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HCMV infection of PDCs deviates the NK cell response into cytokine-producing cells unable to perform cytotoxicity ☆

Madeleine Cederarv[a], Cecilia Söderberg-Nauclér[a,*,1], Jenny Odeberg[b,c,1]

[a]*Department of Medicine, Centre for Molecular Medicine, L8:03, Karolinska Institutet, Karolinska University Hospital Solna, SE-171 76 Stockholm, Sweden*
[b]*Karolinska Institutet, Division of Neurodegeneration, Department of Neurobiology, Care Sciences and Society, Karolinska Institutet, Novum, SE-141 86 Stockholm, Sweden*
[c]*Stockholms Sjukhem-Foundation, Mariebergsgatan 22, SE-112 35 Stockholm, Sweden*

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Abstract

Plasmacytoid dendritic cells (PDCs) are thought to induce natural killer (NK) cell CD69 expression, cytotoxicity, and cytokine secretion. Since human cytomegalovirus (HCMV) interferes with multiple functions of infected cells, we investigated whether the HCMV infection of PDCs affects NK cell activation. Human PDCs infected with HCMV strain VR1814 at multiplicity of infection (MOI) 10 or stimulated with control CpG-A were cocultured with human NK cells in an autologous system. As expected, CpG-stimulation of PDCs increased expression of the NK cell activation marker CD69, enhanced cytotoxicity and stimulated secretion of tumor necrosis factor (TNF)-α and IFN-α, but not IFN-γ, and induced NK cell migration. In contrast, incubation with HCMV-infected PDCs induced CD69 expression, migration and elevated production of both TNF-α and IFN-γ by NK cells, but these cells did not exhibit enhanced cytotoxicity. Also, HCMV-infected PDCs were unable to induce increased intracellular perforin levels. Thus, HCMV infection of PDCs induce NK cells to increase CD69 expression and produce inflammatory cytokines, but infected PDCs are unable to induce NK cell cytotoxicity. This NK cell phenotype with impaired killing abilities, but enhanced production of inflammatory cytokines may instead facilitate reactivation and replication of HCMV. This data indicate that HCMV can target PDCs through novel dual strategies that may result in evasion of the innate immune response at the same time as facilitating virus reactivation and replication early in the infection, through enhanced inflammation.

Keywords: Cell activation; Dendritic cells; Natural killer cells; Viral infection

Abbreviations: PDC, plasmacytoid dendritic cell; HCMV, human cytomegalovirus; MOI, multiplicity of infection; MDC, myeloid dendritic cell; MoDC, monocyte-derived dendritic cell; MDM, monocyte-derived macrophages.

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*Corresponding author. Tel.: +46 8 51779844; fax: +46 8 313147.
E-mail addresses: madeleine.cederarv@ki.se (M. Cederarv), cecilia.naucler@ki.se (C. Söderberg-Nauclér), Jenny.Odeberg@ki.se (J. Odeberg).
[1]Shared senior authorship.

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Introduction

As professional antigen-presenting cells that express lymphocyte co-stimulatory molecules and secrete cytokines, dendritic cells (DCs) are important regulators of the immune system. DCs can prime both innate and adaptive immune responses and may be involved in inducing self-tolerance (Banchereau and Steinman, 1998; Granucci et al., 2004). The two DC subtypes in humans, plasmacytoid DCs (PDCs), and myeloid DC (MDCs), differ in their functional properties (Gerosa et al., 2002). MDCs primarily prime naive T cells, whereas PDCs, through their ability to secrete large amounts of cytokines and chemokines such as interferon (IFN)-α and tumor necrosis factor (TNF)-α, are an important part of the innate immune response to viruses (Zuniga et al., 2004).

Several reports describe the bidirectional cross-talk between DCs and natural killer (NK) cells (Gerosa et al., 2002; Moretta, 2002; Walzer et al., 2005). Activated NK cells can kill autologous immature monocyte-derived DCs (MoDCs), but mature MoDCs seem to be protected against NK cell lysis (Ferlazzo et al., 2002; Pietra et al., 2003). Depending on the maturation state and DC:NK cell ratio, NK cells can also induce MoDC maturation (Gerosa et al., 2002). Both MoDCs and MDCs are believed to trigger NK cell functions, such as cytotoxicity, CD69 expression, migration, and IFN-γ secretion (Ferlazzo et al., 2002; Gerosa et al., 2002; Megjugorac et al., 2004). Several studies have lately described the importance of NK cell cross-talk with PDCs and the important function of PDCs to prime NK cells (Gerosa et al., 2005; Hanabuchi et al., 2006a; Marshall et al., 2006; Romagnani et al., 2005). In response to PDCs activated with Toll-like receptor 9 ligands, autologous NK cells express CD69 and induce their cytotoxicity but secrete low or undetectable levels of IFN-γ and interleukin (IL)-10 (Hanabuchi et al., 2006b; Marshall et al., 2006; Romagnani et al., 2005).

Primary infection with human cytomegalovirus (HCMV), a common herpes virus, is followed by lifelong latency, and 60–100% of healthy adults are carriers of HCMV (Forbes, 1989). The virus can cause severe morbidity in immunocompromised hosts (e.g., transplant recipients and AIDS patients) and in fetuses (Soderberg-Naucler, 2006b). To co-exist with its hosts, HCMV has developed sophisticated immune evasion strategies (Soderberg-Naucler, 2006b). HCMV also affects a range of important DC functions after *in vitro* infection of MoDCs, such as reduced expression of MHC class I and II molecules, inhibited maturation, impaired migration in response to chemokines, and impaired antigen presentation to T cells (Grigoleit et al., 2002; Moutaftsi et al., 2002; Varani et al., 2005). Recently, we found that HCMV-activated PDCs, by interaction with Toll-like receptor 9, release large amounts of TNF-α, as well as induce expression of the activation marker CD83, while the expression of HLA-DR, CD80, and CD86 was not significantly affected. HCMV-infected PDCs could trigger B cell activation and proliferation and, in the presence of T cells, induce B cell antibody production (Varani et al., 2007).

HCMV has several mechanisms for avoiding recognition and elimination by NK cells. The virus affects both the expression of molecules that trigger or inhibit NK cell activation (reviewed in Loenen et al., 2001; Soderberg-Naucler, 2006b). Further, infected cells appear to be resistant to the action of cytolytic proteins (Odeberg et al., 2003). In addition, two HLA class I homologues encoded by HCMV are believed to deliver inhibitory signals to NK cells (Onno et al., 2000; Reyburn et al., 1997; Wills et al., 2005).

Since NK cells and PDCs are crucial for combating viral infections, we investigated whether HCMV infection of PDCs influences the PDC–NK cell cross-talk and the ability of infected PDCs to activate NK cells. We show that CpG-A-stimulated PDCs as expected induce NK cell CD69 expression, perforin production, migration, and cytotoxicity, but not IFN-γ secretion. In contrast, coculture of NK cells with HCMV-infected PDCs, enhances CD69 expression and induce secretion of IFN-γ and TNF-α, but prevents enhanced cytotoxicity, possibly by prohibiting induction of intracellular perforin levels.

Materials and methods

Cell isolation and coculture

Peripheral blood mononuclear cells (PBMCs) from healthy human donors (Karolinska University Hospital) were obtained after separation by Lymphoprep (Axis-Shield PoC AS) as described (Soderberg et al., 1993). NK cells were isolated by negative magnetic separation with an NK cell negative isolation kit (Invitrogen). NK cell purity was >90%, as assessed by flow cytometry (Becton Dickinson FACSCalibur). PDCs were isolated by positive magnetic separation with a BDCA-4 cell isolation kit (Miltenyi Biotech). The purity was ⩾90%. The cells (1×10^6 cells/ml) were cultured in RPMI 1640 (Invitrogen) supplemented with 10% fetal calf serum, 100 U/ml penicillin, and 100 μg/ml streptomycin at 37 °C in a 5% CO_2 atmosphere.

Equal numbers of NK cells and PDCs (total 1×10^6 cells/ml) were cocultured in RPMI 1640 supplemented with 10% fetal calf serum, 100 U/ml penicillin, and 100 μg/ml streptomycin in round-bottom cell-culture tubes at 37 °C in a 5% CO_2 atmosphere. After 24 h, cells were collected for flow cytometry or cytotoxicity assay; supernatants were cleared by centrifugation and frozen