Interferon γ (IFN-γ) Deficiency in Generalized Epstein-Barr Virus Infection with Interstitial Lymphoid and Granulomatous Pneumonia, Focal Cerebral Lesions, and Genital Ulcers: Remission Following IFN-γ Substitution Therapy

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A 26-year-old previously healthy woman developed granulomatous pneumonitis, encephalitis, and genital ulceration during primary Epstein-Barr virus (EBV) infection. EBV DNA was demonstrated by polymerase chain reaction analysis of serum, lung tissue, and genital ulcer specimens. Serology verified primary EBV infection. The patient lacked lymphocytes cytotoxic to autologous EBV-transformed B lymphocytes. No spontaneous or in vitro EBV-induced interferon γ (IFN-γ) production was evident in peripheral blood. The cells had normal IFN-γ production when stimulated with Staphylococcus aureus exotoxin A. In the bone marrow and peripheral blood, the number of large granular CD56+ lymphocytes (natural killer cells) increased 39%–55%, but no CD4 or CD8 cell lymphocytosis was initially found. A partial clinical response was achieved with treatment with acyclovir, corticosteroids, and intravenous γ-globulin. Because of persistent granulomatous central nervous system and lung involvement, subcutaneous IFN-γ therapy was started but was discontinued after 3 months because of development of fever, pancytopenia, and hepatitis. This therapy initiated a complete clinical recovery, which occurred parallel to development of EBV-specific cytotoxic CD8+ T lymphocytes and normalization of natural killer cell lymphocytosis. These findings provide evidence for an EBV-induced lymphoproliferative disorder due to a T lymphocyte dysfunction associated with a selective lack of IFN-γ synthesis.

Case Report

A previously healthy 26-year-old female clerk fell ill with fatigue, dizziness, and neck pain. She was treated with two courses of penicillin therapy for sustained pharyngeal angina. Severe headache, nausea, vomiting, and continuous fever (temperature of ~38°C) then ensued.

Pulmonary roentgenography initially showed a few rounded infiltrations in the proximity of the left hilar region. However, in a fortnight, profuse bilateral rounded and reticular interstitial infiltrations were noted (figures 1 and 2). Spirometry showed restrictive ventilatory impairment with a total lung capacity of ~50% of the predicted value and a single-breath carbon monoxide diffusion capacity of 30% of the predicted value. The patient’s condition deteriorated during the investigations, and she developed exertional dyspnea, dry cough, genital ulcers, and a lethargic mental state.

The differential cell count of bronchoalveolar lavage fluid revealed 87% lymphocytes. Cultures of bronchoalveolar lavage fluid were negative for bacteria, mycobacteria, fungi, Pneumocystis carinii, and viruses. Endobronchial biopsies showed unspecific inflammation. Cerebral MRI demonstrated rounded charged areas compatible with cerebral granuloma (figure 3). Results of CSF analyses including bacterial and viral cultures were all normal. Flow cytometry of bone marrow and peripheral blood samples showed a high percentage of CD56+ natural killer (NK) cells (39% and 55%, respectively) within the lym-
neutrophil or eosinophil infiltration was present. Localized nodules consisting of a mixture of lymphocytes, histiocytes, and giant cells were seen, preferentially in association with small bronchi. Discrete epithelioid granulomas were present, but no necrosis or vasculitis was seen. Immunohistochemical analysis revealed that the lymphoid component of the nodules consisted of a mixture of small T cells and activated blast-transformed B cells (figure 2A). Among the blast-transformed lymphoid cells, EBV latent membrane protein 1 (LMP 1) antigen–positive cells were seen (figure 2B). Thus, partly lymphocytic and partly granulomatous pneumonitis and bronchocentric granulomatosis were diagnosed.

Analysis of acute-phase serum samples showed high titers of IgG and IgM antibodies to the EBV viral capsid antigen (VCA) and early antigen (EA) but no antibodies to EBNA, an antibody pattern compatible with acute progressive EBV infection (figure 5). Immunohistochemical analysis and PCR analysis demonstrated EBNA and EBV DNA, respectively, in genital ulcers and interstitially within cells in the lung parenchyma (figure 4B). Southern blotting revealed the presence of EBV DNA within the lung tissue. PCR analysis of serum was also positive for EBV DNA.

Treatment. The patient was treated with 16 mg of intravenous dexamethasone/d, 3,200 mg of oral acyclovir/d, and 30 g of intravenous γ-globulin on four occasions (figure 6). Her condition improved clinically, and a pulmonary roentgenogram showed a slight reduction in the bilateral infiltrates. Within 10 days, she was discharged; her medications at this time were oral acyclovir and steroids. Her condition improved after 2 months of therapy, and the total lung capacity was now 75% of the predicted value. The pulmonary infiltrates were considerably reduced. Serum antibodies to EBNA appeared temporarily, probably reflecting passive transfer of specific immunity by

Figure 1. Pulmonary roentgenogram of a patient with persistent primary Epstein-Barr virus infection associated with a granulomatous lymphoproliferative disorder that was obtained 14 days after hospital admittance. This posteroanterior chest film demonstrates that the widespread confluent nodular opacities are symmetrical in both lungs.

Figure 2. A. Histological image of an open lung biopsy specimen from a patient with persistent primary Epstein-Barr virus (EBV) infection associated with a granulomatous lymphoproliferative disorder that reveals a lymphocytic and epithelioid giant cell granulomatous infiltration (arrows) in the lung parenchyma (hematoxylin-eosin stain; original magnification, ×100). B. Indirect immunofluorescent antibody staining of the same open lung biopsy specimen for EBV latent membrane protein 1 (LMP 1) antigen–positive cells. There are two EBV LMP 1 antigen–positive cells (arrow) in the lymphoid infiltrations of the lung parenchyma. This biopsy specimen was obtained before treatment (original magnification, ×400).
the patient still had signs of disseminated disease. Therefore, subcutaneous IFN-γ (1.75 × 10^6 U three times per week) was added to the treatment.

After 3 months of combined treatment with acyclovir, corticosteroids, and IFN-γ, the patient was admitted to the hospital because of continuous fever (temperature, 39.5°C), anemia, thrombocytopenia, hypoalbuminemia (albumin level, 20 g/L), and pronounced hepatosplenomegaly. After discontinuation of IFN-γ therapy, her fever subsided, and 2 to 3 weeks later, the liver and spleen returned to their normal sizes. A new cerebral MRI revealed clear reduction of the granuloma-like lesions primary Epstein-Barr virus infection associated with a granulomatous lymphoproliferative disorder that were obtained before treatment (figure 2). Antibodies to EBNA reappeared, and the titer increased steadily during the following months (figure 5). Flow cytometry of a peripheral blood sample showed CD4+ T cell lymphocytosis. The extreme elevation of the NK cell count was normalized during IFN-γ therapy.

Her clinical status and lung function improved significantly (figure 6). Regular menses reappeared, and the vaginal ulcers were reduced, and administration of all medications could be discontinued.

Materials and Methods

Serology. Serology for antibodies to the EBV-specific antigens VCA the D and R complex of EA, and to the EBNAs was performed as previously described [3].

Immunohistochemical analysis for EBV antigen. Immunohistochemical analysis was used to assess the presence of EBV
CHOALVEOlar lavage fluid was examined after no other pretreatment; the specimens then underwent freeze-thawing and boiling. Biopsy specimens were homogenized and treated with proteinase K, and then DNA was extracted by use of a standard chloroform/ethanol method [7].

Cells obtained from peripheral blood. Peripheral blood mononuclear cells (PBMCs) were separated from the patient’s blood by centrifugation of heparinized whole blood over a ficoll-isopaque cushion (Bjørum, Oslo, Norway) at 1,500 rpm for 30 minutes at 20°C. These cells were prepared from the patient’s blood at regular intervals from hospital admittance. After controlled freezing, the cells were kept in liquid nitrogen until thawed and cultured.

Precursor frequency among in vivo EBV-infected B lymphocytes. The thawed lymphocytes that were obtained from peripheral blood were cultured in 10% fetal calf serum for 6 weeks for the presence of spontaneous outgrowth of in vivo EBV-infected B cells [8]. For determination of the precursor frequency among in vitro EBV-infected lymphocytes, a limiting dilution assay with a maximum concentration of 5 × 10^4 to 6 × 10^5 cells per well was used. The transformed cells were also analyzed for EBV antigen by EBNA 1 staining.

Precursor frequency among lymphocytes cytotoxic to autologous EBV-transformed B cells. EBV-specific T cell–mediated immunity was tested by the outgrowth inhibition assay [8, 9]. Lymphocytes were obtained as previously described at indicated times (table 1) during the patient’s treatment. The cells were EBV-infected and cultured at various concentrations (2 × 10^4 to 4 × 10^5 cells per well) in 10% fetal calf serum.

**Figure 5.** Epstein-Barr virus (EBV)–specific serological response in the acute stage of persistent primary EBV infection associated with a granulomatous lymphoproliferative disorder and during the 3-year follow-up period. The patient eventually had a complete normal serological response including development of antibodies to Epstein-Barr nuclear antigens (EBNAs) 1–6. Antibodies to the various EBV-specific antigens were as follows: IgM to viral capsid antigen (VCA) ( ), IgG to the D complex of early antigen ( ), IgM to p107 ( ), IgG to p107 ( ), Ig to EBNA ( ), and IgM to VCA ( ).

**Figure 6.** Clinical symptoms of and therapy given to a patient with persistent primary Epstein-Barr virus infection associated with a granulomatous lymphoproliferative disorder. The onset of disease occurred 4 months before the patient was admitted to the hospital. = onset of symptom to abatement; → persistence of acyclovir treatment for >36 months.

**Symptoms**

- Sore throat
- Clouding of consciousness
- Fatigue/vertigo
- Headache
- Fever
- Dyspnea/dry cough
- Menopause
- Genital ulcers
- Hepatosplenomegaly

**Treatment**

- Steroids
- Acyclovir
- γ-Globulin
- IFN-γ

![Graph showing antibody titer over time](image-url)
Table 1. Frequency of cytokine-producing cells in peripheral blood from a patient with persistent primary Epstein-Barr virus infection associated with a granulomatous lymphoproliferative disorder that was assessed at the single cell level by an immunofluorescence technique.

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Unstimulated</th>
<th>Monoclonal antibody to CD3–stimulated</th>
<th>Staphylococcus aureus exotoxin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Patient</td>
<td>Controls</td>
<td>Patient</td>
</tr>
<tr>
<td></td>
<td>BT</td>
<td>DT</td>
<td>IMs</td>
</tr>
<tr>
<td></td>
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<td>IMs</td>
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<tr>
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<td>0</td>
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</tr>
<tr>
<td>IL-2</td>
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<td>0</td>
<td>10</td>
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</table>

NOTE. Cytokine production per 10⁵ PBMCs was studied in the patient before and after 6 weeks of IFN-γ treatment and was compared with spontaneous reactivity in IMs and HCs. An induced cytokine response was also assessed after stimulation with monoclonal antibody to CD3 or S. aureus exotoxin A. BT = before treatment; DT = during treatment; HCs = healthy controls; IMs = controls with acute infectious mononucleosis; PBMC = peripheral blood mononuclear cell; ra = receptor antagonist.

* Data represent the mean of two repeated experiments per patient.

† Nine IMs.

‡ Four HCs.

for 6 weeks to allow outgrowth of EBV-infected lymphoblastic cell lines. If EBV-immune T cells were present in the patient’s blood, outgrowth of EBV-infected B lymphocytes was inhibited. The strength of this inhibition correlated with the frequency of in vivo precursors of lymphocytes cytotoxic to autologous EBV-transformed B cells [8, 9].

Cytokine detection at the single cell level. The studied cytokines included IL-1α, IL-1β, IL-1ra (receptor antagonist), IL-2, TNF-α, and IFN-γ; cytokine detection was performed as previously described [10, 11]. Mononuclear cells from the patient were also restimulated in vitro for 2 hours to induce cytokine production of in vivo primed cells by immobilized monoclonal antibody to CD3 and by the superantigen Staphylococcus aureus exotoxin A for 48 hours. As previously shown, stimulation for 1–2 hours induces cytokine production in in vivo primed but not in resting T cells, and the method has been used for the detection of normal immune responses in healthy individuals and in patients with acute infectious mononucleosis [12]. Activation by the superantigen S. aureus exotoxin A requires interactions between HLA class II molecules on antigen presenting cells and the variable part of the β chain of T cell receptors [13]. Lymphokine and monokine production in these cultures thus requires a collaboration between antigen presenting cells and T cells. These cell assays therefore offered a way to assess the general cytokine capacity of T cells. Cells from the patient were compared with the reactivity in cells obtained from nine controls with acute infectious mononucleosis and four EBV-seropositive healthy controls.

MRI. MRI of the brain was performed with a Siemens Magnetom SP (Siemens A/G, Frankfurt, Germany) operating at 1.5 T; turbo spin-echo proton density and T₂-weighted as well as spin-echo T₁-weighted images were used. The images were either unenhanced or enhanced following intravenous administration of gadolinium–pentetic acid 0.1 mmol/kg.

Results

Despite persistent generalized EBV infection, the patient had no spontaneous production of TNF-α or IFN-γ, and the levels of IL-1α and IL-1β in the PBMCs were reduced (table 1). The expression of IL-1ra in our patient was higher than those in the controls with acute infectious mononucleosis. In the monoclonal antibody to CD3–stimulated state, the patient’s PBMCs expressed lower levels of IL-1α and IL-1β before treatment but higher levels during treatment with IFN-γ (table 1). No substantial endogenous enhancement of IFN-γ production was noted during IFN-γ therapy. However, the 3 months of IFN-γ treatment were followed by synthesis of TNF-α but not of IFN-γ in the unstimulated PBMCs and an expression of IL-1ra that was four- to sixfold higher than those in controls with acute infectious mononucleosis. S. aureus exotoxin A stimulation of the patient’s PBMCs induced IFN-γ production that was the same magnitude as that of the healthy controls (table 1). This finding indicated no general loss of capacity of the patient’s T cells to produce IFN-γ.

Discussion

Diagnosis. Chronic pneumonitis with a granulomatous element was found in the present case. These features may be seen in other disease states such as stage II–III sarcoidosis, tuberculosis, and fungal infections [14–16]. The sudden onset,
swift progress, and concomitant protracted fever strongly suggest that an infectious agent was the cause of the patient’s disease. Analogous with other cases of chronic serious EBV infections, sustained elevation of titers of antibody to VCA and EAs without development of antibody to EBNA was found [3, 17]. The presence of EBV antigens and the EBV genome in lung, genital tissue, and serum samples was confirmed by immunohistochemical testing, PCR analysis, and Southern blotting, respectively. Thus, the causative infectious agent in the present case in all likelihood was EBV.

One of the important factors prohibiting the proliferation of EBV is the release of IFN-γ from activated T lymphocytes [12, 18–22]. IFN-γ was produced by PBMCs from all controls with acute infectious mononucleosis (table 1). In addition, monoclonal antibody to CD3 stimulation of PBMCs from controls caused a 15-fold increase in IFN-γ production (table 1). By contrast, few IFN-γ-producing cells were noted in unstimulated or monoclonal antibody to CD3–stimulated PBMCs from our patient, while the response to S. aureus A exotoxin stimulation was completely normal (table 1). Therefore, it may be inferred that the patient’s inability to produce IFN-γ was due to a selective EBV-related T cell defect.

NK cell lymphocytosis may normally be seen early in acute infectious mononucleosis. In vitro studies imply that NK cells are an important source of IFN-α, which may be involved in the first line defense against EBV infection [9]. NK cells are also an important source of IFN-γ necessary for activation of T cell cytotoxicity [18]. The patient’s sustained NK cell lymphocytosis may have been a compensation for the lack of IFN-γ production as well as activation of cytotoxic T cells.

The patient may also have had malfunction of the NK cells or monocytes. Monocytes from the patient spontaneously produced only low amounts of IL-1α or IL-1β. Monoclonal antibody to CD3 stimulation generated a significant upregulation of the levels of IL-1α- and IL-1β-producing cells in PBMCs only during IFN-γ treatment. In line with these findings, the patient did have protracted fever, thus production of IL-1 or other proinflammatory cytokines was probably induced from monocytes or other cellular sources during IFN-γ therapy. It has previously been shown that exogenous IFN-γ upregulates the lipopolysaccharide-induced production of IL-1 in peripheral blood monocytes [23]. IFN-γ therapy also generated significant expression of TNF-α; thus, general induction of proinflammatory mediators was achieved by exogenous addition of IFN-γ. The exact mechanisms by which this upregulation induced a specific T cell response in our patient remain to be elucidated.

Pneumonitis in chronic EBV infections has been shown to be associated with profuse lymphocytic infiltration [24, 25]. To our knowledge, this is the first description of EBV pneumonitis associated with the combination of epithelioid and giant cell granulomas and lymphatic infiltration. The granulomatous pattern shown by the brain MRI makes it likely that the histological features of the cerebral lesions were similar to those seen within the lungs. The patient also had EBV-related chronic genital ulceration.

<table>
<thead>
<tr>
<th>Subject(s)</th>
<th>Spontaneous outgrowth</th>
<th>Outgrowth inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Before treatment</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>After treatment*</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Nine controls with infectious mononucleosis’</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Acute phase</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Convalescent phase</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>10 EBV-seropositive healthy controls’</td>
<td>−</td>
<td>+</td>
</tr>
</tbody>
</table>

NOTE. See Materials and Methods section for techniques. Assessment of PBMCs from the patient (who had persistent primary EBV infection associated with a granulomatous lymphoproliferative disorder) was performed when the patient had been ill for 3 months but before treatment with acyclovir, intravenous immunoglobulin, and prednisolone. EBV = Epstein-Barr virus; PBMC = peripheral blood mononuclear cell; + = present; − = absent. * Six months after cessation of IFN-γ treatment. ’ Acute-phase blood samples were obtained at the onset of disease, and convalescent-phase samples were obtained 6 months later. ’ Healthy individuals without clinical history of infectious mononucleosis.

**Treatment.** High doses of intravenous immunoglobulin (0.5 g/kg) together with IFN-α successfully treated five cases of T cell lymphoproliferative disorders and EBV infection [26]. Our patient was initially treated with acyclovir, prednisolone, and intravenous γ-globulin, and partial improvement of the clinical state was achieved. Despite continued oral treatment with acyclovir and prednisolone, her neurological and gynecologic symptoms progressed after 4 to 5 months of therapy.

IFN-γ therapy has been shown to temporarily improve the clinical status in boys with X-linked lymphoproliferative disease and fatal EBV infection [20]. In our case, 3 months of IFN-γ therapy brought about a flare-up of high-grade fever, hepatosplenomegaly, and anemia. We believe that these symptoms may have been associated with development of cytotoxic lymphocyte responses to EBV-infected B cells in the liver and in other organs. Shortly after this episode, the patient’s serum became permanently EBNA-positive. Instead of NK cell lymphocytosis, she developed T cell lymphocytosis, and her clinical status improved, thus indicating the appearance of a specific cellular immune response. In all probability, IFN-γ therapy shifted the cytotoxic response to EBV from the NK to the T cell type (figure 4). A better control of EBV infection was achieved (table 2), and this improved control has lasted for >3 years after cessation of IFN-γ therapy.

**Conclusion**

Our patient thus had a selective immune defect against EBV infection, probably due to a primary or secondary T cell mal-
function. Three months of IFN-γ therapy brought about a shift in her immune response from NK cell dominance toward T cell dominance. After IFN-γ therapy was given, a sustained clinical improvement also ensued. To our knowledge, the effects of IFN-γ therapy on chronic severe EBV infection in subjects without generalized immune defects have not previously been reported. Trials with IFN-γ therapy for patients with severe chronic EBV infection are warranted.

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References