Expression of programmed cell death ligand 1 and programmed cell death 1 in cutaneous warts

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Background: Cutaneous warts have high prevalence and cause significant morbidity. Understanding the mechanisms by which warts evade the immune system could lead to targeted and improved treatments.

Objective: To determine whether cutaneous warts express programmed cell death ligand 1 (PD-L1) and to characterize the expression of programmed cell death 1 (PD-1) within the immune infiltrate of inflamed lesions.

Methods: In total, 44 biopsies of cutaneous warts were retrieved from the Department of Dermatopathology archives of the University of California, San Francisco. Biopsies were stained with hematoxylin and eosin and PD-L1 monoclonal antibody, and biopsies of inflamed lesions were stained with PD-1 monoclonal antibody.

Results: PD-L1 was expressed on keratinocytes in cases of verrucae vulgares (12/30, 40%) and myrmecia (7/14, 50%) and was associated with an interface inflammatory reaction. PD-1 was expressed by the inflammatory infiltrate in verrucae vulgares (21/24, 88%) and myrmecia (5/8, 63%).

Limitations: This was a retrospective observational study conducted at a single institution.

Conclusion: Many cutaneous warts express PD-L1, suggesting that human papillomavirus might use this pathway to promote immune dysfunction. This discovery helps explain the recalcitrance of warts to current therapies and provides a rationale for investigating anti–PD-1 immunotherapy as a potential treatment for warts. (J Am Acad Dermatol 2019;81:1127-33.)

Key words: HPV; human papillomavirus; immunotherapy; interface dermatitis; pathophysiology; PD-1; virology; warts.

Cutaneous warts, also known as verrucae, are extremely common, with a prevalence of up to 30% in the general population and up to 77% among immunocompromised patients.[1,2] Although considered benign, warts cause significant morbidity. Despite multiple available treatment modalities, a sizeable number of warts remain resistant to numerous therapies and impair quality of life for patients.

Human papillomaviruses (HPVs) are the etiologic agents that cause verrucae. During the establishment of warts, HPV is thought to escape detection by the host immune system, but the mechanisms that underlie this process are not completely understood.[3] The program cell death ligand 1 (PD-L1)–programmed cell death 1 (PD-1) pathway has

Abbreviations used:

HPV: human papillomavirus
PD-1: programed cell death 1
PD-L1: programed cell death ligand 1

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emerged as an important mechanism for immune evasion by viruses and cancer, and therapies that block this checkpoint have demonstrated dramatic efficacy in some settings.[4] Some evidence suggests that HPV might induce PD-L1 expression in squamous cell carcinoma of the head and neck and cervical cancer.[5,6] Given that a subset of warts demonstrates remarkable resistance to a variety of treatments, a possible mechanism for this phenomenon might be the induction of immune inhibitory molecules, such as PD-L1, and dysfunction of PD-1–expressing lymphocytes.

CAPSULE SUMMARY

- Cutaneous warts have a high prevalence and cause significant morbidity. Understanding the mechanisms by which warts evade the immune system might lead to targeted treatment.
- Many cutaneous warts express programed cell death ligand 1, suggesting that human papillomavirus infection might promote immune dysfunction. These findings might explain the recalcitrance of some warts to standard treatment modalities and provide a rationale for the use of anti–programed cell death 1 therapy to treat cutaneous warts.

METHODS

This study was approved by the institutional review board at the University of California, San Francisco. Thirty biopsies diagnosed as verruca vulgaris (common wart) and 14 biopsies diagnosed as myrmecia (volar warts exhibiting extreme viral cytopathic effects) were retrieved from the Department of Dermatopathology archives of the University of California, San Francisco. All biopsies were stained with PD-L1 monoclonal antibody (clone E1LN3; Cell Signaling Technology, Danvers, MA) and PD-1 monoclonal antibody (Cell Marque, Rocklin, CA). A dermatopathologist evaluated each stained section for keratinocyte expression of PD-L1 and inflammatory cell expression of PD-L1 and PD-1. Biopsies demonstrating at least 5% membranous expression of PD-L1 or PD-1 were considered positive. The amount and type of inflammation was graded for each specimen and scored as zero (no inflammation), 1 (mild; focal interface, perivascular, or both types of inflammation), 2 (moderate; multifocal but not confluent interface inflammation), or 3 (severe; confluent interface inflammation).

RESULTS

Clinicopathologic features of cutaneous warts are summarized in Tables I and II. In brief, 44 biopsies consisting of 30 cases of verrucae vulgares and 14 cases of myrmecia were retrieved. Patients ranged in age (4-94 years old), and 55% (24/44) were female. Verrucae vulgares were characterized by papillomatosis with prominent parakeratosis at the tips of the digitations, hypergranulosis in the dells between papillations, and mild-to-moderate koilocytic changes in some of the biopsies (Fig 1, *A* and *B*). Myrmecia also had a papillated contour but, in contrast with verrucae vulgares, largely exhibited a deeply endophytic profile and striking eosinophilic nuclear and cytoplasmic inclusions in lesional keratinocytes (Fig 2, *A* and *B*); 75% (18/24) of verrucae vulgares biopsies and 57% (8/14) of myrmecia biopsies demonstrated inflammation that predominantly showed an interface reaction pattern (28/44, 64%).

In verrucae vulgares, PD-L1 was expressed by lesional keratinocytes in 12 of 30 (40%) cases and by the inflammatory infiltrate in 20 of 22 (91%) cases (Table I). Expression of PD-L1 correlated with the intensity of inflammation and with an interface reaction pattern. There were no cases of keratinocyte PD-L1 expression without accompanying inflammation; 57% (12/21) of the specimens with at least moderate inflammation demonstrated epidermal PD-L1 expression. In all cases, PD-L1 expression was membranous, usually confined to the lower layers of the epidermis within foci of inflammation (Fig 1, *C* and *D*). Histologically normal-appearing epidermis at the periphery of each specimen served as an internal negative control to demonstrate that normal-appearing epidermis does not express PD-L1 (Fig 1, *C*).

Myrmecia also demonstrated strong PD-L1 expression in both keratinocytes (7/14, 50%) and the inflammatory infiltrate (7/8, 88%) (Table II). In the subset of specimens with at least moderate inflammation, all myrmecia demonstrated keratinocyte PD-L1 expression. Again, PD-L1 expression was localized to the lower epidermis in areas of interface inflammation and was membranous in quality (Fig 2, *C* and *D*). PD-L1 expression did not correlate with age or sex in either cases of verrucae vulgares or myrmecia.

PD-1 membranous staining was noted on the inflammatory cells in the vast majority of specimens for which there was an infiltrate present (26/32, 81%). Specifically, PD-1–expressing inflammatory cells were localized to areas of interface inflammation (Fig 3).

Table I. Clinical and histopathologic characteristics of verrucae vulgares

Case	Age/sex	Site	Immune status	Epithelial PD-L1 expression (% cells)	Inflammatory PD-L1 expression (% cells)	PD-1 expression	Inflammatory score*	Type of inflammation
1	56/M	Deltoid	Competent	Positive (~5)	Positive (~40)	Positive	3	Interface, perivascular
2	66/F	Calf	Unknown	Positive (~20)	Positive (~50)	Positive	3	Interface, perivascular
3	63/M	Thigh	Unknown	Positive (~10)	Positive (~70)	Positive	3	Interface, perivascular
4	87/F	Neck	Unknown	Positive (~5)	Positive (~40)	Positive	3	Interface, perivascular
5	89/F	Thigh	Unknown	Positive (~5)	Positive (~40)	Positive	3	Interface, perivascular
6	57/M	Cheek	Competent	Positive (~10)	Positive (~50)	Positive	3	Interface, perivascular
7	13/F	Canthus	Unknown	Positive (~40)	Positive (~60)	Positive	3	Interface, perivascular
8	56/F	Back	Unknown	Positive (~20)	Positive (~40)	Positive	3	Interface, perivascular
9	72/M	Forearm	Unknown	Positive (~10)	Positive (~60)	Positive	3	Interface, perivascular
10	93/M	Cheek	Unknown	Positive (~10)	Positive (~60)	Positive	2	Interface, perivascular
11	23/M	Cheek	Unknown	Positive (~40)	Positive (~30)	Positive	2	Interface, perivascular
12	34/F	Cheek	Competent	Positive (~5)	Positive (~30)	Positive	2	Interface
13	77/M	Forearm	Unknown	Negative	Positive (~50)	Positive	3	Interface, perivascular
14	38/F	Thigh	Unknown	Negative	Positive (~50)	Positive	3	Interface, perivascular
15	45/M	Forearm	Unknown	Negative	Positive (~40)	Positive	3	Interface, perivascular
16	86/F	Thigh	Unknown	Negative	Positive (~60)	Positive	3	Interface, perivascular
17	72/M	Forearm	Unknown	Negative	Positive (~30)	Positive	3	Interface, perivascular
18	73/F	Thigh	Competent	Negative	Positive (~20)	Positive	2	Perivascular
19	65/M	Back	Compromised, liver transplant	Negative	Positive (~50)	Negative	2	Perivascular
20	61/F	Back	Unknown	Negative	Positive (~50)	Positive	2	Interface, perivascular
21	94/M	Back	Unknown	Negative	Positive (~40)	Negative	2	Interface, perivascular
22	46/M	Heel	Unknown	Negative	Positive (~10)	Positive	1	Interface
23	34/M	Forehead	Competent	Negative	Negative	Negative	1	Perivascular
24	4/M	Arm	Competent	Negative	Negative	Positive	1	Perivascular
25	59/M	Elbow	Competent	Negative	NA	NP	0	NA
26	45/M	Penis	Compromised, heart transplant	Negative	NA	NP	0	NA
27	11/F	Hand	Unknown	Negative	NA	NP	0	NA
28	54/F	Finger	Unknown	Negative	NA	NP	0	NA
29	54/F	Shin	Unknown	Negative	NA	NP	0	NA
30	24/M	Finger	Unknown	Negative	NA	NP	0	NA

F, Female; *M*, male; *NA*, not applicable; *NP*, not performed; *PD-1*, programmed cell death 1; *PD-L1*, programmed cell death ligand 1.

*A score of 0 indicates no inflammation, 1 mild (focal interface, perivascular, or both) inflammation, 2 moderate (multifocal but not confluent interface) inflammation, and 3 severe (confluent interface) inflammation.

Table II. Clinical and histopathologic characteristics of myrmecia

Case	Age/sex	Site	Immune status	Epithelial PD-L1 expression (% cells)	Inflammatory PD-L1 expression (% cells)	PD-1 expression	Inflammatory score*	Type of inflammation
1	15/F	Foot	Unknown	Positive (~10)	Positive (~10)	Positive	3	Interface
2	18/F	Foot	Unknown	Positive (~10)	Positive (~20)	Positive	2	Interface
3	35/F	Wrist	Unknown	Positive (~5)	Positive (~20)	Positive	2	Interface
4	49/F	Foot	Unknown	Positive (~10)	Positive (~30)	Positive	2	Interface, perivascular
5	17/M	Ankle	Unknown	Positive (~20)	Positive (~20)	Positive	2	Interface, perivascular
6	35/F	Finger	Unknown	Positive (~40)	Negative	Negative	1	Interface
7	38/F	Finger	Unknown	Positive (~10)	Positive (~10)	Negative	1	Interface
8	49/F	Forearm	Unknown	Negative	Positive (~10)	Negative	1	Interface, perivascular
9	15/F	Foot	Unknown	Negative	NA	NP	0	NA
10	19/M	Foot	Unknown	Negative	NA	NP	0	NA
11	47/F	Heel	Unknown	Negative	NA	NP	0	NA
12	67/F	Finger	Unknown	Negative	NA	NP	0	NA
13	19/M	Foot	Competent	Negative	NA	NP	0	NA
14	40/F	Thumb	Unknown	Negative	NA	NP	0	NA

F, Female; *M*, male; *NA*, not applicable; *NP*, not performed; *PD-1*, programmed cell death 1; *PD-L1*, programmed cell death ligand 1.
*A score of 0 indicates no inflammation, 1 mild (focal interface, perivascular, or both) inflammation, 2 moderate (multifocal but not confluent interface) inflammation, and 3 severe (confluent interface) inflammation.

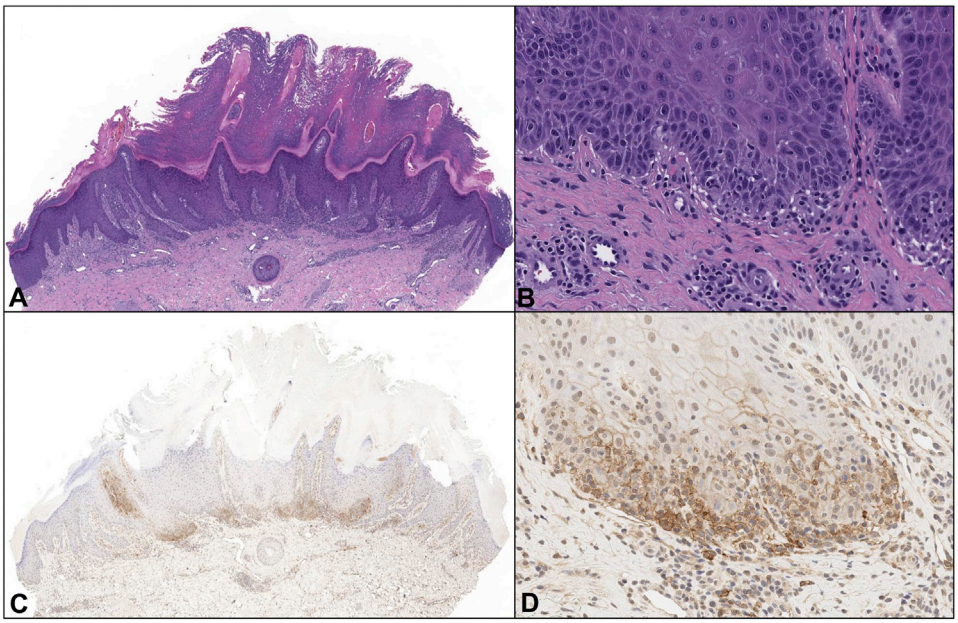

Fig 1. Programed cell death ligand 1 (PD-L1) expression in verruca. **A**, Digitated epidermal hyperplasia with hypergranulosis and parakeratosis at the tips of digitations in a verruca vulgaris. **B**, On higher magnification of **A**, interface dermatitis is apparent, with lymphocytes infiltrating the basal layer of the epidermis. **C**, PD-L1 expression can be seen in the basal layers and is localized to areas of inflammation. **D**, Strong membranous PD-L1 expression is present in both keratinocytes and inflammatory cells. *PD-L1*, Programed cell death ligand 1. (**A** and **B**, Hematoxylin-eosin stain; **C** and **D**, PD-L1 stain; original magnification: **A** and **C**, ×40; **B** and **D**, ×200.)

DISCUSSION

Herein, we demonstrate that cutaneous warts express PD-L1 and the associated inflammatory infiltrate expresses PD-1, suggesting that HPV might utilize this pathway to induce immune dysfunction. We found that ~43% of verrucae express PD-L1 in keratinocytes, which is similar to the proportion of melanomas (35%) and Merkel cell carcinomas (49%) expressing PD-L1.[7,8] In all cases of inflamed warts, PD-L1 expression on lesional keratinocytes was localized to areas of interface inflammation. This pattern is also consistent with studies of PD-L1

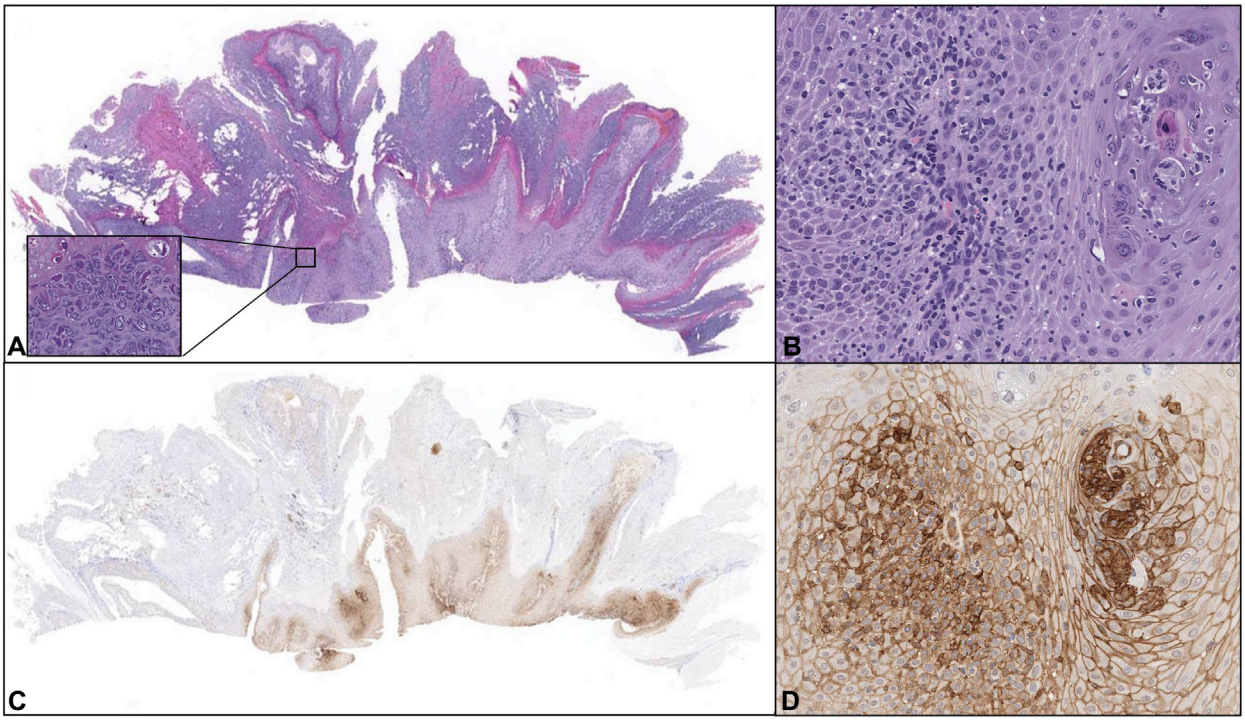

Fig 2. Programed cell death ligand 1 (PD-L1) expression in myrmecia biopsies. **A**, Prominent viral cytopathic changes in the form of dense eosinophilic globules in the granular layer (inset) and notable digitated acanthosis. **B**, High-power view of **A**, showing prominent inflammation with many lymphocytes in exocytosis. **C**, Programed cell death ligand 1 (PD-L1) expression is seen diffusely throughout the basal layer. **D**, Strong membranous PD-L1 expression is present in keratinocytes and inflammatory cells. *PD-L1*, Programed cell death ligand 1. (**A** and **B**, Hematoxylin-eosin stain; **C** and **D**, PD-L1 stain; original magnification: **A** and **C**, ×40; **B** and **D**, ×200.)

expression in melanoma, Merkel cell carcinoma, and head and neck squamous cell carcinoma, suggesting that infiltrating immune cells might promote PD-L1 expression on lesional cells.[7] The expression of PD-L1 was noted on the accompanying inflammatory infiltrate in a significant subset of cases as well. The pattern of PD-L1 expression on inflammatory mononuclear cells is not unlike that observed in urothelial carcinoma, which was shown to have a positive prognostic value in some settings.[9] Taken together, upregulation of PD-L1 on keratinocytes, as well as the inflammatory component might act in concert to protect HPV-infected cells from immune-mediated destruction.

Previous studies have shown PD-L1 expression in high-grade squamous intraepithelial lesions and carcinomas of the cervix and in a subset of head and neck squamous cell carcinomas, which are lesions associated with HPV types 16, 18, 31, and 33.[5,6] On the other hand, cutaneous warts are caused by HPV types 1, 2, 4, 7, and 26-29. This suggests that PD-L1 expression might be a general mechanism of immune evasion for HPV rather than a result of malignancy. As such, intrinsic HPV-mediated upregulation of PD-L1 on lesional cells remains a possibility. The expression of PD-L1 on basal keratinocytes, the reservoir for HPV persistence, is intriguing.

In the present study, a subset of specimens did not express PD-L1. However, PD-L1 expression might be transient or require induction by inflammatory cells. Thus, PD-L1 expression on warts could be more prevalent than is suggested by biopsies that represent a single time-point and a limited area of the cutaneous lesion. Along these lines, warts that are PD-L1 negative might indeed benefit from PD-L1—PD-1 targeted therapy, analogous to observations in melanoma and other cancers.[10,11] Some verrucae might authentically lack PD-L1 expression, which could be due to an inability to upregulate PD-L1 in an HPV-associated, cell-intrinsic manner or the lack of an antiviral immune response that results in PD-L1 expression. It will be intriguing to determine whether

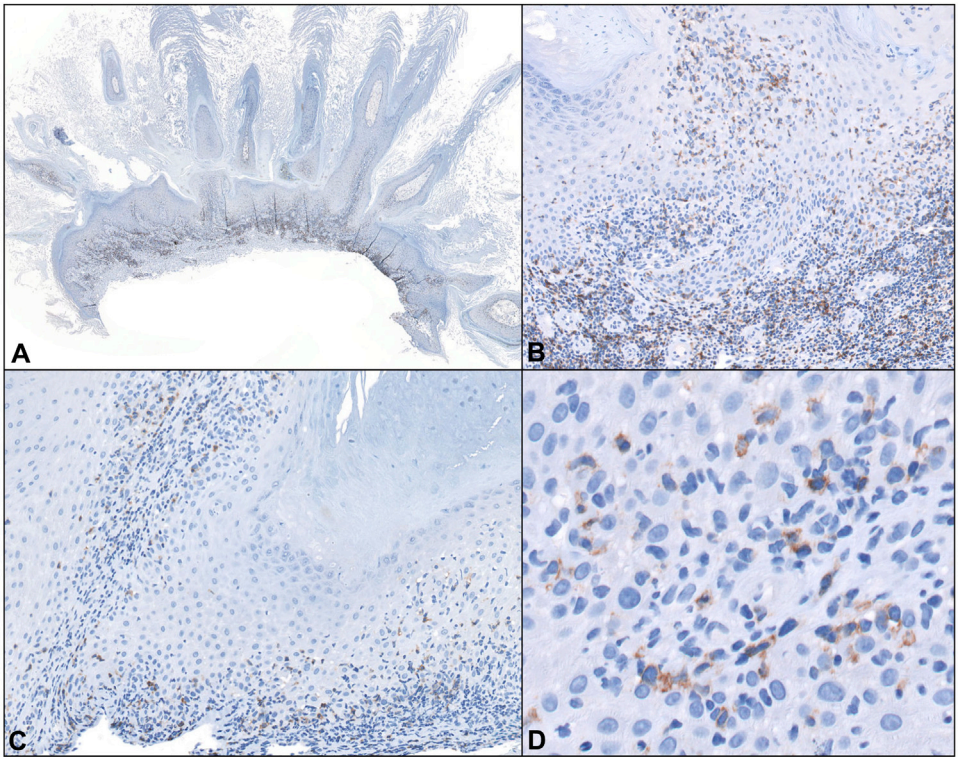

Fig 3. Programed cell death 1 (PD-1) expression in verruca vulgaris and myrmecia infiltrates. **A**, PD-1 staining of the inflammatory infiltrate of a verruca vulgaris lesion shows an interface pattern. **B**, In verruca vulgaris, numerous inflammatory cells along the dermoepidermal junction and in exocytosis express PD-1. **C**, Also in myrmecia, PD-1 expression is present on lymphocytes at the dermoepidermal junction. **D**, In myrmecia, membranous PD-1 expression is present on inflammatory cells in exocytosis. *PD-1*, Programed cell death 1. (**A**-**D**, PD-1 stain; original magnification: **A**, ×40; **B** and **C**, ×100; **D**, ×400.)

recalcitrant warts of this type would benefit from combined therapy with immune-stimulating agents (eg, imiquimod) and PD-L1—PD-1 blockade.

One limitation of this study is that histopathologic attributes of verrucae were relied on as a proxy for HPV infection; investigation of HPV DNA or protein was not undertaken. However, included in our study was an analysis of myrmecia, which are a subtype of verruca caused by HPV types 1, 60, and 65 that have pathognomonic histopathologic findings of large eosinophilic cytoplasmic and nuclear inclusions.[12] In addition, the cases reported as verrucae vulgares demonstrated prominent papillomatosis, hemorrhagic parakeratosis, hypergranulosis, and variable koilocytosis, which are classic histopathologic features strongly suggestive of HPV infection. Another limitation is the possibility of selection bias, considering that most warts are not biopsied, and those that are might be atypical appearing or recalcitrant to treatment. Prospective studies to evaluate a wider spectrum of warts for PD-L1 and PD-1 expression would help validate these results.

This study establishes that PD-L1 and PD-1 are expressed in virus-associated cutaneous lesions outside of the tumor setting, suggesting that this pathway might represent a defense mechanism for chronic HPV infections. Likewise, both Epstein-Barr virus and hepatitis B virus have also been shown to promote PD-L1 expression in chronically infected tissue.[13,14] We note that our findings are preliminary and that further investigation is required to determine whether HPV directly influences PD-L1 expression in infected cells or whether the chronic inflammatory state created by HPV infection indirectly leads to the induction of this pathway. Overall, upregulation of the PD-L1—PD-1 pathway offers a potential explanation for the recalcitrance of some warts to standard therapies and provides a rationale for investigating anti—PD-1 treatment as a potential immunotherapy against verrucae.

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