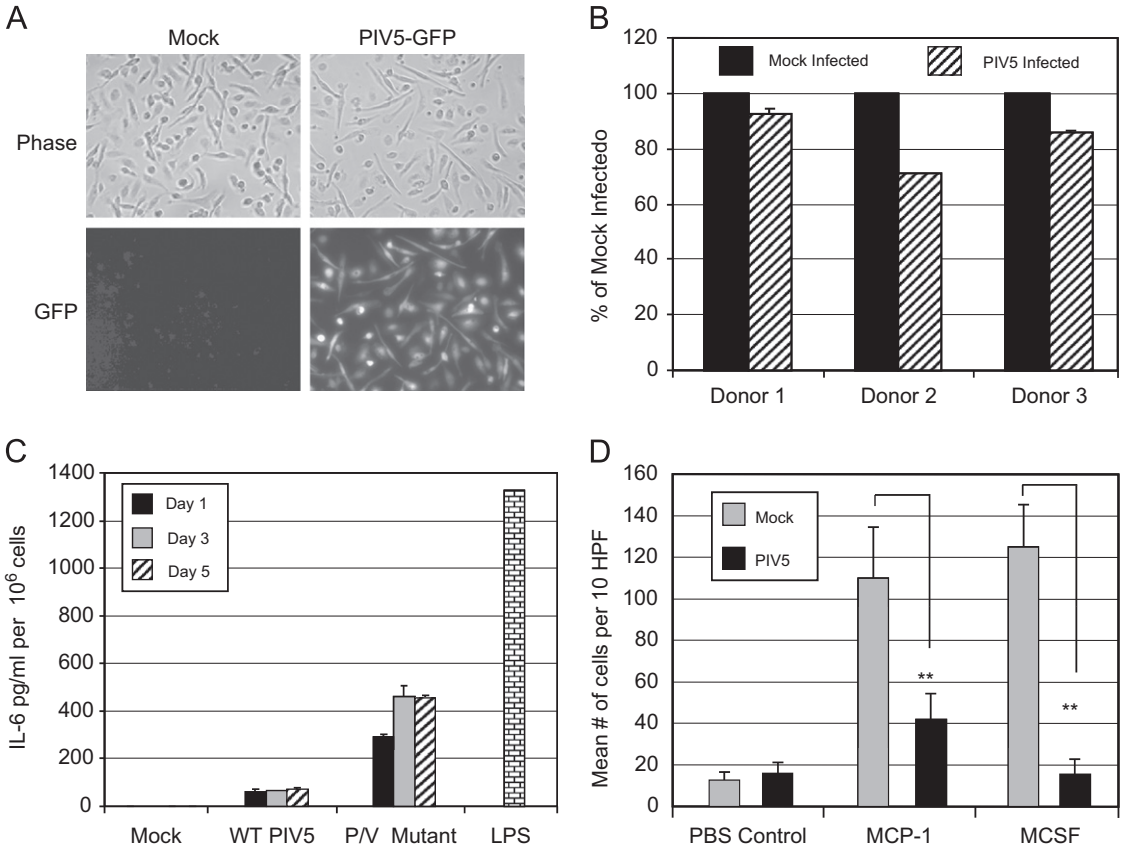

Fig. 1. *PIV5 infected primary human macrophages are inhibited in chemotaxis.* (A and B) Infection of primary human macrophages. Macrophages were mock infected or infected with WT PIV5-GFP at an moi of 10. At 24 h pi, cells were examined for GFP expression (panel A) or for viability using an MTT assay (panel B). (C) Cytokine production. Primary human macrophages were mock infected or infected at high moi with WT PIV5-GFP or the P/V-CPI- mutant. At the indicated days pi, media were assayed by ELISA for production of IL-6. Results are the mean values (with bars indicating standard deviation) for three samples from a representative donor. (D) Chemotaxis. Macrophages were mock infected (gray bars) or infected with WT PIV5-GFP at an moi of 10 (solid bars), and 24 h pi cells were used in a migration assay toward the chemokine MCP-1 or MCSF as described in Materials and methods. Results are expressed as the mean number of cells in 10 high power fields (with bars indicating standard deviation) for three samples from a representative donor. Data are representative of the results obtained from multiple experiments with cells from different donors. $^{**}p < 0.005$.