

Case Report

Clinical presentation of tuberculoid leprosy in an epidermodysplasia verruciformis patient

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Abstract

Epidermodysplasia verruciformis (EV) is triggered by a variety of mechanisms that at least partly include genetic background. We present a Brazilian man with a 30-year history of flat, wart-like lesions with clinical, histopathological, and evolutive aspects consistent with papillomavirus (HPV)-associated EV. Histological analysis of the wart lesions showed epidermis with hyperkeratosis, regular acanthosis, hypergranulosis, and cells with abundant basophilic cytoplasm. Moreover, a perivascular lymphocytic infiltrate was found in the superficial dermis, consistent with a viral wart. Type-2-HPV DNA was detected in various fragments of skin-wart lesions using the polymerase chain reaction (PCR). Two years after the EV diagnosis, the patient presented with an anesthetic well-demarcated, erythematous and mildly scaly plaque on his right forearm. A histopathological analysis of this lesion demonstrated the presence of a compact tuberculoid granuloma. Ziehl-Neelsen staining demonstrated the presence of rare acid-fast bacilli and confirmed the tuberculoid leprosy diagnosis. The patient's Mitsuda Intradermal Reaction was positive. To elucidate the possible mechanism involved in this case of EV, we genotyped the HLA genes of this patient. DQB genotyping showed the polymorphic HLA alleles DQB1*0301 and 0501. The patient was treated with a paucibacillary multi-drug therapy scheme, and the disease was cured in six months. This report describes an EV patient with an *M. leprae* infection, confirming that tuberculoid leprosy patients possess a relatively specific and efficient cell-mediated immunity against the bacillus and, therefore, localized forms of the disease. Moreover, we show the possible involvement of the polymorphic HLA alleles DQB1*0301 and 0501 in EV induction mechanisms.

Key words: Epidermodysplasia verruciformis; papillomavirus; *Mycobacterium leprae*; DQB1*0301/ 0501HLA alleles

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Introduction

Epidermodysplasia verruciformis (EV) presents a variety of symptoms and is triggered by a several mechanisms, including – at least partly – by genetic background. EV is associated with depressed cell-mediated immunity, which facilitates human betapapillomavirus (HPV)-skin infections [1,2,3]. The genus betapapillomavirus comprises more than 40 types of virus that are ubiquitous in the general population. Massive viral replication only occurs in EV-patients. Approximately 30% to 60% of patients experience the malignant transformation of flat wart lesions, especially those living in areas exposed to high levels of solar radiation [4,5,6]. Extreme cases of *Mycobacterium leprae* infection may induce the

polar tuberculoid form of leprosy, which is characterized by a massive Th1 cell-mediated immune response that helps to kill the bacteria. At the other extreme is the polar lepromatous form of leprosy, a highly infectious form of the disease that is characterized by a Th2-type humoral immune response [7]. Herein, we describe the case of an EV patient who developed a susceptibility to HPV and presented with a relatively efficient and specific cellular immune response against *M. leprae*.

Case report

A 54-year-old Brazilian man presented with a 30-year history of the progressive development of verrucous lesions on the skin. A dermatological

examination showed multiple erythematous plaques formed by the coalescence of hyperkeratotic lesions in the extremities of his superior and inferior limbs (Figure 1A) and non-regular depigmented macules similar to pityriasis versicolor on his abdomen and trunk.

Epidermodysplasia verruciformis (EV) diagnostic investigation

The microscopic examination of a biopsy obtained from the skin lesion on the patient's right hand showed epidermis with hyperkeratosis, regular acanthosis (Figure 1B), hypergranulosis (Figure 1C), and cells with abundant basophilic cytoplasm (Figure 1D). Moreover, a perivascular lymphocytic infiltrate was found in the superficial dermis. The clinical, histopathological, and evolutive aspects were consistent with EV. He also had several tumors distributed throughout different regions of his body. A histopathological examination of the excised lesions showed that the vast majority of these lesions were squamous cell carcinoma. The patient has no known family history of similar skin problems and no history of consanguineous marriage in his family.

HPV detection and typing

Type-2-HPV DNA was detected by DNA amplification (PCR) and electrophoresis from various fragments of skin lesions. HPV detection and DNA extraction methods were performed as follows: The skin fragment was digested with 400.0 µg/mL proteinase K for 12 hours at 56°C. After digestion, we extracted the total DNA using the Illustra Kit (GE Healthcare, Little Chalfont, Buckinghamshire, UK) according to the manufacturer's instructions. The quality of the DNA extraction procedure was verified by the amplification of the beta-globin gene using the primers PCO4 and GH20. The sample was positive for beta-globin and considered suitable for analysis. PCR and electrophoresis were performed as follows: all master mixes were prepared in a pre-PCR room sterilized by ultraviolet light for 15 minutes. The final reaction volume of 25.0 µL contained 10 x PCR buffer (Invitrogen, Foster City, California, USA); 2.0 µL MgCl₂; 0.5 µL 20.0 nM DNTP mix; 0.25 µL Platinum Taq DNA Polymerase (Invitrogen, Foster City, California, USA); 1.25 µL of each primer (stock concentration 10.0 µM); 15.25 µL of autoclaved Milli-Q water (Milli Q Plus; Millipore) and 2.0 µL of each sample to be analyzed. The incubation was carried out in the Eppendorf Mastercycler Personal

thermal cycler (Eppendorf North America, New York, USA).

Each sample was screened for the presence of HPV using the standard nested PCR approach for the MY09/11 primer set (primary PCR) and the GP5+/6+ primer set (secondary PCR). Initially, 2.0 µL of each sample was amplified with the MY9/11 primers. The second PCR consisted of the amplification of 2.0 µL of the primary PCR product using the GP5+/6+ primers. The amplification cycle for the GP5+/6+ primer set was as follows: denaturation at 95°C for 45 seconds, primer annealing at 47.7°C for 45 seconds, and amplification at 72°C for 1 minute. This protocol was repeated for a total of 45 cycles and was followed by an extension step of seven minutes at 72°C and storage at 4°C. A negative control that used 2.0 µL of Milli-Q water (EMD Millipore Corporation, Billerica, Massachusetts, USA) in place of DNA was included. A positive control using HPV DNA isolated from HeLa cells was also amplified. The amplified PCR products were resolved by electrophoresis on a 1.5% agarose gel and visualized and photographed using Gel Red™ (GelRed Nucleic Acid Gel Prestaining Kit, Hayward, CA, USA) staining. A DNA marker was included on the gel to aid in interpretation of product size. Bands were recorded as either present (positive) or absent (negative).

HPV typing was performed by restriction fragment length polymorphism analysis (RFLP) after PCR amplification. The 450 base pair products obtained from the PCR were cleaved with the following restriction enzymes according to the manufacturer's instructions: BamHI, DdeI, HaeIII, HinfI, PstI, RsaI and Sau3AI (New England BioLabs, Inc, Ipswich, MA, USA). The digested fragments were separated on an 8% acrylamide gel and stained with silver nitrate [8]. The HPV types identified were clustered into species groups as described in Bernard *et al.* [9].

Leprosy diagnosis

Two years after the initial diagnosis, the patient presented with an anesthetic well-demarcated, erythematous and mildly scaly oval-shaped plaque of approximately 10 cm on his right forearm (Figure 2A). The histopathological analysis of this lesion demonstrated the presence of large epithelioid cells arranged in a compact granuloma in addition to Langhans' giant cells, neurovascular bundles, and a dense peripheral lymphocyte accumulation. Ziehl-Neelsen staining demonstrated the presence of rare

acid-fast bacilli and confirmed the tuberculoid leprosy diagnosis. Although a patient's response to the Mitsuda Intradermal Reaction is not useful in making a diagnosis of leprosy, a positive result in a leprosy patient indicates the patient's ability to develop a granulomatous response with efficient memory CD4⁺ T cell participation and the ability to completely eliminate the bacilli [7]. To confirm the cellular immune response against *M. leprae*, an intradermal injection of 0.1 mL of lepromin was administered into the volar surface of the forearm (Mitsuda Intradermal Reaction, concentration of *M. leprae* = 40,000 bacilli/mL) (ISEP-Paraná, Brazil), and one erythematous papule with a 7-mm diameter was found 28 days later (Figure 2B).

Lymphocyte subset determination

Defects in cell-mediated immunity such as a decreased CD4⁺/CD8⁺ T-cell ratio are common in immunocompromised subjects such as EV patients. Peripheral white blood cell counts taken from this patient at 30-day intervals (n = 3) showed leukocytosis followed by lymphocytopenia. The CD3⁺, CD4⁺, and CD8⁺ T-cell counts were as follows: CD3⁺ = 1465 ± 55.5 cells/mm³ (normal counts = 1609±454 cells/mm³); CD4⁺ = 713.33±17.07 cells/mm³ (normal counts = 837 ± 262); and CD8⁺ = 651.40 ± 49.37 cells/mm³ (normal counts = 660 ± 231). The ratio of CD4⁺/CD8⁺ cells was 1.0 (1.04 ± 0.5). The patient was treated with a paucibacillary multi-drug therapy scheme, and the disease was cured in six months.

EV triggering mechanism

The underlying genetic factors found in EV patients include mutations in the EVER1 and EVER2 genes, which have an autosomal recessive mode of transmission. This transmission mode is supported by the fact that 10% of patients are the offspring of consanguineous marriages and that X-linked inheritance has also been described for at least two genes on two different chromosomes [1,2]. The disease is also linked to certain histocompatibility leukocyte antigen (HLA) haplotypes [10]. EV is associated with depressed cell-mediated immunity as well as defects in antigen presentation by the MHC, both of which can facilitate HPV-skin infections. To identify the mechanism that triggered EV in this patient, we performed human HLA typing and DQB genotyping (PCR-SSP) (One Lambda, Canoga Park, CA, USA). The genotype of this patient was as

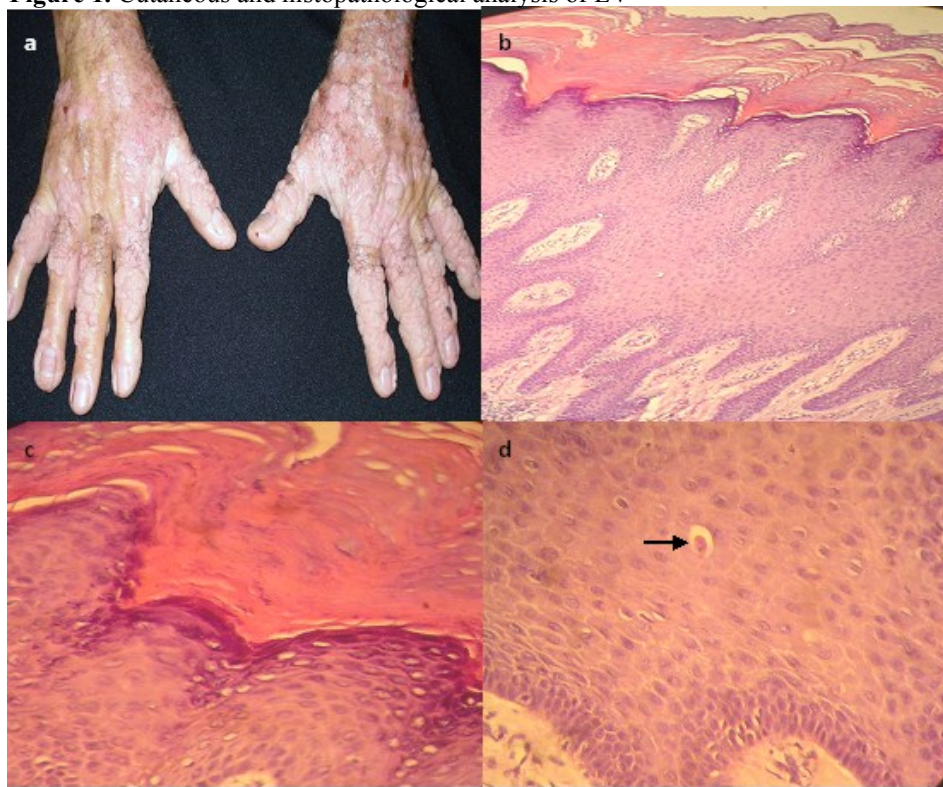
follows: A*03,23; B*1402,4201; DRB1*0102,0804; and DQB1*0301,0501.

To investigate the possibility of EV comorbidities in this patient, we performed serologic tests against hepatitis C (HCV), hepatitis B (HBV), human immunodeficiency virus (HIV), and Epstein-Barr virus (EBV). All of these tests were negative. The renal and hepatic functions of the patient were also within normal limits.

Discussion

Although approximately 75% of EV-patients carry an autosomal recessive defect in the EVER1 and EVER2 genes, there are EV patients who do not have these mutations, suggesting the involvement of other genetic loci [10]. Our patient exhibits the polymorphic HLA alleles DQB1*0301 and 0501, which are highly prevalent in individuals with long-lasting and persistent cutaneous common warts induced by HPV [10]. The HLA system plays a critical role in host defense against pathogens. HLA molecules, which are highly polymorphic, present foreign peptides to antigen-specific T cell receptors. The DQB1*0301 allele may be associated with susceptibility to certain types of HPV-induced warts, enhances the risk of cervical cancer, and may modulate other tissue-specific tumor susceptibilities such as EV [10,12]. A study conducted by Barzegar *et al.* (1998) [4] showed a close association between HIV-EV and the HLA-DQB1*0301 allele, suggesting that this allele could be an EV susceptibility allele. The present case suggests that this allele may be associated with non-HIV associated EV as well. Some authors [10,12] speculate that the immunogenic viral epitopes of some HPV strains may not be presented efficiently by DQB1*0301 alleles, which bind to the peptide with low affinity and thus induce tolerance in T cells. These alterations could be implicated in changes in the T cell levels and the CD4⁺/CD8⁺ T cell ratio, a change observed in our patient and demonstrated in other patients [5]. A type-2-HPV genotype was found in our patient. Studies in the European population have shown that common warts are induced predominantly by HPV-2, HPV-27, and HPV-57 and that EV patients are commonly linked to the oncogenic HPV-5 and HPV-8 types. However, regional differences in HPV distribution and pathogenesis have also been demonstrated [13]. We did not find any studies correlating HPV-genotype and EV in Brazilian populations.

Figure 1. Cutaneous and histopathological analysis of EV



A. Cutaneous wart lesions on the upper side of the hands
B. Microscopy image showing hyperkeratosis and regular acanthosis. (hematoxylin and eosin, 40x)
C. Microscopy image showing hyperkeratosis and hypergranulosis (hematoxylin and eosin, 100x)
D. Detail image showing cells with abundant basophilic cytoplasm and a dyskeratotic cell (arrow) (hematoxylin and eosin, 100x)

Figure 2. Skin lesion and cellular immune response against *M. leprae*



A. Erythematous lesion measuring about 10 cm at its greatest extent, with slightly atrophic center and well-defined borders, located at the dorsal surface of the right forearm
B. Mitsuda Intradermal Reaction site showing a papule measuring 7-mm

Impaired immune responses have been found in EV patients [5,14]. However, in this case, the patient did not block the HPV infection but reacted efficiently to *M. leprae*. The converse situation to our case report (two African sisters suffering from lepromatous leprosy who had EV) has already been described [15]. In contrast to tuberculosis, clinical leprosy does not appear more frequently in immunosuppressed individuals such as HIV-positive subjects [7]. However, immune reconstitution inflammatory syndrome (IRIS) may occur in HIV-infected individuals after starting highly active antiretroviral therapy (HAART). Increasing numbers of leprosy-associated IRIS diagnoses have been reported in countries in which HAART is widely available and HIV and EV are endemic [16].

In conclusion, to our knowledge, this is the first description of an EV patient developing a tuberculoid leprosy infection. The report confirms previous findings that EV patients are capable of mounting a relatively specific and efficient cell-mediated immune response against the bacillus and, therefore, localized forms of the disease. Moreover, we show the possible involvement of the polymorphic HLA alleles DQB1*0301 and 0501 in EV induction mechanisms. Because the number of EV patients is increasing worldwide, doctors should become familiar with the clinical manifestations of this infection to allow for a prompt diagnosis.

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