Orthopoxvirus infection does not induce maturation of mouse FLT3L-derived dendritic cells

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INTRODUCTION

Ectromelia virus (ECTV) and vaccinia virus (VACV) are DNA viruses, that belong to the genus *Orthopoxvirus* from the *Poxviridae* family. The genus *Orthopoxvirus* also includes the variola virus (VARV), which causes smallpox in humans. ECTV is the causative agent of mousepox, while VACV was used to vaccinate Edward Jenner against smallpox in the 18th century. Both viruses provide a suitable model for studying the immunobiology of orthopoxviruses. Dendritic cells (DCs) are professional antigen-presenting cells (APCs) whose function is to control the pericellular environment, capture antigens, and present them to T lymphocytes in secondary lymphoid organs. DCs as APCs determine the induction of the immune response as well as being involved in the induction of immune tolerance. DCs are divided into two main populations - conventional DCs (cDCs), which are mainly involved in antigen presentation, and plasmacytoid DCs (pDCs), which have a relatively minor role as APCs and are mainly involved in the production of type I interferons (IFN-I). DCs, upon recognition of the infectious agent, undergo a maturation process that is necessary for the stimulation of the immune response. During maturation, expression of major histocompatibility complex class II (MHC II), costimulatory molecules (CD) such as CD80, CD 86, and chemokine receptors increase on the surface of DCs, which enhances DC migration to lymphoid organs. **Since DC maturation underlies the immunoregulatory functions of DCs, we evaluated how ECTV and VACV infection affect the maturation of FLT3L-derived dendritic cells.**

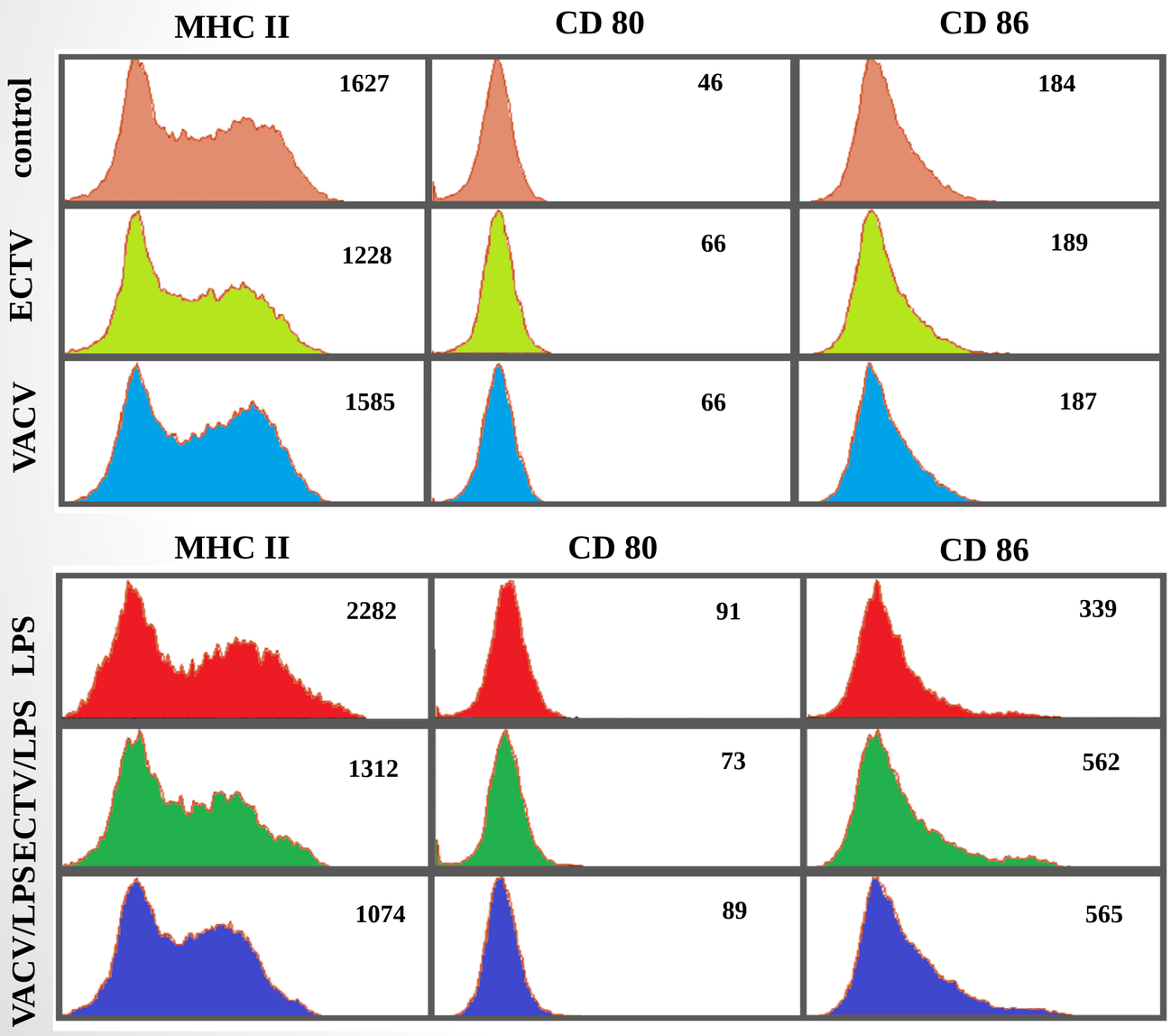

Figure 1. ECTV and VACV infection inhibits the maturation of DCs. Representative histograms showing the major histocompatibility complex class II (MHC II), CD80, and CD86 expression on control, ECTV-infected or VACV-infected DCs, untreated or treated with lipopolysaccharide (LPS) at 18 hpi. Numbers represent of mean fluorescence intensity (MFI) value for a given marker.

RESULTS

Our results show that DCs ($CD11c^+$, $XCR1^+$, $Sirp1\alpha^+$, $CD11b^+$) were unable to fully mature after infection with ECTV and VACV. For this purpose, the levels of MHC II molecules and CD80 and CD86 were analyzed on control, ECTV-infected or VACV-infected DCs, untreated or treated with lipopolysaccharide (LPS) at 18 hpi.

In ECTV-infected or VACV-infected cells, the MFI for MHC II decreased and the MFI for CD86 and CD80 did not change significantly compared to uninfected control cells (**Fig. 1, Fig 2.**). Treatment of uninfected cells with LPS for 18 hours induced cell maturation, as the MFI for all analyzed markers increased significantly on the surface of DCs compared to control cells cells (**Fig. 1, Fig 2.**). ECTV-infected and VACV-infected cells, stimulated with LPS were not able to increase the expression of MHC II molecules to the levels observed in cells treated with only LPS, while CD80 expression did not change significantly and CD86 expression increased compared to cells treated with only LPS (**Fig. 1, Fig. 2**). **In summary, DCs after ECTV and VACV infection are unable to fully mature.**

METHODS

For this purpose, primary cultures of mouse tyrosine-protein kinase ligand (FLT3L)-derived dendritic cells (DCs) were used in the experiments properly. FLT3L controls DC development and is particularly important for pDCs and cDCs (cDC1 and cDC2). Cells were infected with ECTV strain Moscow and VACV strain Western Reserve at MOI=1 and stimulated with LPS as a control for cell maturity. At 18 hours of infection (hpi), cytometric analysis was performed to determine cell maturity. DCs were stained with the following mAb: anti-MHC II-PerCP-Cy5.5, anti-CD80-APC, anti-CD86-BV711, anti-CD11c-BV421, anti-CD11b-BV605, anti-XCR1-BV786 and anti-Sirp1α-PE.

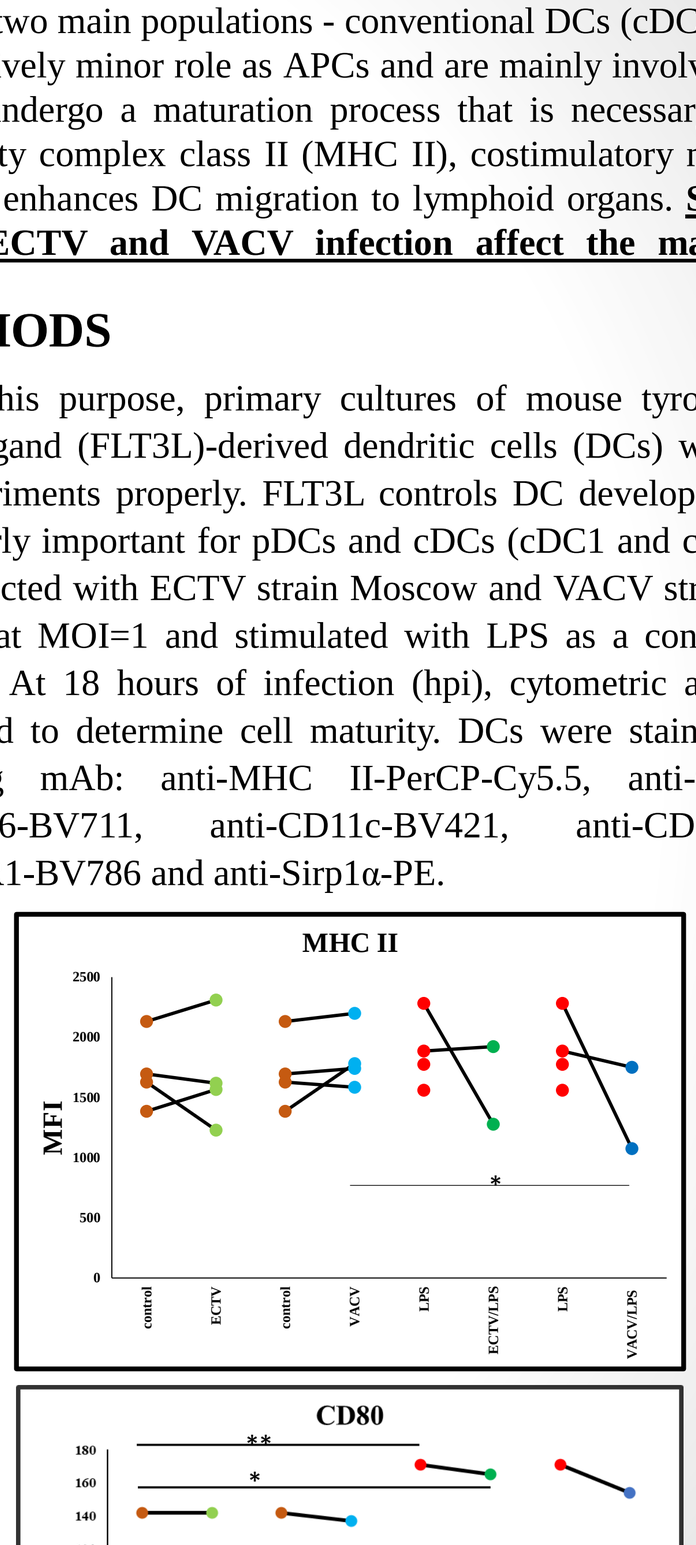

Figure 2. Graphs show individual data of MFI of MHC II, CD80, and CD86 molecules from four independent experiments. Statistical significance were indicated by horizontal bars between two sets of data (* $p<0.5$, ** $p<0.005$).

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