

CLINICAL REPORT

A Case of Exaggerated Mosquito-bite Hypersensitivity with Epstein-Barr Virus-positive Inflammatory Cells in the Bite Lesion

TOKUYA OHSAWA¹, TSUKASA MORIMURA¹, YOSHITAKA HAGARI¹, TETSUJI KAWAKAMI¹, MOTOYUKI MIHARA¹, KAZUMITSU HIRAI², KAZUFUMI IKUTA³, MASANAO MURAKAMI³, TAKESHI SAIRENJI³ and TOMOCHO MATSUSE⁴

Departments of ¹Dermatology, ²Medical Zoology and ³Biosignaling, Faculty of Medicine, Tottori University, Yonago, and ⁴Department of Biodefence Medicine, Faculty of Medicine, Toyama Medical and Pharmaceutical University, Toyama, Japan

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.

(Accepted September 3, 2001.)

Acta Derm Venereol 2001; 81: 360–363.

T. Ohsawa, Department of Dermatology, Faculty of Medicine, Tottori University, Nishi-cho, Yonago, 683-8504, Japan.

Mosquito-bite hypersensitivity (MH) is a disorder which has cutaneous symptoms such as local erythema, swelling, bullae and ulcers with accompanying systemic high fever (1). The disorder is extremely rare, with only 41 cases, including our case, reported so far (2). Here we report a case of MH in which Epstein-Barr virus (EBV) infection was detected in the infiltrated T cells in the large intractable ulcers developed by mosquito-bites.

CASE REPORT

Clinical course

A 17-year-old Japanese boy visited our clinic in August 1997, for large deep ulcers on his bilateral legs following mosquito-bites 3 weeks prior to consultation. Since the age of 6, he had noticed that, following mosquito-bite, erythematous to papular lesions developed on the bitten areas and that this was associated with systemic fever. The lesions subsequently necrotized and then healed with residual scars. In the middle of July, 1997, he was bitten by mosquitoes on the legs and feet, and again developed high fever after a few hours. The bitten sites became erythematous and then ulcerated. On examination, three large irregularly shaped ulcers were observed on the medial aspect of the right lower leg near the foot joints, and on the outer dorsal surface of the right foot (Fig. 1). The

ulcers were covered partly with brown to black eschars and partially with granulation tissues. The borders of the ulcers were fringed with necrotic grey eschars. His forehead and upper and lower extremities had many depressed mosquito-bite scars.

A biopsy specimen was obtained from the border of the ulcer and the histopathology showed a striking pseudoepitheliomatous hyperplasia with moderate perivascular and periadnexal infiltration of lymphocytes and histiocytes without eosinophils (Fig. 2).

Laboratory results (the normal ranges in parentheses) were as follows: leukocyte count 1.33×10^{10} cells/l ($0.33\text{--}0.88 \times 10^{10}$ cells/l) with 66% (13–57%) lymphocytes (atypical lymphocytes were not detected) and 0% (< 8%) eosinophils; erythrocyte 4.54×10^{12} cells/l ($4\text{--}5.7 \times 10^{12}$ cells/l); hemoglobin 132 g/l (120–170 g/l); hematocrit 36% (36–51%); total serum protein 67 g/l (60–80 g/l); albumin 30 g/l (35–50 g/l); total bilirubin 3 mg/l (2–12 mg/l); aspartate aminotransferase 26 IU/l (5–40 IU/l); alanine aminotransferase 20 IU/l (5–47 IU/l); T-cell subsets: CD4 (+) cells 12.5% (25–56%); CD8 (+) cells 10.2% (17–44%); CD4/CD8 ratio 1.23 (0.6–2.9). Immunoglobulins: IgM 1.58 g/l (0.7–1.6 g/l); IgA 5.53 g/l (1.29–3.17 g/l); IgG 1.5 g/l (1.14–1.72 g/l); IgE 769 IU/ml (< 457 IU/ml). EBV antibody titers: anti-viral capsid antigen (IgG) 1:5,120; anti-early antigen (IgG) 1:1,280; anti-EBV-associated nuclear antigen 1:1,280. The Mantoux test was negative and sensitization with dinitrochlorobenzene (DNCB) was 0.1% positive but 0.01% negative. The computed tomography scan showed slight splenohepatomegaly.

Despite topical treatment with sulfadiazine silver, the ulcers became deep, and required debridement and grafting. In mid-July 1998, his left upper extremity was again bitten by mosquitoes and erythema was observed at the bitten areas. Immediately after prednisolone 50 mg/day was given orally, and triamcinolone acetonide 20 mg was injected locally into the sites of bites. Fever abated and the lesions healed spontaneously without ulceration.

Laboratory studies

Immunohistochemistry for the inflammatory cells in bite lesion. An avidin-biotin complex immunoperoxidase technique was performed for the paraffin-embedded sections of the biopsy specimen. As primary antibodies, mouse anti-human T-cell (CD45-R0, UCHL-1; Dako, Kyoto, Japan) and mouse anti-human B-cell (MB1; Bio-Science Products, Emmenbrucke, Switzerland) monoclonal antibodies were used. Almost all

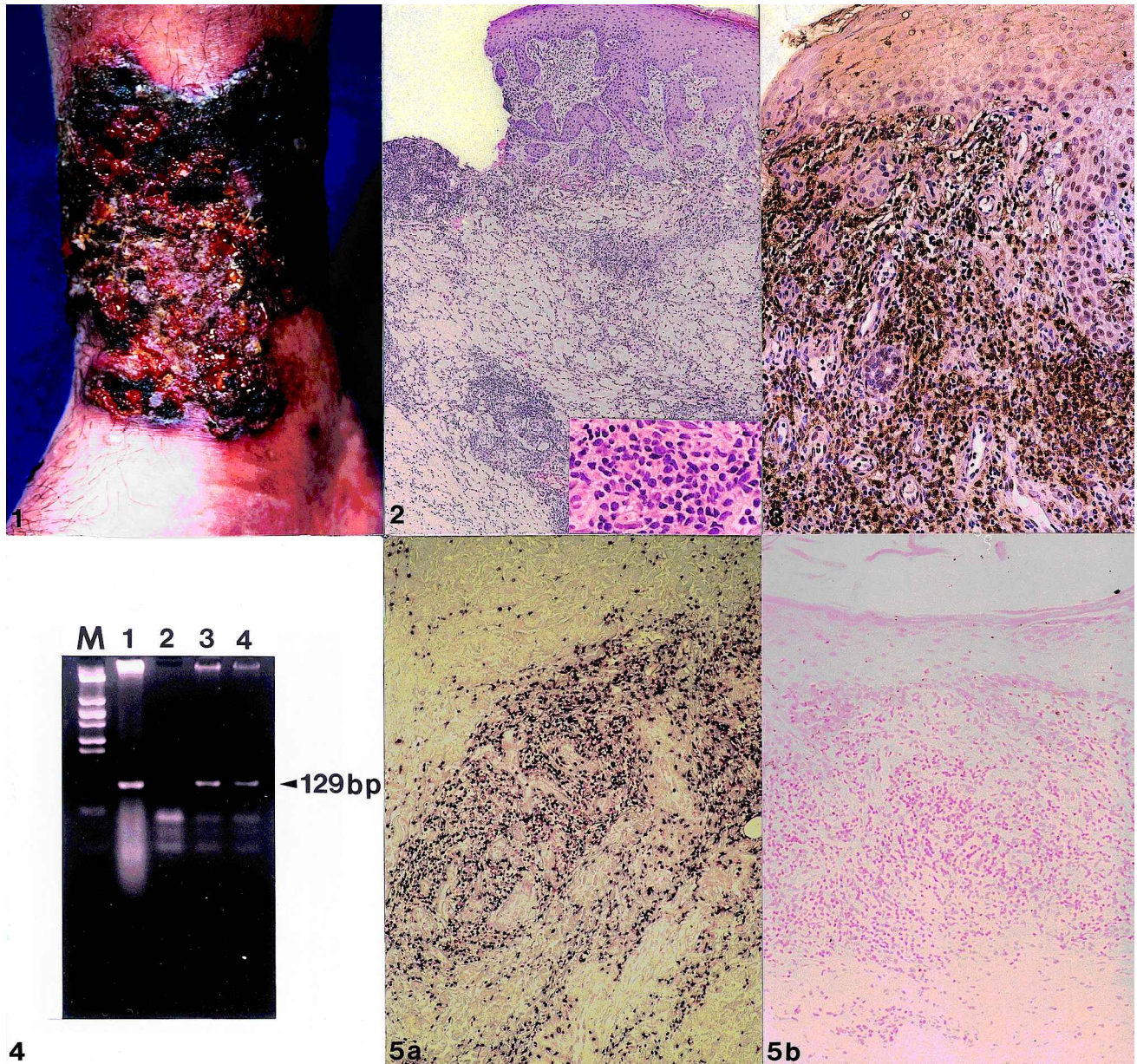


Fig. 1. The ventral aspect of an intractable ulcer on the right lower leg of the patient.

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

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) ($\times 50$).

Fig. 4. Detection of EBV DNA by PCR. The PCR product (129 bp) of EBV *Bam*HI-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 ($\times 33$); (b) EBER was not detected in infiltrating cells of lichen planus (negative control $\times 33$).

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 orthophenylenediamine (Ortho Diagn. Syst. Ratian, 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.

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. Burlingame, 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 *Bam*HI-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 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 DNCB 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 pseudoepitheliomatous 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.

ACKNOWLEDGEMENTS

We are grateful to Dr. Gen-i Hazama for his helpful discussions, and to Akie Koseki for her excellent technical assistance and Sayuri Matsumoto for typing the manuscript.

REFERENCES

1. Sugihara K, Yamada Y, Dekio S, Jidoi J. A case of mosquito hypersensitivity showing abnormality of peripheral monocyte function. *J Dermatol* 1989; 16: 142-145.
2. Ishihara S, Ohshima K, Tokura Y, Yabuta R, Imaishi H, Wakiguchi H, et al. Hypersensitivity to mosquito bites conceals clonal lymphoproliferation of Epstein-Barr viral DNA-positive natural killer cells. *Jpn J Cancer Res* 1997; 88: 82-87.

3. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 1970; 227: 680–685.
4. De Bruin PC, Jiwa NM, van der Valk P, Van Heerde P, Gordijn R, Ossenkoppele GJ, et al. Detection of Epstein-Barr virus nucleic acid sequences and protein in nodal T-cell lymphomas: relation between latent membrane protein-1 positivity and clinical course. *Histopathology* 1993; 23: 509–518.
5. Boyum A. Separation of white bloods cells. *Nature* 1964; 204 793–794.
6. Ikuta K, Satoh Y, Hoshikawa Y, Sairenji T. Detection of Epstein-Barr virus in salivas and throat washings in healthy adults and children. *Microbes and Infection* 2000; 2: 115–120.
7. Chang KL, Chen YY, Shibata D, Weiss LM. Description of an *in situ* hybridization methodology for detection of Epstein-Barr virus RNA in paraffin-embedded tissues, with a survey of normal and neoplastic tissues. *Diagn Mol Pathol* 1992; 1: 246–255.
8. Tokunaga M, Uemura Y, Tokudome T, Ishidate T, Masuda H, Okazaki E, et al. Epstein-Barr virus related gastric cancer in Japan: a molecular patho-epidemiological study. *Acta Pathol Jpn* 1993; 43: 574–581.
9. Brown A, Griffiths THD, Erwin S, Dyrenforth LY. Arthus's phenomenon from mosquito bites: report of a case with experimental studies. *South Med J* 1938; 31: 590–596.
10. Tokura Y, Tamura Y, Takigawa M, Koide M, Satoh T, Sakamoto T, et al. Severe hypersensitivity to mosquito bites associated with natural killer cell lymphocytosis. *Arch Dermatol* 1990; 126: 362–368.
11. Rickinson AB. Chronic, symptomatic Epstein-Barr virus infection. *Immunol Today* 1986; 7: 13–14.
12. Perry HO, Brunsting LA. Pyoderma gangrenosum. A clinical study of nineteen cases. *Arch Dermatol* 1957; 75: 380–386.
13. Wolff K, Stingl G. Pyoderma gangrenosum. Rook/Wilkinson/Ebling. In: Champion RH, Burton JL, Burns DA, Breathnack SM, eds. *Textbook of dermatology*, vol. 2, 6th ed. Blackwell Science, 1998: pp. 1140–1148.
14. Blotnick S, Peoples GE, Freeman MR, Eberlein TJ, Klagsbrun M. T lymphocytes synthesize and export heparin-binding epidermal growth factor-like growth factor and basic fibroblast growth factor, mitogens for vascular cells and fibroblasts: differential production and release by CD4+ and CD8+ T cells. *Proc Natl Acad Sci USA* 1994; 91: 2890–2894.
15. Weed RI. Exaggerated delayed hypersensitivity to mosquito bites in chronic lymphocytic leukemia. *Blood* 1965; 26: 257–268.
16. Ishihara S, Okada S, Wakiguchi H, Kurashige T, Hirai K, Kawa-Ha K. Clonal lymphoproliferation following chronic active Epstein-Barr virus infection and hypersensitivity to mosquito bites. *Am J Hematol* 1997; 54: 276–281.
17. Iwatsuki K, Ohtsuka M, Harada H, Han G, Kaneko F. Clinicopathologic manifestations of Epstein-Barr virus-associated cutaneous lymphoproliferative disorders. *Arch Dermatol* 1997; 133: 1081–1086.