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# Plasmacytoid dendritic cells and type I interferon in the immunological response against warts

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## Summary

**Background.** Plasmacytoid dendritic cells (pDCs) are the most potent producers of type I interferons (IFNs), and are involved in the pathogenesis of several cutaneous infectious (especially viral), inflammatory/autoimmune and neoplastic entities. Their role in the pathogenesis and regression of human papilloma virus (HPV)-induced skin lesions has not been well studied.

**Aim.** To investigate pDC occurrence and activity in HPV-induced skin lesions, including inflamed and uninflamed warts as well as epidermodysplasia verruciformis (EDV)-associated lesions.

**Methods.** In total 20 inflamed and 20 uninflamed HPV-induced skin lesions (including 7 EDV lesions) were retrieved from our database, and the tissue was immunohistochemically tested for pDC occurrence and activity using anti-BDCA-2 and anti-MxA antibodies, respectively.

**Results.** pDCs were present in all 20 inflamed warts and absent from all 20 uninflamed cases. MxA expression was also diffuse and strong in 75% (15/20) inflamed warts, but not in any of the uninflamed warts.

**Conclusions.** pDCs constitute a central component of the inflammatory host response in inflamed warts, possibly contributing to their regression through production of type I interferons.

## Introduction

Plasmacytoid dendritic cells (pDCs) are a specialized DC population that exhibit plasma cell morphology.[1–4] They express CD4, CD123, human leucocyte antigen-DR, blood-derived dendritic cell antigen (BDCA)-2, Toll-like receptor (TLR)7 and TLR9 within endosomal compartments, and are capable of producing large quantities of type I interferons (IFNs) against pathogenic agents or danger signals.[1–4] In addition to providing antiviral resistance, pDCs link the innate and adaptive immunity by controlling the function of myeloid DCs, T cells, B cells and natural killer (NK) cells. pDCs are not found in normal skin, but have been found to infiltrate the skin in several pathologies, where they appear to contribute to the pathogenesis of several infectious (especially viral infections), inflammatory/autoimmune and neoplastic entities.[1–4]

Human papilloma virus (HPV) induces benign and malignant mucocutaneous diseases, including common and genital warts, myrmecia, bowenoid papulosis and epidermodysplasia verruciformis (EDV), among others.[5] Although HPV-encoded antigens can be detected in epidermal layers, HPV-infected cells generally evade the host immune surveillance using mechanisms that are still not completely clear. However, HPV-related warts sometimes regress spontaneously, a phenomenon commonly associated with acute inflammatory changes, and mediated mainly by CD8+ T cells, together with the migration of Langerhans cells (LCs).[6,7] A similar process occurs with molluscum contagiosum virus (MCV)-induced skin lesions, which have recently been shown to be mediated by the recruitment of pDCs and the

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production of IFNs.[8] In addition, pDCs have been shown to be involved in cervical carcinomas induced by HPV.[9]

Based on this evidence and the known antiviral role of pDCs, our objective was to investigate the effect of these cells and their product, type I IFNs in inflamed versus uninflamed HPV-induced skin lesions.

## Methods

The study was approved by the institutional review board of the American University of Beirut Medical Center. The requirement for informed consent was waived, as the study was a retrospective study involving immunohistochemical staining of previously collected tissue.

### Patient samples

Archival materials with a diagnosis of inflamed and uninflamed HPV-induced skin lesions (common or genital warts) were retrieved from the dermatopathology database at the Dermatology Department, American University of Beirut Medical Center.

In total, 20 patients (13 men, 7 women; mean age 46 years; range 9–81) with inflamed HPV-induced skin lesions and 20 patients (12 men, 8 women; mean age 41 years; range 6–81) with uninflamed HPV-induced skin lesions (including 7 EDV cases) fitted the criteria for inclusion in the study.

### Histological analysis

The histological sections of all cases were re-reviewed and the diagnosis confirmed by a dermatopathologist (OA). Only straightforward cases of verruca were included in the study, based on the presence of the following characteristic microscopic features: cytopathic koilocytic changes, epidermal hyperplasia with vertical tiers of parakeratosis on top of the exophytic component, a prominent granular layer and increased papillary dermal vascularity. Criteria for diagnosis of inflamed HPV-induced skin lesions included dense inflammatory host reaction associated with histological evidence of keratinocyte cell death.[7,8]

### Immunohistochemical analysis

Immunohistochemical analysis was performed on 5-mm thick sections obtained from formalin-fixed, paraffin wax-embedded tissue. Following dewaxing and rehydration steps, antigen retrieval was performed using citrate buffer and steam. Slides were treated with 3% hydrogen peroxide in methanol for 10 min to block the endogenous peroxidase, and then incubated with appropriate blocking serum.

BDCA-2 mouse IgG1 antibody (clone 124B3.13; Dendritics, Lyon, France) and anti-human myxovirus protein A (MxA) monoclonal antibody (M143; Professor Haller, University of Freiburg, Freiburg, Germany) were used to detect the presence and activity, respectively, of pDCs. Anti-BDCA2 is a specific marker of pDCs, while anti-MxA can be used to indirectly assess for pDC activity as it is an IFN-α/β-inducible intracellular protein that is well established as a surrogate marker for local type I IFN production.[8,10]

pDC content was scored on the BDCA2-stained tissue sections stained for BDCA2 as a percentage of the total mononuclear infiltrate, using a four-point score: 0 (very rare positive cells), 1 (1–10% positive cells), 2 (10–50% positive cells) or 3 (> 50% of positive cells).[11] MxA staining was scored on a three-point score: 0 (negative), 1 (weak/patchy staining) and 2 (intense staining). Normal skin tissue served as negative control and cases of inflamed MCV lesions served as positive control.[8]

### Statistical analysis

Statistical analysis was performed by using the Mann–Whitney test to analyse statistical differences in pDC and MxA scores between the two groups. Two-tailed $P < 0.05$ was considered statistically significant. Spearman rank test was used to test for the association/correlation between pDC and MxA scores.

## Results

### Patients and lesions

Of the 20 patients with inflamed warts, 10 (50%) had lesions located on the head, 2 (10%) had lesions on the trunk and 8 (40%) had lesions on the extremities, while of the 20 patients with uninflamed warts, 9 (45%), 3 (15%) and 8 (40%) had lesions on the head, trunk and extremities, respectively.

### Plasmacytoid dendritic cells in inflamed versus uninflamed human papilloma virus-induced warts

pDCs were identified as medium-sized round cells. BDCA-2+ pDCs were present in all 20 inflamed HPV-induced warts (Fig. 1b), but in none of the uninflamed HPV-induced warts (Fig. 1e,h) including the 7 EDV cases (Table 1). pDC density in inflamed HPV-induced warts was also relatively high, with all cases having a pDC score of either 2 or 3 (Fig. 1b).



 

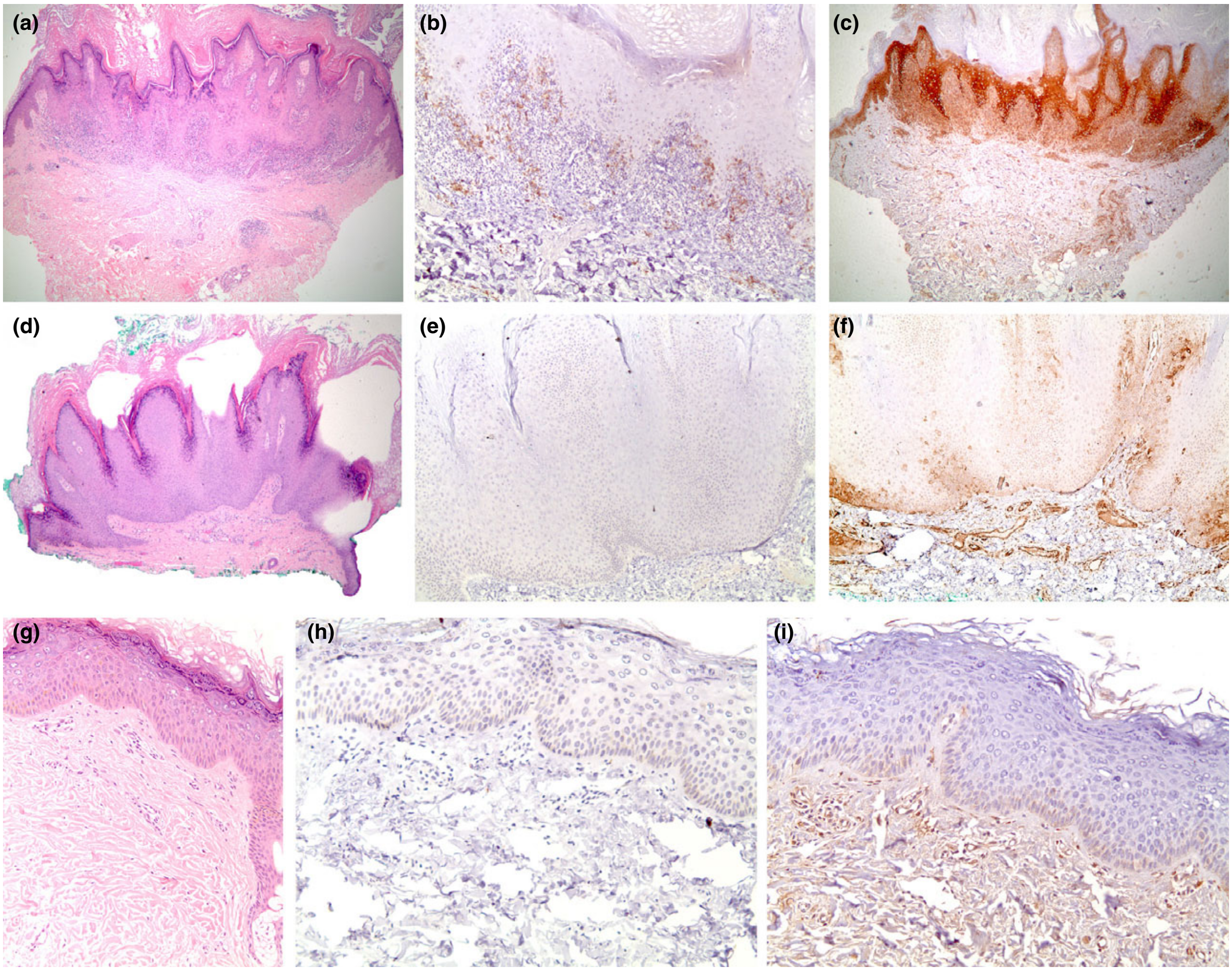


**Figure 1** (a–c) Inflamed wart: (a) representative case with classic findings of verruca and a dense lichenoid inflammatory response (haematoxylin and eosin, original magnification × 40); (b) BDCA-2 immunostaining highlighted numerous plasmacytoid dendritic cells (pDCs) (original magnification × 100); (c) MxA immunostaining was diffuse and intense in the epithelium and inflammatory cells (original magnification × 40). (d–f) Uninflamed wart: (d) representative case with classic findings (haematoxylin and eosin, original magnification × 40); (e) BDCA-2 immunostaining showed absence of pDCs (original magnification × 100); (f) MxA immunostaining was weak and patchy (original magnification × 100). (g–i) EDV lesion: (g) representative case with classic findings (haematoxylin and eosin, original magnification × 100); (h) BDCA-2 immunostaining shows absence of pDCs (original magnification × 100); (i) MxA immunostaining was negative (original magnification × 100).

In addition to being distributed as a rim of cells surrounding islands of infected hyperplastic epithelium in inflamed HPV-induced warts, pDCs also appeared as variable-sized clusters within dermal inflammatory nodules and even infiltrated the hyperplastic epithelium.

### MxA expression

When MxA expression was compared in inflamed versus uninflamed HPV-induced warts, it was found to be significantly higher in inflamed warts ($P < 0.001$). In inflamed HPV-induced skin lesions, a diffuse and strong cytoplasmic expression of the protein was detectable in keratinocytes and inflammatory cells in most cases (15/20) (Fig. 1c), whereas uninflamed HPV-induced warts, including EDV lesions, were either negative (15/20 cases, 75%), or, less commonly, had focal/patchy (5/20 cases; 25%) reactivity (Fig. 1f,i). None of the uninflamed HPV-induced warts showed diffuse strong MxA expression.

### Correlation between BDCA2 and MxA staining

The association between BDCA2 and MxA staining was found to be statistically significant ($r = 0.64$;

**Table 1** pDC and MxA scores in inflamed vs uninflamed warts (%).

| Assessment of cases with: | Inflamed wart ($n$ = 20) | Uninflamed wart ($n$ = 20)‡ | $P$§ |
|---|---|---|---|
| pDC infiltration, $n$ | 20 | 0 | < 0.001 |
| pDC score, $n$* | | | |
| 0 | 0 | 20 | < 0.001 |
| 1 | 0 | 0 | |
| 2 | 9 | 0 | |
| 3 | 11 | 0 | |
| pDC clusters, $n$ | 11 | 0 | < 0.001 |
| Epithelium-infiltrating pDCs, $n$ | 14 | 0 | < 0.001 |
| MxA score, $n$† | | | |
| 0 | 0 | 15 | < 0.001 |
| 1 | 5 | 5 | |
| 2 | 15 | 0 | |

EDV, epidermodysplasia verruciformis; MxA, myxovirus protein A; pDC, plasmacytoid dendritic cell. *Scored as a percentage of total mononuclear infiltrate: 0 (no positive cells), 1 (1–10% positive cells), 2 (10–50% positive cells), 3 (> 50% positive cells); †MxA staining was scored as: 0 (negative), 1 (weak/patchy) and 2 (intense); ‡including 7 EDV cases; §Mann–Whitney $U$-test to analyse statistical differences in pDC and MxA scores between entities, with two-tailed $P < 0.05$ considered statistically significant.

Spearman rank test), another indicator that pDCs are the main source of type I IFNs.

## Discussion

As natural IFN-producing cells, pDCs are the most potent type I IFN producers, secreting up to 1000 times more type I IFNs than do other cell types.[1–4] Signalling of Type I IFNs through various pathways (Janus-activated kinase, signal transducer and activator of transcription, mitogen-activated protein kinase p38, phosphatidylinositol 3 kinase cascades)[11] usually leads to induced expression of hundreds of genes that lead mainly to an antiviral state by preventing virus replication, limiting its spread to adjacent cells and indirectly activating natural killer/CD8+ T-cell cytotoxicity.[12]

Our hypothesis in this study concerning a role for pDCs in the immunological inflammatory response against warts, ultimately leading to wart regression, was based on several observations. First, all types of warts are HPV-induced, and as the main function of pDCs is antiviral resistance, their action against warts would not be surprising.[1,5] Second, several reports have described the effectiveness of intralesional IFN-alfa in the treatment of warts and EDV, and the endogenous counterpart of this drug is produced by pDCs.[13–16] Third, imiquimod, an immunomodulator known to be a potent pDC activator through its effect on TLR-7, is approved by the US Food and Drug Administration for the treatment of genital warts.[17] Finally, studies suggest that regression of warts is dependent mainly on the immune response mediated by cytotoxic T lymphocytes (CD8+),[6,7] which can be stimulated by pDCs.[1–4]

Our study confirmed the hypothesis. Not only were pDCs present consistently and abundantly in all inflamed wart cases, but they were also present in an active state, as was demonstrated indirectly by the diffuse and intense MxA expression in most of the inflamed wart lesions. The role and activity of pDCs in inflamed wart lesions established in our study is comparable to the recent demonstration of their role in other cutaneous viral infections. pDCs and their product, type I IFNs, have been shown to play a central role in anti-MCV responses that ultimately contribute to the spontaneous regression of MC lesions.[8] A similar role for pDCs has also been demonstrated in herpetic infections.[18,19]

The few previous studies on the role of pDCs against warts have provided conflicting results. Tomasini *et al.* looked at inflamed warts among other cutaneous entities (such as lupus erythematosus, Jessner lymphocytic infiltrate, lichen planus, psoriasis, eczema and granulomatous disorders) and noted the complete absence of pDCs in inflamed warts,[20] attributing this to the immune evasion strategies adopted by the HPV virus. However, a clear definition of inflamed wart was not provided in that study. In another study, Nakayama *et al.* compared the immune response in different HPV-induced skin lesions, and found that inflamed warts were infiltrated by different subsets of DCs, including some pDCs, while HPV-induced Bowen disease showed no pDC infiltration.[7] However, the authors pointed out that no further characterization of the infiltrating DCs including pDCs was performed, owing to technical limitations. In addition, MxA expression as a reflection of type I IFN production was not assessed. Finally, Tassaone *et al.* assessed WHIM syndrome (warts, hypogammaglobulinaemia, infections and myelokathexis), an autosomal dominant genetic disease characterized by severe persistent chronic warts refractory to treatment.[21] In that study, quantitative analysis of pDCs in peripheral blood taken from patients with WHIM and compared with healthy controls showed that pDC number was significantly decreased in affected patients and that they were unable to secrete IFN-α.[21] In addition, pDCs were not found in biopsy specimens of WHIM warts and there was no MxA reactivity.[21] Based on these findings, the authors ascribed the intractable warts to the inability of pDCs to mount an antiviral response via TLR9.

Similar to WHIM syndrome, EDV, which is a rare autosomal recessive genodermatosis, carries an increased susceptibility to HPV infection, particularly types 5 and 8.[22,23] The lesions present as discrete flat papules or pityriasis rosea-like macules over the body, mostly on the hands and feet. Lesions on sun-exposed sites have a propensity to progress to cutaneous squamous cell carcinoma (SCC).[22,23] Previous reports have shown response of EDV-associated warts to IFN injections.[13] In our study, the patients with EDV were devoid of pDCs. This may raise the possibility that a genetic defect contributing to a failure in recruiting pDCs and/or their IFN secretion may be one of the immune abnormalities that lead to the persistence of warts in patients with EDV. Future studies should investigate this possibility.

## Conclusion

In summary, we have shown that pDCs constitute a central component of the inflammatory host response in inflamed warts, and that their active production of type I IFNs ultimately contributes to wart regression. These findings also have therapeutic implications. Future research should focus on identifying immunomodulators that may be even more potent than imiquimod in activating pDCs, leading ultimately to better treatment of HPV-induced skin lesions.

### What's already known about this topic?

- pDCs infiltrate the skin in several infectious, inflammatory and neoplastic conditions, mainly providing antiviral resistance and linking the innate and adaptive immune responses.
- pDCs and their product, type I IFNs, have been shown to play a central role in anti-MCV and antiherpetic responses.
- The few previous studies have provided conflicting results on pDC role against warts.

### What does this study add?

- pDCs constitute a central component of the inflammatory host response in inflamed warts.
- pDC may contribute to wart regression through type I IFN production.

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