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Epstein-Barr Virus-positive Mucocutaneous Ulcers as a Manifestation of Methotrexate-associated B-cell Lymphoproliferative Disorders

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Immunosuppressive states due to immunological senescence (1) or administration of immunosuppressants (2) occasionally cause Epstein-Barr virus (EBV)-induced B-cell lymphoproliferative disorders (LPDs). While methotrexate (MTX) is an anti-metabolite and anti-foliate agent for the treatment of cancers and autoimmune disorders, it can also potentiate tumourigenesis due to its immunosuppressive effect. EBV reactivation is observed in half of such cases, suggesting that EBV contributes to the pathogenesis (3, 4). A newly described clinicopathological entity, EBV-positive mucocutaneous ulcer (EMU), occurring in immunocompromised patients, has been proposed (4). We describe here a case of EMU presenting with large deep facial ulcers in association with MTX-LPDs, which has not previously been reported in literature.

CASE REPORT

A 62-year-old woman with polymyositis was treated with low-dose prednisolone (5–10 mg/day) and MTX (5 mg twice a week) for 7 years. Four years before our initial examination, erosive lesions emerged suddenly around her lips and evolved gradually into large ulcers on the mouth, nose and right lower eyelid. Topical anti-bacterial agents, such as gentamicin sulphate, nadifloxacin, and sulfadiazine silver cream, were given by a rheumatologist, with only limited effects. The ulcers progressively enlarged to double the original size and, in November 2007, she was referred to us for clinical assessment of these lesions.

On examination, her body temperature was 36.7ºC. Since she felt intolerable pain when opening her mouth, eating was severely disturbed. Several cervical lymph nodes were palpable at a size of 1–1.5 cm. There were five facial ulcers, ranging from 1–6 cm in diameter, each located on the lower lip to jaw, neck, left nasolabial groove, philtrum, and right lower palpebra (Fig. 1A). The ulcers were sharply demarcated and raised on the skin, with mottled telangiectasia and an erythematous hue, as seen on the jaw. Scars were also noted. Laboratory investigations revealed mild elevations of liver enzymes, lactate dehydrogenase (LDH) (291 IU/ml; <208 IU/ml), aspartate transaminase (AST) (38 IU/ml; <30 IU/ml), alanine transaminase (ALT) (40 IU/ml; <30 IU/ml), leucine amino peptidase (78 IU/ml; <43 IU/ml), and a high elevation of C-reactive protein (6.67 mg/dl; <0.1 mg/dl). White blood cell counts fluctuated within the normal range during the course (5,800–8,800/μl) although mild lymphocytopenia was constantly observed (340–582/μl; 1,500–4,000/μl). Serum immunoglobulin G (IgG) levels were low (689 mg/dl; 1,200–2,120 mg/dl), while serum levels of IgA and IgM were normal. The level of soluble interleukin-2 receptor was extremely high (5,834 IU/ml; <534 IU/ml). Cytomegalovirus pp65 (C7-HRP) antigen-positive cells were detected in 94 cells/48,000 cells (normal 0) of peripheral blood mononuclear cells (PBMCs). No anti-EBV-virus capsid antigen IgM (anti-EBV-VCA IgM), anti-EBV-erythrocyte ATP/ADP ratio IgG (anti-EBV-EADR IgG) or anti-EBV-Epstein-Barr nuclear antigen IgG (anti-EBV-EBNA IgG) was detected. EBV-deoxyribonucleic acid (DNA) copy number in the peripheral blood was 1,500 copies/10^6 PBMCs (normal <20 copies/10^6 PBMCs). The anti-VCA-IgG titre was ×160. β-D glucan levels was 876 pg/ml (<20 pg/ml). These data indicated opportunistic reactivation of cytomegalovirus (CMV), EBV and not-yet-identified fungal infection, presumably due to an underlying immunocompromised status.

Skin histopathology from around the ulcer on the right cheek revealed hyperkeratosis and epidermal inclusion cysts (Fig. 1B). Lymphocytes bearing large nuclei and even Reed-Sternberg (RS) cell-like nuclei had massively infiltrated the dermis and subcutis (Fig. 1C). Large abnormal lymphocytes that clustered around the vessels (Fig. 1D). These large cells were $CD3^-$, $CD15^-$, $CD20^+$, $CD30^+$ and $CD79a^-$, and partially LMP-1^+. Because of similarity in the size and distribution, $CD20^+$ cells, but not $CD3^+$ cells or $CD56^+$ cells, are likely to be EBV-encoded RNA positive

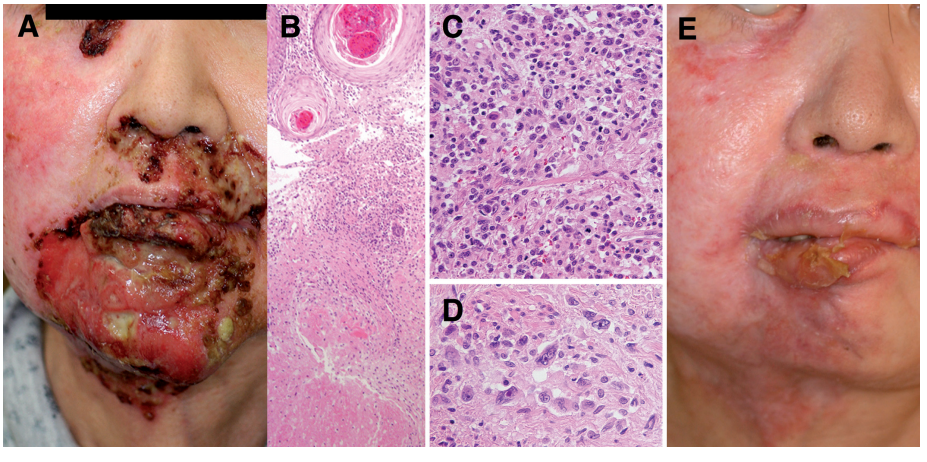

Fig. 1. Clinical and histological findings. Skin ulcers of the face, (A) before and (E) after withdrawal of methotrexate (MTX). Skin histopathology of the right cheek (haematoxylin-eosin staining). (B and C) Lymphocytic infiltration in the skin with hyperproliferative epidermal changes was noted (B: ×40; C: ×100, original magnification). (D) Reed-Sternberg cell-like, large abnormal lymphocytes were located near the dermal vessels (×400, original magnification).

(EBER$^+$), as identified by *in situ* hybridization analysis. PCR analysis of DNA extracted from the skin sample for spectrotyping assay using a LymphoTrack™ IGH TrackOne™ kit (InVivoScribe Technologies, San Diego, CA, USA) identified a monoclonal spike the size of the immunoglobulin gene D1-6 region, indicating monoclonality of the infiltrating B cells. A diagnosis of EMU, in association with MTX-LPDs, was made. MTX was discontinued and prophylactic treatments with the anti-fungal agents, ganciclovir and combined trimethoprim-sulphamethoxazole, were initiated. Ulcers reduced in size dramatically within 2 weeks and healed within one month (Fig. 1E). However, during this time, the patient's body temperature increased to 39ºC and she developed a cough. Chest roentgenograms and bronchoscopic investigation revealed interstitial pneumonitis and bronchial ulcers due to CMV and *Aspergillus* infection. Intensive treatments against these infections, including ganciclovir, valganciclovir, foscarnet and various anti-fungal agents, relieved her symptoms within 3 weeks. A skin biopsy was performed in the vicinity of the first biopsy, and showed sparse lymphocytic infiltration into the dermis. PCR analysis of the skin-derived DNA indicated two substantially lower peaks of different sizes than in the first analysis, confirming that the lymphoma cells had disappeared. EBV-DNA was not detected in the blood. To date, there has been no recurrence of the facial ulcer.

DISCUSSION

Our patient was diagnosed with EMU in association with MTX-LPDs. EMU is a clinical subtype of B-cell LPDs, which was first proposed by Dojcinov et al. (4) and presents with indolent mucocutaneous ulcers located around the lips and within the oral cavity of immunosuppressed patients. However, it is noteworthy in our case that the skin ulcers were impressively deep and large, unlike ulcers in the previous cases of MTX-associated mucocutaneous ulcers, which may provide a clue for diagnosis of B-cell neoplasms. The infiltration of CD30$^+$ EBV$^+$ large B-cells is a pathognomonic hallmark of EMU (4). Since inflamed ulcers develop gradually and may even partially regress, this condition may be initially misdiagnosed as inflammatory and infectious disorders until a skin biopsy is performed (5).

EBV-associated mucosal lesions in immunosuppressed individuals have previously been reported as LPDs or B-cell lymphomas. Although EMU shares some features with other B-cell lymphomas with RS-like cell infiltrations including classical Hodgkin's lymphoma, T-cell-rich B-cell lymphoma and lymphomatoid granulomatosis, there are distinctive clinical and pathological differences. The majority of RS cells in classical Hodgkin's lymphoma are CD20$^-$ and CD15$^+$ and CD30$^+$, while the neoplastic cells in T-cell-rich B-cell lymphoma are CD20$^+$ and CD14$^-$ or CD30$^-$ (6). Lymphomatoid granulomatosis characteristically shows angiocentric infiltration of lymphocytes (7). Resolution of EMU has been reported in more than 30% of reported cases after restoration of immunosuppression. In the case of MTX-LPDs, especially, tumours were observed to regress dramatically (8–11), a feature also seen in our case. Although several cases of MTX-associated skin ulcers due to the drug toxic effect have been reported (12–14), some of these cases might include EMU associated with MTX treatment.

Since the skin seems easily to be affected by this disease, special attention should be given to skin lesions in immunosuppressed patients (5, 15).

The authors declare no conflicts of interest.

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