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Rapid Communication

Plasmacytoid dendritic cells are present in cervical carcinoma and become activated by Human Papillomavirus type 16 virus-like particles

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Abstract

Objectives. Plasmacytoid dendritic cells (PDC) play an important role in the innate immune response to viral infections through the secretion of high levels of IFNα. We investigated whether PDC play a role in Human Papillomavirus (HPV) associated cervical carcinoma.

Methods. Frozen sections of 18 cervical carcinomas were analyzed for the presence of myeloid and plasmacytoid DC. To study whether the HPV virus can activate PDC, expression of putative VLP receptors (CD49f and CD16) was analyzed on PDC in peripheral blood mononuclear cells of healthy donors. Furthermore, CD83 induction and IFNα production by purified blood-derived PDC was measured after incubation with HPV 16 virus like particles (VLP).

Results. PDC were detected in 83% of the CxCa cases, primarily in the stroma. PDC express one of the putative VLP receptors (CD49f). IFNα production but no CD83 expression was induced in PDC upon incubation with VLP.

Conclusion. Our data suggest that PDC, which are at hand locally in the cervix, play a role in the natural immune response against HPV and identify PDC as possible targets for VLP-based vaccines.

Keywords: Plasmacytoid dendritic cells; HPV; VLP; Cervical carcinoma

Introduction

The dendritic cell (DC) is the most potent antigen-presenting cell known to date and appears to be the only cell type capable of inducing primary T cell responses. DCs are considered to be the sentinels of the body and play a key role in delivering antigen from the periphery to the secondary lymphoid organs. After antigen uptake, DCs mature, co-stimulatory molecules (e.g., CD40, CD80, CD86) are up-regulated, and expression of CD83, a specific marker for mature DCs, is induced. Mature DC migrate to the lymphoid tissues where they activate effector T cells. Two types of myeloid, CD11c-positive DC have been described: interstitial DC found in peripheral tissues and the dermis and Langerhans cells (LC) found in epithelia. In vitro, myeloid DCs are generated from monocytic $CD14^+$ precursors. Monocytes differentiate into $CD1a^+$ DC (MoDC) after culture for 1 week in medium containing GM-CSF and IL-4 (reviewed in Ref. [1]). A third type is the CD11c-negative plasmacytoid DC (PDC) found in lymphoid organs and abnormal skin [2–4]. CD1a and Langerin are typical LC markers, although CD1a-positive cells are frequently observed in the dermis as well. PDC are characterized by high expression of CD123, and expression of the blood DC markers BDCA2 and BDCA4 [5]. Myeloid DC and PDC differentially express toll-like receptors (TLR). TLR are innate immune receptors that recognize conserved motifs on microbes and induce inflammatory signals. TLR4 is a receptor for LPS and is expressed on myeloid DC, while

Abbreviations: CIN, cervical intraepithelial neoplasia; CxCa, cervical cancer; DC, dendritic cell; GM-CSF, Granulocyte Macrophage-colony stimulating factor; HPV, Human Papillomavirus; IFN, interferon; LC, Langerhans cell; LPS, lipopolysaccharide; MoDC, monocyte derived dendritic cell; PDC, plasmacytoid dendritic cell; TLR, Toll-like receptor; ΔVLP, heat-inactivated virus-like particle; VLP, virus-like particle.

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PDC express TLR7 and TLR9 [6]. Upon triggering of PDC expressed TLR7 by imiquimod and TLR9 by bacterial DNA, high levels of IFNα are produced. Recognition of bacterial DNA is based on the presence of unmethylated CG dinucleotides in particular sequence contexts (CpG motifs). PDC are susceptible to stimulation by synthetic oligodeoxynucleotides that contain such CpG motifs (CpG ODN) through TLR 9. PDC produce high levels of IFNα upon viral interaction, which has direct anti-viral effects and enhances cytotoxic effects of macrophages and natural killer cells. PDC infected by influenza virus stimulate allogeneic T cells [7] and influenza-specific T cells [8].

Cervical cancer (CxCa) and its precursor lesions cervical intraepithelial neoplasia (CIN) are associated with high risk or oncogenic Human Papillomavirus (HPV) infection [9]. HPV capsid protein derived virus like particles (VLP) induce myeloid DC activation [10], indicating that the virus can induce maturation and migration of local myeloid DC during a productive infection. Since PDC are activated upon interaction with viruses such as the influenza and herpes simplex viruses, we asked whether HPV can also activate PDC. Furthermore, local administration of imiquimod has been shown to result in an IFNα-mediated regression of CxCa precursor lesions but serious side effects prohibited further use of this treatment [11]. However, because this effect is IFNα-mediated, which is an important molecule for the activation of several immune cells, it is important for the development of immunotherapeutical strategies against HPV-induced CxCa to further elucidate the mechanism behind the induction of regression of CxCa precursor lesions. Since TLR7 is expressed on human PDC [6], we wondered whether local production of IFNα by PDC might play a role in the clinical effects of imiquimod. The aim of this study is therefore to investigate whether PDC are present in cervical carcinoma tissue and whether PDC are, like MDC, activated upon interaction with VLP.

Materials and methods

Analysis of DC subsets in cervical carcinoma tissue

Clinical samples were used according the guidelines provided by the ethical committee of the VU University Medical Center. Immunohistochemical analysis was done on freshly snap-frozen tumor samples. Acetone-fixed sections were pre-incubated with blocking serum (normal rabbit serum 1:50; Dako, A/S, Denmark). All reagents were diluted in PBS containing 1% bovine serum albumin (Roche Diagnostics, Mannheim, Germany). The following antibodies were used: BDCA-2 (clone AC144, murine IgG1, 1:10; Miltenyi Biotec, Bergisch Gladbach, Germany), CD123 (clone 9F5, murine IgG1, 1:25; Pharmingen, San Diego, CA), CD83 (clone HB15a, murine IgG2b, 1:25; Immunotech, Marseilles, France), CD1a (clone MTB1, murine IgG1, 1:25; Monosan, Uden, The Netherlands) and keratin markers (clones AE1/AE3, murine IgG1 1:100; Boehringer Mannheim, Almere, The Netherlands). For all samples, one slide was incubated with an isotype control antibody (ICN Biomedicals, Aurora, OH). After incubation with the primary antibody, slides were incubated with a biotinylated rabbit anti mouse Fab_2 fragment (1:150, Dako) and subsequently incubated with streptavidin–alkaline phosphatase (1:100; Dako). Bound alkaline phosphatase was visualized as described previously [12]. Finally, the slides were counterstained with hematoxylin and mounted. We observed CD123-positive endothelial cells but these could be discriminated from infiltrating PDC on the basis of morphology. In addition, CD123 is also known to be expressed by granulocytes but no CD123-positive cells with polymorphic nuclei were observed.

The preparation of a singe cell suspension from tumor samples was done as previously described [13]. Subsequent FACS analysis was done on the single cell suspension of cervical carcinoma tissue with the aforementioned FITC or PE conjugated antibodies as well as with Langerin (clone DCGM4, murine IgG1, CD107, Immunotech).

DC isolation and culture

Peripheral blood mononuclear cells were isolated from buffy coats of normal human volunteer donors by density centrifugation over Lymphoprep (Nycomed AS, Oslo, Norway). PDC were isolated (>90% pure as shown by BDCA2 and CD123 double staining) using the $BDCA4^+$ cell MACS isolation kit (Miltenyi Biotec) according to the manufacturer's recommendations. Isolated $BDCA4^+$ cells were cultured at 3×10^5 to 5×10^5 cells per milliliter in Yssels medium [14] supplement with 2% human pooled serum and 10 ng/ml IL-3 (R&D Systems, Abingdon, Oxon, UK). MoDC of the same donor were generated as described previously [15].

Stimulation of DC

$BDCA4^+$ PDC or MoDC were incubated for 24 h with 10μg/ml intact or heat inactivated (10 min at 95°C [16]) HPV16VLP (a generous gift from John T. Schiller, NCI, Bethesda, MD) or with 3 μg/ml CpG2216 (a generous gift from Dr. A. Krieg, Coley Pharmaceutical Group, MA) as a positive control. Stimulation was done in the presence of 10 ng/ml IL-3 for the PDC and in the presence of 1000 U/ml IL-4 (Centraal Laboratorium van de bloedtransfusiedienst, Amsterdam, The Netherlands) together with 100 ng/ml GM-CSF (Schering-Plough, Madison, NJ) for the MoDC.

Flow cytometry

Cells were incubated at 4°C for 30 min in PBS with 0.1% BSA and 0.01% NaN_3, in the presence of appropriate dilutions of FITC- or PE-labeled isotype controls and mouse mAbs to BDCA-2 and BDCA-4 (Miltenyi Biotec), CD1a,