Selective Defect in Plasmacyoid Dendritic Cell Function in a Patient with AIDS-Associated Atypical Genital Herpes Simplex Vegetans Treated with Imiquimod

Lillian Abbo,1,2 Vladimir Vincek,1,2 Gordon Dickinson,1,2 Niraj Shrestha,2 Susanne Dobiecki,1,2 and Patrick A. J. Haslett 1,2

1The Miami Veterans Administration Medical Center and 2University of Miami Miller School of Medicine, Miami, Florida

We report a case of acquired immunodeficiency syndrome (AIDS)–associated, acyclovir-refractory genital herpes infection treated with topical imidazoquinoline therapy. The patient’s plasmacytoid dendritic cells made a robust interferon-α response following in vitro stimulation with imidazoquinoline but not with herpes simplex virus. We hypothesize that disease resulting from defective herpes simplex virus–stimulated interferon-α may be overcome by stimulating intact alternative pathways.

In patients with advanced HIV-1 infection, unusual and severe manifestations of herpes simplex virus (HSV) infection are common and are included among the opportunistic processes that define AIDS [1]. The use of long-term immunosuppressive therapy to control recurrent HSV episodes may lead to drug resistance in these patients. Immune system–stimulating agents that promote IFN-α production may represent an alternative or adjunct approach to treatment with antiviral agents. IFN-α is a key component of the innate immune response, which plays a critical role in the control of viral infections, including HSV infection. Recently, a specialized cell, the plasmacytoid dendritic cell (pDC), was shown to be the principal producer of IFN-α in peripheral blood [2]. However, this function is deficient in patients with AIDS, a deficiency that may predispose them to opportunistic infections [3]. IFN-α production by pDCs occurs in response to ligation of pathogen-recognition receptors called Toll-like receptors (TLRs). Of the 10 known human functional TLRs, TLR7 and TLR9 are expressed by pDCs and recognize viral RNA and DNA motifs, respectively [4]. HSV is known to stimulate IFN-α production via TLR9, as well as by TLR-independent pathways [5]. TLR7, on the other hand, is stimulated by RNA motifs, such as those present in HIV-1, as well as by synthetic ligands, such as the imidazoquinolines [6]. Here, we present the case of a patient with advanced AIDS and disabling HSV infection that did not respond to prolonged acyclovir therapy but resolved upon topical application of the imidazoquinoline drug imiquimod. We demonstrate the application of a novel assay of TLR-induced IFN-α in peripheral blood pDCs and discuss the apparent paradox of successful therapeutic immune stimulation in a patient whose disease is a manifestation of immune system failure.

Clinical case report. A 42-year-old man with chronic, advanced HIV-1 infection presented to the hospital with a 5-week history of multiple painful ulcers of the penis and scrotum. Before this presentation, the patient had been admitted to another hospital with a diagnosis of Cryptococcus neoformans meningitis, for which he was treated with liposomal amphotericin B for 2 weeks, followed by fluconazole, with clinical improvement. The genital ulcers had developed during that hospitalization and were treated empirically with intravenous acyclovir; however, acute renal failure, attributed to amphotericin toxicity, developed, and acyclovir therapy was discontinued after 4 days. The patient was discharged with unhealed genital ulcers and was subsequently admitted to our institution (Miami Veterans Administration Medical Center, Miami, FL) with worsening pain in the genital area and large, tender hyperkeratotic and exophytic lesions on the penis and scrotum and in the inguinal area (figure 1A). The patient had been poorly adherent to antiretroviral therapy. His peripheral blood CD4+ T cell count was 17 cells/mm³, and his plasma HIV-1 level was 12,685 RNA copies/mL.

In the past, the patient had attended the clinic with recurrent genital HSV infection. On 1 occasion, 2 years previously, a skin biopsy was performed because of the unusual fleshy appearance of the lesions and concerns about possible granuloma inguinale or malignancy. However, histopathological findings were consistent with HSV infection, although with an atypical eosinophilic infiltrate. He was successfully treated with oral acyclovir, followed by suppressive doses of this drug until the onset of cryptococcosis.

On this occasion, genital Herpes simplex vegetans was diagnosed and confirmed by culture of HSV type 2. Skin biopsy
Figure 1. A, Scrotal herpetic lesions displaying hyperproliferative vegetative changes, unchanged after 2 weeks of intravenous acyclovir therapy. Similar lesions were present on the penis and in the inguinal creases. B, Hematoxylin-eosin stain of a skin punch biopsy specimen. The arrow indicates herpes virus inclusions (original magnification, ×400). C, Hematoxylin-eosin stain of a skin punch biopsy specimen showing periadnexal and perivascular lymphohistiocytic infiltrate and increased numbers of eosinophils (arrows) (original magnification, ×400). D, Healing with near-complete epithelialization following 14 days of treatment with 5% imiquimod applied perilesionally and oral famciclovir. E, Frequency of peripheral blood plasmacytoid dendritic cells (pDC) expressing intracellular IFN-α following treatment of whole blood with resiquimod (Resiq), CpG DNA, or herpes simplex virus (HSV). Frequency is expressed as the percentage of PBMCs multiplied by 10^3. HC, simultaneously analyzed healthy control subject; PT, patient; US, unstimulated control subject.

revealed typical herpes inclusions (figure 1B) in inflamed ulcerated skin. Periadnexal and lymphohistiocytic infiltrates with increased numbers of eosinophils were observed (figure 1C). The patient began empiric treatment with intravenous acyclovir, dosed appropriately for renal function. The results of laboratory tests for syphilis and gonorrhea were negative. There was no clinical improvement after 14 days of acyclovir therapy. Drug susceptibility testing was not performed, so the clinical impression of acyclovir resistance could not be confirmed. To avoid treatment with nephrotoxic second-line antiviral agents, the patient was given a trial regimen of topical 5% imiquimod cream (each dose equivalent to ~250 mg of imiquimod), applied around the lesions 3 times per week, together with oral famciclovir (500 mg twice daily), following a report of success with this regimen in a case of AIDS-associated genital HSV infection refractory to treatment with antiviral drugs [7].

Within 6 days after initiation of imiquimod and famciclovir therapy, the hyperkeratotic areas began to separate, revealing new granulation tissue. By day 14, there was almost complete epithelialization of the lesions, as shown in figure 1D. Of note, no constitutional symptoms were reported by the patient to suggest significant systemic activation of IFN-α production.

**Laboratory analysis.** Because imiquimod therapy was aimed at stimulating the patient’s IFN-α response, we were interested in evaluating this function in vitro. Informed consent was obtained from the patient, and we performed a novel flow cytometric assay to measure IFN-α production by the patient’s peripheral blood pDCs following various stimuli, as described elsewhere [8]. In brief, aliquots of 180 μL of heparin-anticoagulated blood were stimulated with 10 μM of resiquimod, which is a potent analog of imiquimod, 50 μg/mL of immunostimulatory CpG DNA (obtained from Coley Pharmaceutical Group), or live HSV type 1, strain 2931 (cultured in our laboratory) at an estimated multiplicity of infection of 5 infectious units per PBMC. Resiquimod is also a ligand for TLR8, expressed by myeloid dendritic cells. However, resiquimod does not stimulate significant IFN-α production by myeloid dendritic cells [8]. Following 3 h in culture at 37°C, RBCs were lysed, and cells were permeabilized for 4-color fluorescent antibody staining to identify CD123-bright, HLA-DR–positive, lineage-negative pDCs, as well as intracellular IFN-α. We recently described this technique in detail elsewhere [8]. As a control, we performed a simultaneous assay using blood samples obtained from an age-matched healthy donor.

As expected, we observed reduced numbers of pDCs in the patient, compared with the control subject (0.03% vs. 0.08% of PBMCs). However, following stimulation with resiquimod, there were comparable numbers of IFN-α–positive pDCs in the samples from the patient and the control subject (figure 1C), reflecting a greater percentage of pDCs expressing IFN-α in the patient than in the control subject (21.7% vs. 12.8%). In contrast, there was a negligible response to either HSV or TLR9 stimulation with CpG in the patient, compared with the control subject (figure 1C). The levels of response in the healthy control subject are similar to those we have observed in a cohort of healthy individuals that we have analyzed recently [8] (unpublished data). Our findings suggest that the patient was unable to mount an IFN-α response to HSV infection, perhaps...
because of a defect in TLR9 signaling, whereas there was a relatively preserved response to TLR7 stimulation with resiquimod.

**Discussion.** Exuberantly proliferative HSV lesions, as seen in this patient, have been reported in patients with AIDS and are thought to be a reflection of the duration of the disease rather than the result of any inherent change in the pathogenicity of the herpesvirus [9, 10]. It has been suggested that local cytokine production may result in enhanced proliferation of keratinocytes [9]. Of interest, our patient had presented with a similar hyperproliferative clinical picture of HSV infection 2 years earlier, in which an eosinophil infiltrate was also observed, although the infiltrate was less striking than on this occasion. The consistency of the unusual clinical and histological pattern on both occasions suggests that the same dysregulated host response was contributing to pathogenesis. Following clinical failure with acyclovir, and in the absence of formal drug-susceptibility data, we chose to treat this patient’s severe disease with both famciclovir and imiquimod, because it was important to avoid nephrotoxic agents, and this combination had been reported by Danielsen et al. [7] to be effective in a similar case. We cannot exclude the possibility that our patient’s disease would have responded to famciclovir alone. In the case reported by Danielsen et al. [7], there was clinical resistance to acyclovir and famciclovir when these agents were administered alone, despite in vitro drug susceptibility. More recently, Brummitt [11] reported success with topical imiquimod in a case of non-HIV-associated recurrent genital HSV infection with documented acyclovir resistance. We think that our experience confirms the potential role of imiquimod in the treatment of HSV infections that are unresponsive to standard antiviral agents.

The presence of eosinophils in the patient’s lesions is consistent with a TH-2 cytokine response, which is characteristic of advanced HIV-1 disease. Imiquimod is known to promote TH-1 immunity, presumably via IFN-α production [12], and this immunomodulatory effect may, therefore, have corrected the local immunopathological process. Using a novel assay, we observed that our patient’s imidazoquinoline-induced IFN-α response was relatively preserved, while the response induced by HSV, or the TLR9 pathway stimulated by HSV DNA, was defective. Thus, we speculate that the clinical efficacy of imiquimod was the result of in vivo stimulation of IFN-α, via TLR7, in a patient in whom TLR9 activation by endogenous HSV was failing. Other investigators have noted a loss of HSV-induced IFN-α production in patients with advancing HIV-1 infection, which seems to progress more rapidly than the decrease in pDC numbers [3] (F. P. Siegal, personal communication). It is possible that differential defects in TLR signaling can explain these discrepancies. However, the mechanism for a selective failure of IFN-α induction by HSV/TLR9 is unknown. Further studies of TLR function and IFN-α induction in chronic, recurrent HSV infection in patients with and patients without HIV-1 infection will be necessary to elucidate these observations.

**Acknowledgments**

We thank 3M Pharmaceuticals for kindly providing resiquimod.

**Financial support.** Dana Foundation Human Immunology Program grant (to P.A.J.H.).

**Potential conflicts of interest.** All authors: no conflicts.

**References**