We describe a unique patient with mosquito-bite hypersensitivity who had extremely high titres of Epstein-Barr virus antibodies. For many years he developed intractable ulcers on the sites of mosquito-bite. Epstein-Barr virus infection was detected in almost all inflammatory cells in the ulcers and in the peripheral blood lymphocytes by using in situ hybridization to Epstein-Barr virus-encoded small ribonucleic acids and by polymerase chain reaction to Epstein-Barr virus DNA. The inflammatory cells in the ulcers were positive for T-cell marker. Our results suggest that the Epstein-Barr virus infection in T cells may participate in the pathogenesis of exaggerated mosquito hypersensitivity and in delayed healing of ulcers on the sites of mosquito-bite.

Key words: chronic active Epstein-Barr virus infection; mosquito hypersensitivity; skin ulcer; T cells.

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Fig. 1. The ventral aspect of an intractable ulcer on the right lower leg of the patient.

Fig. 2. Histopathologic feature of the border of the ulcerated lesion showing a striking pseudoepltheliomatous hyperplasia with a moderate perivascular and periadnexal infiltration of inflammatory cells (×13.2). Inset shows the inflammatory cells consisting mostly of lymphocytes and histiocytes without eosinophils (×100).

Fig. 3. Immunohistochemical analysis of the ulcerated lesion. Most of the infiltrating cells in the dermis are positive (dark brown) for anti-human T-cell antibody (CD45-R0, UCHL-1) (×50).

Fig. 4. Detection of EBV DNA by PCR. The PCR product (129 bp) of EBV BamHI-W was detected in DNA extracted from the tissue sections and lymphocytes in the peripheral blood of the patient. Lanes: Molecular-weight markers (M), Raji cells as the positive control (1), distilled water as the negative control (2), the tissue sections of the ulcerated lesion (3), and lymphocytes in the peripheral blood of the patient (4).

Fig. 5. In situ hybridization of EBV-encoded Small RNAs (EBER) on the ulcerated lesions. (a) EBER positive dark-brown cells were detected in many infiltrating cells (×33); (b) EBER was not detected in infiltrating cells of lichen planus (negative control ×33).

inflammatory cells in the dermis were positive for the anti-human T-cell antibody (Fig. 3), but were negative for the anti-human B cell antibody (data not shown).

Identification of the causative mosquito species by enzyme-linked immunosorbent assay (ELISA) and immunoblot analysis. Four species of mosquitoes, Culex (C.) pipiens pallens, C. tritaeniorhynchus, Aedes (A.) togoi and A. albopictus were studied. ELISA was performed as described previously (1). The antigen-antibody complex was detected with ortho-phenylenediamine (Ortho Diagn. Syst. Rantian, NJ, USA). In the patient’s serum, the titer to A. albopictus was still detected at 1:6,400. The antibody to A. togoi was detected at 1:3,200 but not at 1:6,400. C. tritaeniorhynchus and C. pipiens pallens were detected only at 1:100, respectively. The antibodies in the control serum were detected only at 1:100 for all mosquitoes.

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Proteins in the mosquito extracts were separated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) (3). Immunoblotting was performed with the ABC (avidin-biotin complex) method using Vectastain ABC-AP Kit (Vector Lab. Buringame, CA, USA). Colour development was performed by the alkaline phosphatase method. Proteins with molecular weight of 16, 19, 26 and 31 kDa were detected in the extracts of A. albopictus and proteins with 17 and 31 kDa were detected in the extracts of A. togoi. Proteins were not detected in the extracts of either C. pipiens pallens or C. tritaeniorhynchus. ELISA and immunoblot analysis suggest that the causative mosquitoes are probably Aedes species, which are common in Japan.

Detection of EBV DNA by polymerase chain reaction (PCR).

Samples of the patient were obtained from the cutaneous lesions of mosquito-bite on his first visit to our clinic in August 1997 and from the peripheral blood 8 months later. DNA extraction from paraffin-embedded sections of the biopsy specimen and peripheral blood lymphocytes were performed as previously described (4). Lymphocytes were separated by Ficoll-Conray (5). The primers were designed to amplify a 129-base pair segment in the BamHI-W region of EBV genome, as previously described (6). PCR consisted of 34 cycles of denaturation at 94°C for 1 min, annealing at 58°C for 1 min, and extension at 72°C for 1 min. DNA extracted from an EBV-infected cell line Raji and distilled water were used as the positive and negative controls, respectively. The PCR product (129 bp) was detected in DNA extracted from the sections (lane 3), lymphocytes of the patient (lane 4) and Raji cells (lane 1), but not detected in the negative control (lane 2) (Fig. 4).

In situ hybridization of EBV-encoded small RNAs (EBER).

In situ hybridization was carried out in paraffin-embedded sections of the biopsy specimen using a digoxigenin labelled EBER-1 30 base oligonucleotide probe. The sequence used was 5'-AGA CAC CGT CCT CAC CCG GGA CTT GTA-3' as previously described (7, 8). Briefly, deparaffinized sections were dehydrated and pretreated with pepsin, then hybridized with the fluorescein-conjugated EBER oligonucleotides. Colour solution was prepared with bromochloroindolylphosphate and nitroblue tetrazolium (Nichirei Co., Tokyo, Japan). The sections were counterstained with Kernechtrot. As a negative control, paraffin-embedded sections of lichen planus were used. EBER was detected in almost all inflammatory cells of the bite lesion (Fig. 5a) but not in the inflammatory cells of lichen planus (Fig. 5b).

DISCUSSION

The patient suffered from typical MH with clinical features of high fever and erythematous to papular lesions followed by ulceration a few hours after being bitten by mosquitoes. According to previous papers (1, 9, 10), histology of the lesion shows epidermal necrosis, extravasation of erythrocytes and infiltration of eosinophils and neutrophils in the early stage after the mosquito-bite. These findings suggest that MH may be induced by an Arthus reaction. Apart from the Arthus reaction, the patient showed not only an increase in IgA and IgE levels but also negative reactions to the Mantoux test and DNBC sensitization. This implies a down-regulation of cell-mediated hypersensitivity.

Interestingly, the patient had extremely high antibody titers against EBV, indicating that he suffered from chronic active EBV infection (11). Furthermore, EBV DNA was detected in the cutaneous lesion of mosquito-bite as well as in the peripheral blood lymphocytes. EBER positive cells were observed in the inflammatory infiltrate in the ulcerated lesion. The inflammatory cells were positive for T-cell marker. These data suggest a significant relationship between MH and EBV infection in T cells. It remains to be studied why EBV-infected T cells infiltrate the ulcerated lesion of mosquito-bite.

It was a striking clinical feature of this patient that the skin ulcers were resistant to common topical treatments. Histologically, the edges of ulcers showed pseudoplasmatous hyperplasia and the ulcers did not show specific granulomatous changes. These clinical and histologic findings are similar to those of pyoderma gangrenosum (PG). However, our patient did not show a progressive enlargement of the ulcers, as in PG, and the borders of the ulcer were not undermined. Furthermore, although the etiology of PG remains unknown, pathergy has been proposed in the pathogenesis, which means the development of new PG lesions or aggravation of existing ones following trivial trauma (12, 13). Our patient did not have any clinical manifestations for pathergy and mosquito-bite was a definite etiologic factor. We therefore believe that the pathogenesis of ulcer formation in MH is different from that in PG.

T cells produce cytokines, such as epidermal growth factors and fibroblast growth factors, that directly stimulate the proliferation of vascular cells and fibroblasts, suggesting that T cells play an important role in promoting wound healing (14). Thus, delayed healing of the ulcers can result from dysfunction of T cells impaired by EBV infection.

In our patient, combined treatment with oral and local administration of corticosteroids was very effective if given immediately after the mosquito-bites, thus confirming a previous report by Tokura et al. (10).

Exaggerated mosquito-bite reactions may be associated with chronic lymphoproliferative disorders such as lymphocytic leukemia (15). Furthermore, many patients with MH have died of hemophagocytic syndromes, such as malignant histiocytosis and virus-associated hemophagocytic syndrome (16). On the other hand, EBV is closely associated with T-cell and natural killer cell proliferation (2) and lymphomas (17). MH may therefore be one of the prodromal phenomena of EBV-associated lymphoproliferative diseases or histiocytic proliferative diseases.

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