Herpes Simplex Virus Type-I and Pyogenic Granuloma: A Vascular Endothelial Growth Factor-Mediated Association?

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Abstract
Pyogenic granuloma (PG) is a vascular endothelial growth factor (VEGF)-related neoangiogenic process. Minor trauma, chronic drugs and pregnancy may favor PG. Viral triggers have not been reported up to date. A 52-year-old woman with hairy-cell leukemia presented because of a 3-month history of a giant pseudotumoral lesion on her left cheek. All prior antibacterial, antifungal and anti-inflammatory treatments had failed. Histology revealed PG with sparse and isolated epithelial cell aggregates. Immunohistochemistry (IHC) identified herpes simplex virus type-I (HSV-I) antigens in the nuclei and cytoplasm of normal-appearing as well as cytopathic epithelial cells, suggesting a chronic, low-productive HSV infection. No HSV-I signal was evidenced in the endothelial cells of the PG. Furthermore, IHC revealed VEGF in the HSV-I infected epithelial cells as well as within the PG endothelial cells. These results incited oral treatment with valaciclovir, and the PG promptly resolved after 2 weeks. These findings suggest that a chronic HSV-I infection might play an indirect, partial role in neoangiogenesis, presumably via HSV-I infection-related stimulation of keratinocytic VEGF production.
Introduction

Pyogenic granuloma (PG) is a common reactive inflammatory and pseudotumoral neo-angiogenic process [1-3]. Occasionally, the lips and face may also be affected. PG may present as a single or multiple lesion(s) and sometimes develops as a giant tumor [1]. Histology reveals turgid endothelial cells and capillary proliferation of variable size. The precise pathomechanisms and triggers of PG are still unclear. PG usually follows minor trauma, sometimes related with chronic irritation [1, 2]. It may also be drug-induced or is observed during pregnancy [1]. To this day, infectious triggers of PG are not reported.

As far as we know, this case report is the first to suggest a partial link between chronic herpes simplex virus type-I (HSV-I) infection and cutaneous PG.

Case Description

A 52-year-old woman was diagnosed with hairy-cell leukemia in 1999 and treated with cladribine [2-chlorodeoxyadenosine (2CDA)], which led to complete remission. Two recurrences occurred in 2005, both successfully treated with 2CDA and lenograstim, a recombinant granulocyte colony-stimulating factor. In 2006, another recurrence was treated with lenograstim without obtaining complete remission. In 2007, due to persisting grade-2 pancytopenia and medullar infiltration, interferon alpha-2a was initiated. In 2008, remission was obtained with the chimeric anti-CD20 monoclonal antibody rituximab (8 cures), but leuconeutropenia persisted. Since June 2011, no further treatments have been administered, although the patient still presents moderate leuconeutropenia (without any infectious complications, however).

Three months previously, a slow-growing vascular lesion appeared on the lower lip following an episode of labial herpes. Clinical examination revealed a large, unilateral, annular, ulcerated, painful, indurated and easily bleeding lesion on her left cheek (fig. 1a, b). No locoregional lymphadenopathies were evidenced. The treatment consisted of aldacantine once daily, elthyrone 100 gamma/day, lutenyro 100 gamma/day and bisoprolol 2 × 2.5 mg. The laboratory counts were as follows: red blood cells 3.26 × 10^6/mm³ (3.90–4.90); platelets 146,000/mm³ (150,000–353,000); white blood cells 2.33 × 10^9/mm³ (4.60–10); neutrophils 31.1% (42.2–71.0); lymphocytes 67.2% (17.5–43.5); sedimentation rate 57 mm/h (<21); C-reactive protein 21.6 mg/l (0.0–6.0), and IgM <0.17 g/l (0.40–2.48). The T-cell population did not reveal any aberrant phenotype, and the CD4/CD8 ratio was 0.91. Immunophenotyping revealed the virtual absence of B cells, and the tricholeucocyte phenotype CD103 percentage was 0.2%. The serological status for HSV was IgM- and IgG+. The renal and hepatic functions were unremarkable. Various treatments, including topical antifungals, antibiotics, antiseptics and corticosteroids, were unsuccessful. Systemic antibiotics and antifungals were also inefficacious. After 3 months, a dermatologic advice was requested. Histology confirmed PG, revealing a vascular neoplasm characterized by small vessel ectasia with thin walls, normal endothelial cells and a dense lymphocytic and neutrophilic inflammatory infiltrate. The conjunctive stroma was severely edematous (fig. 2a). On serial sections, some isolated epithelial cell islands were intermingled in the PG. These epithelial cells sometimes showed signs of cytopathic effects (CPE), including intranuclear inclusions and giant syncytial cell formation, suggesting an alpha-herpesviridae infection (fig. 2b, c). Immunohistochemistry (IHC) was performed according to an earlier published protocol [4] with the antibody panel shown in table 1. A strong nuclear and cytoplasmic signal for HSV-I was evidenced in some epithelial cells (fig. 2d), whereas the HSV-II and varicella zoster virus...
stainings remained negative. Some of the HSV-I-positive cells presented CPE, whereas others did not (fig. 2d). No immunohistochemical signal for HSV-I was evidenced in the endothelial cells or vessel walls of the PG. IHC using the Ulex europaeus lectin revealed a strong signal on the cell membranes of the HSV-I-infected epithelial cells as well as on the endothelial cells of the PG (fig. 3a, red signal). Mac 387 immunostaining was positive for HSV-I-infected epithelial cell membranes. The vascular endothelial growth factor (VEGF) immunostaining provided a positive signal in the HSV-infected keratinocytes as well as in endothelial cells (fig. 3b, red signal). The epidermal growth factor receptor (EGFR) immunostaining revealed intense membranous expression on HSV-I-infected epithelial cells (fig. 3c, brown signal). The Ki67 marker revealed a strong signal in the nuclei of some epithelial and endothelial cells (fig. 3d, red signal). IHC with antibodies against CD45 evidencing the leucocyte common antigen and CD3 T-cells provided an intense staining of the inflammatory infiltrate of the PG, but no signal surrounding the HSV-I-infected epithelial cells was observed. In contrast, IHC with CD45R0 demonstrating activated T cells revealed a very dense infiltrate among the epithelial cells. The CD45R B cell marker was negative. The markers CD68 and Mac 387 were positive in the inflammatory infiltrate surrounding the epithelial cells and the PG. Using the identical antibody panel for a post-traumatic labial PG of a patient without recurrent herpes labialis, the staining patterns were similar, except for the absence of signals for HSV-I, EGFR and CD45R0. A viral culture remained negative.

Following the discovery of the HSV-I antigens in the PG, oral treatment with valaciclovir (1,000 mg, thrice daily for 15 days) was initiated, and the lesion promptly resolved after 15 days (fig. 1c). No search for viral thymidine kinase (TK) resistance was performed. After 1 month, a slightly erythematous postinflammatory pigmentation persisted (fig. 1d). Until now, the patient has remained recurrence free.

**Discussion**

PG consists of a pseudotumoral neoangiogenic process and is often linked to pregnancy, various drugs (cyclosporine, lamivudine, indinavir, isotretinoin, docetaxel and the EGFR inhibitors), traumatism, preexisting vascular lesions, food impaction or total periodontosis [1–3]. FLT4, a tyrosine kinase receptor associated with pathological angiogenesis, is also specifically related to PG [3]. The rapid vascular growth during PG is related to angiogenesis enhancers such as VEGF [5] and fibroblast growth factor, and angiogenesis inhibitors including thrombospondin-1 and angiotatin [5]. In addition, the vessel morphogenic factors Tie-2 (tyrosine kinase with immunoglobulin-like and EGF-like domains 1), angiopoietin-1/2, ephrin B2 and B4 are upregulated in PG lesions [6]. These data sustain that PG is predominantly a reactive neoangiogenic process.

There are no previous reports on HSV-I infections related to PG in the literature. However, other members of the human herpesviridae group, the Epstein-Barr virus and the Kaposi’s sarcoma-associated herpesvirus (HHV-8), are also capable of inducing neoangiogenesis and neolymphangiogenesis by directly stimulating various signalling pathways via the VEGF protein family and their receptors [7]. In the eye, HSV-I may cause a chronic immunoinflammatory process with secondary corneal neoangiogenesis called stromal keratitis, potentially leading to blindness [8]. In addition, herpetic stromal keratitis may even progress without the actual presence of replicating HSV-I. In fact, HSV-I DNA persistence in tissue and HSV-IgG complexes may upregulate VEGF and MMP-9 production as both intervene in the neoangiogenic process [9].
In parallelism, a similar pathogenic role of HSV-I in the development of PG could be advanced. The nuclear and cytoplasmic HSV-I staining patterns in non-CPE-presenting epithelial cells are in line with a chronic, low-productive type of HSV infection, as observed in other chronic HSV infections [10]. The strong expressions of EGFR and Ki67 in the epithelial cells suggest that these cells are still replicating. The rapid response to antiviral treatment, the ≥3-month evolution and the failure of previous non-antiviral treatments favor the hypothesis of a chronic but active HSV replication rather than the presence of persistent viral DNA or IgG complexes in the PG. In fact, the prior corticosteroid-based anti-inflammatory therapy was not successful, although it is known to be efficacious for treating granulomatous reactions linked with persistent herpetic glycoproteins [11].

The fact that no HSV-I antigens were detected in the PG endothelial cells but only in the epithelial cell aggregates favors an indirect stimulation of neoangiogenesis by HSV-1 infection. VEGF, a potent pro-angiogenic factor, is sourced by histiocytes but is also expressed at stand-by levels in epidermal keratinocytes [12]. Following upregulation during psoriasis, skin cancers and wound healing, keratinocytes release VEGF [12]. Hence, the presence of VEGF in HSV-I-infected keratinocytes may play a stimulating role of the PG angiogenesis. Yet, VEGF is also produced by histiocytes, but the rapid regression after antiviral therapy suggests that the HSV-I-infected keratinocytes are a predominant source of VEGF.

This case shows some parallelism with anogenital herpes vegetans, a chronic condition due to HSV-II that is usually associated with HIV infection and occasionally with other immunodeficiencies [13]. Some anogenital HSV lesions may present as PG-like lesions [1, 13]. In contrast, atypical HSV-I oro-lingual-facial lesions are far more exceptional and always associated with immunosuppression [14].

Although TK-dependent antiviral resistance is a common event in chronic anogenital HSV infection, this patient responded well to oral valaciclovir. TK-dependent antiviral resistance does not seem common in oro-facial chronic HSV-I infections [14].

This report possibly illustrated a new face of HSV infection besides the common cytopathic infection as well as other, more unusual HSV-related cutaneous patterns, including verrucous [13], granulomatous [11], lichenoid [10] and follicular [15] reactions (table 2).

Conclusion

The presence of HSV-I antigens in epithelial cell islands scattered throughout the PG, the particular pattern of HSV-I antigen distribution in epithelial cells suggesting a low-productive HSV infection, the simultaneous VEGF expression in the PG endothelial cells and in HSV-I-infected keratinocytes as well as the prompt regression following oral valaciclovir after repetitive failure of all prior non-antiviral treatment options raised the hypothesis that, under certain circumstances, HSV-I might play an indirect role in the triggering of PG, presumably via an VEGF-dependent pathway.

Disclosure Statement

The authors declare that there are no conflicts of interest.
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Table 1. Antibody panel

| Antibody | Type | Clone | Antigen | Target | Source |
|----------|------|-------|---------|--------|--------|
| Mac387   | Mouse, IgG1, kappa | Mac387 | L1H + L1L (calprotectin) | Myeloid cells/hiystocytes | DAKO |
| CD68     | Mouse, IgG1, kappa | KP1 | CD68 | Reactive human monocytes, macrophages and myeloid cells | DAKO |
| HSV-I    | Rabbit | NA | HSV-I | HSV-I | DAKO |
| HSV-II   | Rabbit | NA | HSV-II | HSV-II | DAKO |
| VZV      | Mouse, IgG1, kappa | VL9 | gE | VZV gE envelope glycoprotein | Virol Dept |
| Ulex europaeus-I | Rabbit | NA | Anti-lectin | Endothelial cells, epithelial cells | Sigma Aldrich |
| EGFR     | Mouse, IgG1, kappa | E30 | EGFR | 170 kDa wild-type EGFR and EGFR variant | Sigma Aldrich |
| VEGF     | Mouse, IgG1, kappa | V91 | VEGF | VEGF-121, VEGF-165, and VEGF-189 isoforms | DAKO |
| Ki67     | Mouse, IgG1, kappa | MIB-1 | Ki67 | All active phases of the cell cycle (G1, S, G2 and M phases) | DAKO |
| CD45     | Mouse, IgG1, kappa | 2B11 + PD7/26 | LCA | CD45 | DAKO |
| CD8      | Rabbit | NA | Chain of CD3 | Pan T-cell | DAKO |
| CD45Ro   | Mouse, IgG2a, kappa | UCHL1 | CD45Ro | Activated T-cells | DAKO |
| CD45RA   | Mouse, IgG1, kappa | 4KB5 | CD45RA | B-cells | DAKO |

NA = Not applicable.
**Table 2.** Different HSV-I and HSV-II-related mucocutaneous reactive patterns

| Pattern        | Frequency | Type of viral infection | TK-resistance | Clinical pattern | Histology                  | Ref. |
|----------------|-----------|-------------------------|---------------|-----------------|----------------------------|------|
| Vesicular      | Usual     | Productive             | Exceptional   | Labial/genital herpes | Intraepithelial vesiculation; CPE: +++ | 8    |
| Granulomatous  | Rare      | Non-productive         | NA            | Granulomatous lesions  | Mid/deep dermal granuloma; CPE: absent | 9    |
| Verrucous/ulcerated | Rare | Non- or very low-productive | Frequent | Wart-like lesions with central ulceration | Hyperacanthosis, hyperkeratosis; CPE: rare | 14   |
| Lichonoid      | Rare      | Low-productive         | Rare?         | Lichen planus-like lesions | Lichenoid infiltrate; CPE: rare or absent | 15   |
| Follicular     | Rare      | Productive             | Rare?         | Inflammatory or pustular folliculitis | Superficial or deep folliculitis; CPE: rare | 15   |
| Angiogenic     | Rare      | Low-productive         | Rare?         | PG-like lesions     | Neo-angiogenesis; CPE: rare | 15   |

NA = Not applicable; Ref. = reference.

**Fig. 1.** a Large pseudotumoral neoangiogenic lesion of the left perioral area. b Magnification of the pseudotumoral neoangiogenic process. c Complete crusting after 2 weeks of antiviral therapy. d Residual erythematous post-inflammatory pigmentation at 4 weeks.
Fig. 2. a Histology evidencing PG (HE ×10). The black arrow indicates epithelial cell islands in the PG. b, c High-power magnification illustrates some keratinocytes exhibiting CPE. d HSV-1-specific immunostaining (red signal) in giant epithelial cells (black arrows), some exhibiting cytopathic signs (×20).
Fig. 3. **a** Ulex europaeus lectin immunohistochemical expression in HSV-I-infected epithelial cells (=>) as well as in endothelial cells (–>) (red signal, ×20). **b** VEGF immunohistochemical expression in HSV-I-infected epithelial membranes (red signal, ×40). **c** EGFR immunohistochemical expression in HSV-I-infected epithelial membranes (brown signal, ×20). **d** Ki67 immunohistochemical expression in epithelial and endothelial cells, suggesting proliferation (red nuclear signal, ×40).