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Case report

A pathological and immunohistological case report of fatal infectious mononucleosis, Epstein-Barr virus infection, demonstrated by in situ and Southern blot hybridization

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Abstract. We present an autopsy case of 20-month-old boy who had a fulminant course of infectious mononucleosis, with severe hepatic failure. Autopsy revealed marked infiltration of immunoblasts in the lymph nodes, liver, spleen, thymus and kidneys. We identified a large number of Epstein-Barr virus (EBV) genomes in the immunoblasts of the lymph nodes, liver and spleen by in situ hybridization. EBV genomes were also detected in the liver and spleen by Southern blot hybridization. Histology of the liver revealed diffuse feathery degeneration of the hepatocytes. However, EBV genomes were not detected in the hepatocytes by in situ hybridization and monoclonal antibody studies. Immunostaining of the autopsy liver specimen revealed a large number of suppressor/cytotoxic T cells (Leu2a positive) in the portal areas and of natural killer (NK) cells (Leu7 positive) in the portal areas and sinusoids of the liver. We therefore suggest that the hepatocellular damage was not caused by the viral replication in the hepatocytes but was mainly caused by the abnormal killer cell activity of the suppressor/cytotoxic T cells and NK cells.

Key words: Infectious mononucleosis – Epstein-Barr virus – In situ hybridization – Suppressor/cytotoxic T cell – Natural killer cell

Introduction

Infectious mononucleosis (IM) is usually a benign self-limited disease caused by Epstein-Barr virus (EBV). The majority of patients with IM recover uneventfully from the infection, and fatal cases are very rare. Since Penman (1970) first reported 20 patients with fatal IM, 200 fatal cases have been reviewed in the English literature. In 1975 Purtilo et al. reported a family with immunodeficiency exclusive in males who were highly susceptible to IM. This disorder was designated as X-linked lymphoproliferative syndrome (Duncan's disease).

In this study, we present an autopsy case on a 20-month-old boy who had a fulminant course of IM. We confirmed EBV genomes in the lymph nodes, liver and spleen by in situ and Southern blot hybridization.

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Case report

The patient was a 20-month-old, previously healthy boy who had no family history of immunological disease. He was taken to his family doctor's clinic because of rhinorrhoea, cough, sore throat and high fever of 39° C. Five days later, he had a general convulsion, lasting for about 80 min. Physical examination at that time showed swelling of infra-auricular lymph nodes and hepatosplenomegaly. Laboratory examination revealed jaundice (3.3 mg/dl of total bilirubin) and marked elevation of SGOT and SGPT (7735 IU/l and 1650 IU/l). He was immediately transferred to Tsukuba University Hospital in a semi-comatose condition. Lung radiography showed bilateral pulmonary hilar shadows, which were suggestive of pulmonary oedema. On admission, in the peripheral blood, red blood cell count was $3.40 \times 10^6/mm^3$; the white blood cell count was $14900/mm^3$ with 17% lymphocytes and 31% atypical lymphoid cells. Blood lymphocyte analysis showed a CD4/CD8 ratio of 0.25 (CD4 cell count of $1147/mm^3$ CD8 cell count of $4554/mm^3$). As he was in severe hepatic failure, daily plasmapheresis and intensive conservative therapy were given from the 6th to 9th day of the disease. On the 9th day pneumoperitoneum and ascites appeared. The following day he underwent a partial jejunectomy with end-to-end anastomosis for perforation of the jejunum. Post-operative coagulopathy developed complicated by melaena and haematuria. From the 13th day plasmapheresis was repeated, but it was ineffective. The patient gradually developed dyspnoea and concentrated oxygen was given continuously from the 8th day. Finally the patient died of respiratory failure caused by massive lung haemorrhage.

Materials and methods

Liver and bone marrow biopsy specimens taken from the patient on the 10th day were fixed in formalin solution and the paraffin sections were examined histologically. Autopsy was performed 2 h after death, and routine histologial examination was performed on the autopsy specimens.

For the purpose of EBV detection in situ DNA hybridization using biotinylated EBV probe (Enzo Biochemical, New York, USA) was performed on several specimens from the lymph nodes, spleen and liver. Southern blot analysis was also done using the ^{32}P-labelled *Bam*-HI W fragment of EBV DNA (kindly supplied by Dr. K. Takada) after *Bam*-HI digestion of DNA, which was extracted from the liver and spleen.

Furthermore, EBV-membrane antigen was immunohistochemically examined by using anti-EBV-membrane antigen antibody (Biosoft, Paris, France). Immunohistochemical analysis of lymphocyte subset was done on the specimens of frozen liver and lymph node tissue using the following cell surface markers; L26 (pan B) (Dako, California, USA), Leu 1 (CD5), Leu 3 (CD4), Leu 2a (CD8) and Leu 7 (CD57) (Becton Dickinson, Mountain View, California, USA).

Results

Histologically the hepatic portal areas were greatly expanded, due to a dense accumulation of mononuclear cells which were mainly composed of immunoblasts and plasma cells. Small lymphocytes and histiocytes were also admixed. Bile ducts in the portal areas had almost disappeared. The remaining epithelial cells of bile ducts were markedly swollen. The limiting plates of hepatocytes were not destroyed; most of the hepatocytes showed feathery degeneration (Fig. 1). However, the reticulin framework of hepatocytes was not disrupted. Immunoblasts were scanty in sinusoids of the liver. There was no spotty necrosis or any single cell necrosis.

The bone marrow appeared normocellular but many immunoblasts were present. Haemophagocytic histiocytes were occasionally observed.

At autopsy the boy was highly icteric. Multiple lymph nodes of the axillary, cervical, mediastinal, retroperitoneal and mesenteric regions were swollen, up to 2 cm in diameter. Lymph nodes showed a diffuse proliferation of immunoblasts (Fig. 2), resulting in the disappearance of normal architecture. No follicles were seen. Small, well-circumscribed necrotic foci were occasionally present in the subcapsular area of the lymph nodes. Enlarged histiocytes with phagocytosis were frequently found in the sinuses. Infiltration of immunoblasts was also observed in the thymus, with moderate atrophy of the cor-

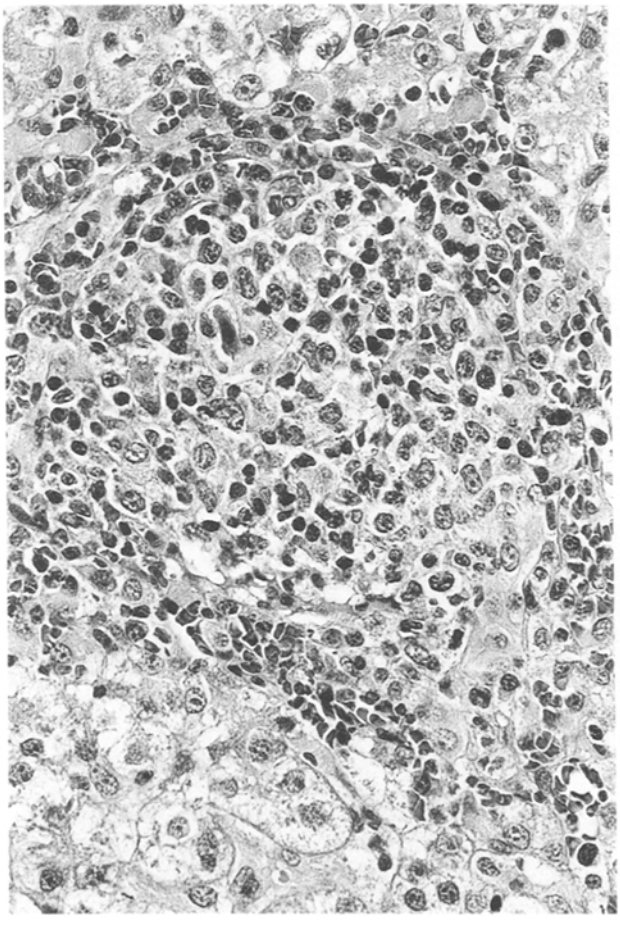

Fig. 1. Histological feature of the liver biopsy specimen showing extension of the portal area with diffuse infiltration of immunoblasts and feathery degeneration of hepatocytes. H & E, ×150

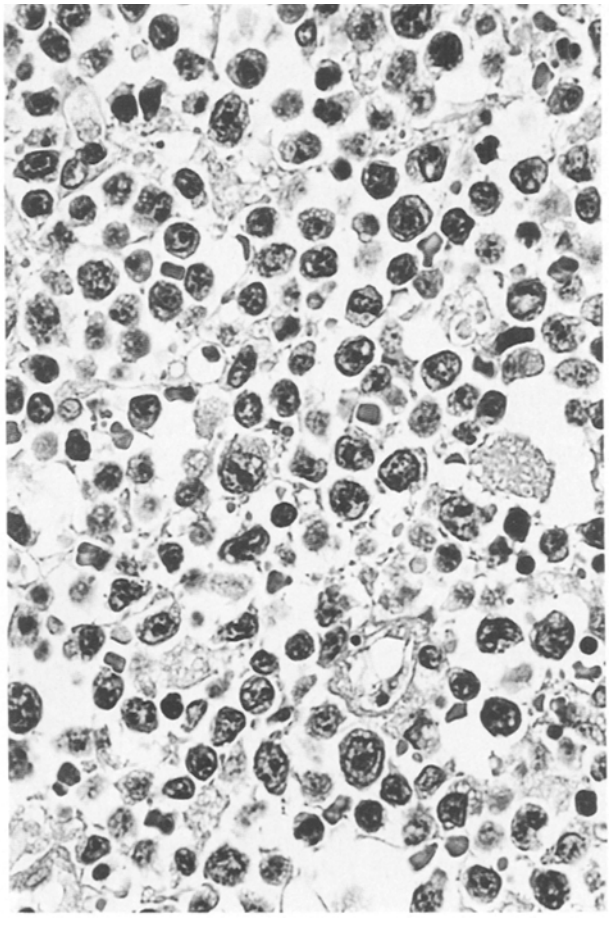

Fig. 2. Diffuse proliferation of immunoblasts in the lymph node. H & E, ×600

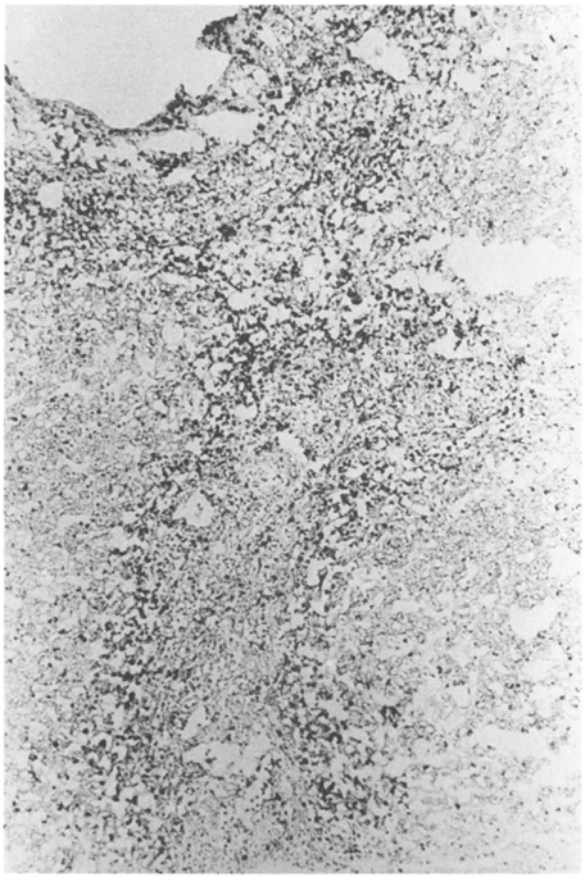

Fig. 3. Immunohistological staining of Leu 2a (CD8) showing many positive cells in the portal areas and sinusoids of the liver. ×60

Fig. 4. Immunohistological staining of Leu 7 showing many positive cells in the portal areas and sinusoids of the liver. ×60

tex and medulla. The bone marrow was hypocellular and a small number of immunoblasts were present.

The liver was greatly enlarged. Infiltrating immunoblasts in the portal areas of the autopsy liver were much more pronounced than those of the biopsy liver. Bile ducts in the portal areas were almost completely destroyed. The limiting plates of hepatocytes were focally destroyed and replaced by mononuclear cells. Other hepatocytes appeared vesicular with numerous small fat droplets. The spleen was markedly enlarged. Heavy infiltrates of immunoblasts were present in the white pulp, resulting in expansion of that area. Several necrotic foci, up to 1 cm in diameter, were present in an irregular shape. Severe acute congestion in the red pulp was present. In both kidneys, immunoblasts infiltrated the interstitium, mainly around small veins. However, tubular epithelia were intact. Both lungs showed severe interstitial haemorrhage in the interlobular septa, extending into alveolar spaces. The peripheral lung showed features of diffuse alveolar damage with alveoli lined by regenerative cuboidal epithelial cells.

Many B lymphocytes (L26 positive) were diffusely distributed in lymph nodes. Suppressor/cytotoxic T lymphocytes (Leu 2a positive) (Fig. 3) and natural killer (NK) cells (Leu 7 positive) (Fig. 4) predominated in the liver while B lymphocytes were scanty there.

Anti-EBV-membrane antigen antibody demonstrated positive reactivity in many immunoblasts and plasma cells at the portal areas of the liver (Fig. 5). The viral DNA was identified as one to three dots in the nuclei of lymphoid cells at the lymph nodes (Fig. 6A) and white pulp of the spleen (Fig. 6B) by in situ DNA hybridization. Southern blot hybridization with EBV probe demonstrated a single 3.1kb band, identical to an EBV-positive control (Raji cell), in the autopsy liver and spleen (Fig. 7).

Discussion

Mroczek et al. (1987) reported detailed morphological features of fatal IM. They described a prominent proliferation of immunoblasts admixed with plasma cells and histiocytes in the lymph nodes, spleen, bone marrow, thymus, liver, lungs, brain, kidneys, gastrointestinal tract and heart. The findings in our case were quite consistent with theirs. We thus diagnosed this case as fatal IM.

In situ and Southern blot hybridization studies for detection of EBV genomes further confirmed our diagnosis. The former showed dot-like positive reactivities in the nuclei of the majority of the immunoblasts in

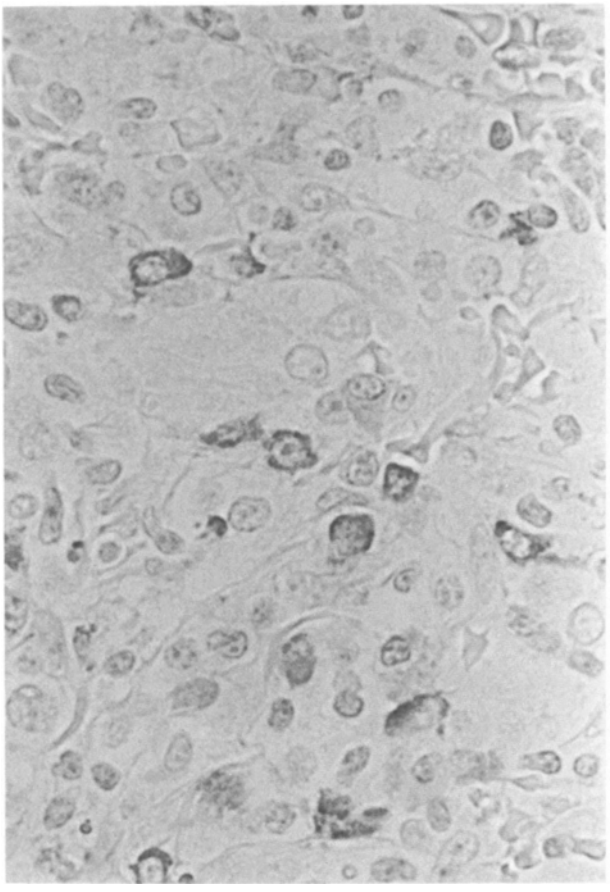

Fig. 5. Immunohistological staining for Epstein-Barr virus (EBV)-membrane antigen revealed positive reactivity in the cytoplasm of some immunoblasts and plasma cells in the portal area of the liver. ×600

the lymph nodes and spleen of the patient. The latter revealed a large amount of EBV genome in the liver and spleen. These results indicated massive replication of virus genome in the immunoblasts infiltrating various organs. Krueger et al. (1987) and Kornstein et al. (1989) identified EBV genomes in cases of fatal EBV infection by in situ hybridization. However, they observed only a small number of positive cells in their studies. We consider that this is the first report to demonstrate clearly massive replication of EBV genomes in fatal IM.

The mechanism of hepatocellular damage is unknown, although there have been several reports indicating hepatic failure as the main cause of death in fatal IM (Markin et al. 1987; Mroczek et al. 1987). The present case showed severe damage of the entire liver. Histologically a large number of immunoblasts and plasma cells were found to infiltrate in and around the portal areas but were not present in sinusoids of the liver. The marked hydropic swelling of hepatocytes observed in our case cannot be attributed only to these immunoblasts and plasma cells distributed locally.

We suggest two possibilities for the hepatocellular damage. First, EBV itself is assumed to have infected hepatocytes and caused degeneration. Chang and Campbell (1975) reported that they found herpes-like virus particles electron microscopically in the hepatocytes of a patient with IM. In situ hybridization on the autopsy liver specimens in our case revealed positive reactivity for EBV genomes in some immunoblasts, but in none of the hepatocytes. Immunostaining on the biopsy liver specimen with the monoclonal antibody for EBV-membrane antigen revealed positive reactivity in some of the infiltrating immunoblasts, but none of the hepatocytes showed positivity. These results could not prove that the hepatocellular damage might be caused by direct involvement of EBV in the hepatocytes. Secondly, the hepatocellular damage is assumed to be caused by suppressor/cytotoxic T cells and NK cells. Sullivan et al. (1985) demonstrated abnormal killer cell activity in the suppressor/cytotoxic T cells from patients with X-linked lymphoproliferative syndrome. These suppressor/cytotoxic T cells are known to show anomalous killer cell activity against a variety of lymphoid cell lines, autologous fibroblasts and hepatocytes. Our immunohistochemical study on the autopsy liver specimen revealed that a large number of NK cells (Leu 7 positive) and suppressor/cytotoxic T cells (Leu 2a positive) were present in the lobules of the liver as well as in the portal areas in addition to B cells (L26 positive). We therefore suggest that the hepatocellular damage in the present case may be partly caused by suppressor/cytotoxic T cells and NK cells.

In this case obstructive jaundice occurred due to a decrease of the number of bile ducts. Here, we present two possibilities to account for the decrease of bile ducts in the portal areas. First, the mononuclear cells markedly infiltrated in the portal areas. We thus suggest that these are the cause of the decrease of bile ducts. Secondly, we cannot neglect the possibility that the mechanism of injury of bile duct was the same as that of injury to the hepatocytes, namely, that the epithelial cells of the bile duct were injured by the abnormal suppressor/cytotoxic T cells which infiltrated in the portal areas.

In the transplant recipient who is administered immunosuppressants, lymphoproliferative syndromes have a high incidence. The EBV-infected B cells are not destroyed by the T cells because they are decreased in number by immunosuppressants and the virus-infected B cells readily proliferate. Our case did not suffer from immunodeficiency. However, EBV-infected B cells were abundant in this case and we suggest that T cells have restricted abnormality to respond to the EBV, as suggested by Purtilo et al. (1975, 1976).

We found larger numbers of NK cells in the present case than Mroczek et al. (1987) reported in cases of fatal IM. A large number of NK cells in the portal areas and sinusoids of the liver may be mobilized to kill B cells infected by EBV, thus compensating for insufficient killer function of suppressor/cytotoxic T cells. The present liver damage may be caused by a functional abnormality of suppressor/cytotoxic T cells similar to that re-

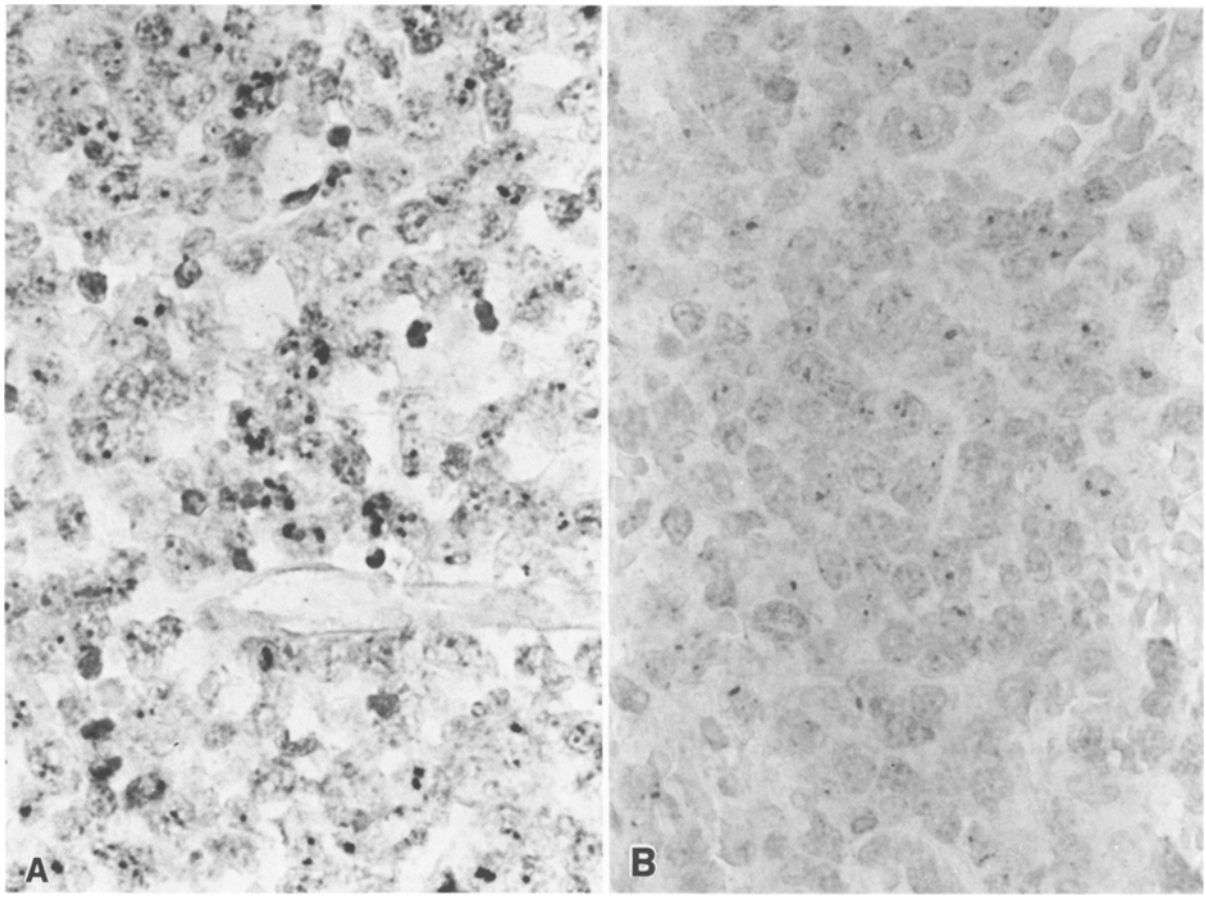

Fig. 6. Detection of EBV genomes in the nuclei of immunoblasts of the lymph node (**A**) and white pulp of the spleen (**B**) by in situ hybridization. × 600

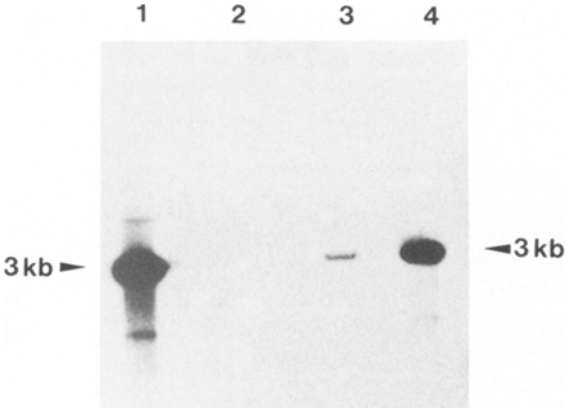

Fig. 7. Southern blot hybridization with the use of a probe specific for EBV-DNA. *Lane 1* contains DNA from a positive control (Raji cell). *Lane 2* corresponds to negative control (DNA from liver of the untreated case). *Lanes 3 and 4* contain DNA from the patient's spleen and liver specimens, respectively. Bands are found in the identical location in lanes 1, 3 and 4, indicating the presence of EBV sequences

ported by Purtilo et al. (1975, 1976) in X-linked lymphoproliferative syndrome. Functional abnormality of suppressor/cytotoxic T cells in fatal IM should be studied further.

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