Brief report

Defect of plasmacytoid dendritic cells in warts, hypogammaglobulinemia, infections, myelokathexis (WHIM) syndrome patients

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Warts, hypogammaglobulinemia, infections, myelokathexis (WHIM) syndrome is a genetic disease that is caused by heterozygous mutations of the CXCR4 gene. These mutations confer an increased leukocyte response to the CXCR4-ligand CXCL12, resulting in abnormal homeostasis of many leukocyte types, including neutrophils and lymphocytes. Analysis of the myeloid and plasmacytoid dendritic cell blood counts in WHIM patients revealed a striking defect in the number of plasmacytoid dendritic cells as well as a partial reduction of the number of myeloid dendritic cells, compared with healthy subjects. Moreover, the production of interferon-α by mononuclear cells in response to herpes simplex infection, or after stimulation with the Toll-like receptor 9 ligand CpG, was undetectable in WHIM patients. Because plasmacytoid dendritic cells play a key role in the defense against viruses and their generation and motility are in part dependent on CXCR4, we hypothesized that the susceptibility of WHIM patients to warts is related to the abnormal homeostasis of plasmacytoid dendritic cells. (Blood. 2010;116(00):000-000)

Introduction

Warts, hypogammaglobulinemia, infections, myelokathexis (WHIM) syndrome is an autosomal dominant genetic disease that is characterized by severe/moderate neutropenia and leukopenia (despite the retention of mature neutrophils in the bone marrow, eg, myelokathexis), hypogammaglobulinemia, recurrent respiratory infections, and severe verruca, which are caused by common human papillomavirus (HPV) strains. WHIM is caused by heterozygous mutations in the CXCR4 gene. These mutations affect the intracellular signaling of the receptor in response to the ligand CXCL12.1-7 Because leukocytes that express CXCR4 have an increased chemotactic response to the CXCL12 ligand, these cells are retained in the bone marrow and lymphoid compartment, thereby reducing their blood counts.6-8 Although several studies have provided insight into the pathogenesis of WHIM syndrome, some clinical features of this genetic disorder remain unexplained. In particular, it is still unclear why WHIM patients develop verruca that affects both the hands and genitalia, which are extremely refractory to treatment.7,8

Recent studies suggest that plasmacytoid dendritic cells (pDCs) might have a role in protecting against HPV infection, as pDCs were present in the epidermis of patients diagnosed with chronic genital HPV infections. pDCs secrete the antiviral cytokine interferon-α (IFN-α) in response to stimulation with HPV virus-like particles9,10 and abnormalities in DC homeostasis have been implicated in various human diseases, including cancer, autoimmune disease, allergy, and infection.11-14 Because pDC generation and trafficking among tissues is regulated by CXCR4,15-17 we investigated the number of circulating pDCs and their ability to secrete IFN-α in WHIM patients.

Methods

Informed consent was obtained from all WHIM patients in accordance with the Declaration of Helsinki according to a protocol approved by the Hospital Ethical Committee (Spedali Civili, Brescia, Italy). Ethylenediaminetetraacetic acid-treated blood samples were collected from 5 WHIM patients (patients 1-5)8 and healthy subjects, who were age-matched with the patients. Immunophenotyping was performed by flow cytometry (FACScan, BD Biosciences) using the following antihuman antibodies (BD Biosciences): anti-CD14/anti-CD15/anti-CD19/anti-CD20/fluorescein isothiocyanate isothiocyanate (lineage cocktail), anti-CD1c-phycocerythrin, anti-CD4-peridinin chlorophyll protein, and anti–BDCA-2-allophycocyanin. The DC subsets were then identified and gated based on CD1c and BDCA-2 surface marker expression. Myeloid DCs were defined as CD4+CD1c+BDCA-2+ cells, and plasmacytoid DCS were defined as CD4+CD1c–BDCA-2+ cells.18

Heparin-treated blood samples were collected from patient 1, patient 2, patient 3, and the controls, and peripheral blood mononuclear cells (PBMCs) were isolated using Lympholyte H density gradient centrifugation.

Formalin-fixed, paraffin-embedded skin biopsies were obtained from a WHIM patient and 10 control cases. For immunohistochemical staining, primary antibodies to the following antigens were used: CD1a (mouse IgG1, clone 010, Dako Denmark), CD207/Langerin (mouse IgG1, clone 010, Dako Denmark), CD1c (mouse IgG1, clone 010, Dako Denmark), and anti-CD4-peridinin chlorophyll protein, and CD1a (mouse IgG1, clone 010, Dako Denmark), and anti-CD4-peridinin chlorophyll protein, and anti–BDCA-2-allophycocyanin. The DC subsets were then identified and gated based on CD1c and BDCA-2 surface marker expression. Myeloid DCs were defined as CD4+CD1c+BDCA-2+ cells, and plasmacytoid DCS were defined as CD4+CD1c–BDCA-2+ cells.18

A 2-tailed Mann-Whitney U test (nonparametric analysis) was used for statistical comparison of the patients to the healthy controls. A P value less than .05 was considered significant.
Results and discussion

To assess the effect of CXCR4 mutations on pDC homeostasis in WHIM patients, we performed a quantitative evaluation of the DC subpopulations (myeloid DCs [mDCs] and pDCs)\(^\text{20}\) in the peripheral blood of 24 healthy subjects and 5 WHIM patients bearing p.Arg334X or p.Gly336X mutations. As shown in Figure 1A, we observed a significant reduction in both DC subsets in the blood of WHIM patients compared with healthy subjects. In particular, the percentages of pDCs were markedly decreased in the patients (mean ± SE, 0.032% ± 0.016%; range, 0.01%-0.05%) compared with the healthy subjects (mean ± SE, 0.53% ± 0.278%; range, 0.22%-1.11%, \(P < 0.05\)). In addition, the numbers of mDCs were reduced in WHIM patients (mean ± SE, 0.2% ± 0.15%; range, 0.06%-0.42%), although to a lesser extent, compared with healthy subject levels (mean ± SE, 0.99% ± 0.466%; range, 0.37%-2.39%, \(P < 0.05\); supplemental Table 1, available on the Blood Web site; see the Supplemental Materials link at the top of the online article).

Because of the paucity of circulating mDCs, we have evaluated cytokine production in DCs derived in vitro from the monocytes isolated from blood of a WHIM patient and from a control subject. Because pDCs are the most potent secretors of IFN-\(\gamma\), they are essential for the antiviral response.\(^\text{22,23}\) To study the effect of the reduced pDC number in WHIM patients, we analyzed PBMC IFN-\(\gamma\) expression. We infected PBMCs derived from 2 WHIM patients and 2 control subjects with an increasing number of type 1 herpes simplex virus particles (HSV1, from 10 up to 10 000 pfu/mL). Analysis of the IFN-\(\gamma\) supernatant concentration after 24 hours of culture showed that, with the lowest number of HSV1 copies, cytokine release by healthy PBMCs was already detectable. In contrast, WHIM-PBMC cytokine production remained undetectable, even after infection with 10 000 pfu/mL of HSV1 (Figure 1B). We subsequently analyzed the WHIM-PBMC IFN-\(\gamma\) production levels in response to CpG (5\(\mu\)M), which stimulates pDCs by activating Toll-like receptor 9. Even under these experimental conditions, we observed a severe defect in IFN-\(\gamma\) production by WHIM-PBMCs compared with the healthy, control PBMCs (Figure 1C). These data suggest that WHIM patients have a severely impaired capacity to produce IFN-\(\gamma\) in response to Toll-like receptor 9 stimulation, which is probably the result of the reduction of the pDC count.

Next, we analyzed the dermal mononuclear infiltrate of HPV-associated Verruca vulgaris from one WHIM patient (Figure 2A-B) and from control subjects (supplemental Figure 2A). Dermal BDCA2\(^\text{+}\) pDCs were not detected in multistep sections of skin biopsies of the WHIM patient but were present at variable extent in warts from control subjects (Figure 2E-F; supplemental Figure 2B). Because IFN-\(\alpha\) secretion by pDC results in the expression of the antiviral protein MxA, skin biopsies were stained with anti-MxA monoclonal antibody. Remarkably, all control cases showed variable intraepithelial reactivity for MxA, whereas Verruca vulgaris from the WHIM patient was completely negative (Figure 2G-H; supplemental Figure 2C-D). In contrast, intraepidermal
Figure 2. DCs in Verruca vulgaris. Sections of Verruca vulgaris were obtained from a WHIM patient (A-D,F,H) and a control case (E,G) stained for hematoxylin and eosin (A, HPV; brown, B), CD1a (blue in C; and brown D), CD207 (brown, C), BDCA2 (brown, E-F), and MxA (brown, G-H). Verruca vulgaris from WHIM patient 4 showed a moderate dermal mononuclear infiltrate (A) and contained HPV-infected keratinocytes (B). By immunohistochemistry, intraepidermal CD1a showed a moderate dermal mononuclear infiltrate (A) and contained HPV-infected BDCA2 (brown, E-F), and MxA (brown, G-H). Verruca vulgaris from WHIM patient 4 completely negative in WHIM patient biopsy (H). Original magnifications are as observed in keratinocytes from a control biopsy (G and inset), whereas it was not seen in the patient biopsy (F). Strong reactivity for MxA was cases (E) but not seen in the patient biopsy (F). Strong reactivity for MxA was observed in keratinocytes from a control biopsy (G and inset), whereas it was completely negative in WHIM patient biopsy (H). Original magnifications are as follows: A,G,H, ×40 (scale bar represents 50 μm); B, ×600 (scale bar represents 20 μm); and C-F, inset in G, ×400 (scale bar represents 50 μm).

References


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Authorship

Contribution: L.T. performed the studies with the dendritic cells and wrote the manuscript; D.M. performed the flow cytometric analysis; W.V. and F.F. performed immunohistopathology studies; M.D.F. studied the response of cells to herpes infection; L.D.N., F.P., V.L., and A.P. were in charge of the patients’ follow-up; and R.B. supervised the project and helped write the manuscript.

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