Fulminant Infectious Mononucleosis
and Recurrent Epstein-Barr Virus
Reactivation in an Adolescent

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We describe a unique case of fulminant infectious mononucleosis and recurrent Epstein-Barr virus reactivation presenting in an adolescent. Detailed assays of Epstein-Barr virus–specific T cell immunity revealed defects in the patient’s T cell receptor signalling pathway characterized by a lack of interleukin-2 and CD25 expression, which may have contributed to her clinical course. Allogeneic stem cell transplantation reversed the clinical and laboratory phenotype.

Fulminant infectious mononucleosis (FIM) is a rare, life-threatening disorder characterized by fever, massive hepatosplenomegaly, atypical lymphocytosis, and pancytopenia to primary Epstein-Barr virus (EBV) infection. It is generally seen in either X-linked lymphoproliferative syndrome (XLPS) or severe combined immunodeficiency syndrome (SCID). Survivors frequently develop hypogammaglobulinaemia and lymphoma [1]. We describe a unique case of FIM in an adolescent female with an undefined autoimmune enteritis. Detailed assays of EBV-specific T cell immunity revealed defects in her T cell receptor signalling pathway characterized by a lack of interleukin (IL)–2 and CD25 expression, which may have contributed to her clinical course. Allogeneic stem cell transplantation (HSCT) reversed the clinical and laboratory phenotype. To our knowledge, there are no previously reported cases.

Case report. An 11-year-old white girl presented with severe malnutrition, weight loss, and profuse watery diarrhea. Prior history was unremarkable, and there was no family history of autoimmunity. Multiple biopsies from the gastric antrum and throughout the bowel showed a lymphocytic infiltrate in the lamina propria, with severe villous atrophy of the small bowel. Features were those of a focally erosive active colitis, which were atypical for chronic inflammatory bowel disease, microscopic colitis, or collagenous colitis. There was no evidence of infectious etiology determined by histological examination or stool culture. The results of tests for endomyosal, gastric parietal, smooth muscle, anti-nuclear and anti-thyroid autoantibodies were negative. Peripheral blood showed a lymphocyte count of $0.8 \times 10^9$ cells/L (normal range, 1.6–3.3 $\times 10^9$ cells/L) but essentially normal T, NK, and B cell percentages. The ratio of CD4 cells to CD8 cells was 1.82 (normal range, 1.0–3.2). Total immunoglobulin (Ig) G level was 7.27 g/L (normal range, 6.24–14.4), with a reduced IgG, subclass (IgG1,3,4 normal), an IgA level of 2 g/L (normal range, 0.74–2.28 g/L), and an IgM level of 6.67 g/L (normal range, 0.48–2.57 g/L). Antibody responses to the vaccines diphtheria, tetanus, and Haemophilus influenzae B (HIB) were present but reduced. The clinical symptoms were unresponsive to a gluten-free diet, and a presumptive diagnosis of an undefined autoimmune enteropathy was made. Weight gain and normalization of bowel motions occurred with cyclosporin and prednisolone immunosuppression, which was subsequently switched to tacrolimus. Repeated endoscopy revealed resolution of inflammatory changes. Her growth and development were normal. The patient exhibited no other autoimmune symptomatology, except mild eczema of the hands and feet. Her clinical condition remained stable, except for one flare secondary to a H. influenzae B upper respiratory infection and a Staphylococcus aureus skin infection that settled with antibiotic therapy and an increased dose of tacrolimus.

At the age of 17 years, the patient presented with cervical lymphadentitis, fever, sore throat, and hepatosplenomegaly. At symptom onset, her IgG level was 7.79 g/L (normal range 6.24–14.4 g/L). The results of assays for EBV and cytomegalovirus IgG and IgM antibodies and of a monospot test were negative, but circulating EBV DNA levels were elevated. A complete blood cell count revealed atypical lymphocytosis (CD3+ cell percentage, 87%; ratio of CD4 to CD8 cells, 1:1; NK cell percentage, 6% [normal range, 5%–15%]; and B cell percentage, <1%). A lymph node biopsy showed a 95% T cell infiltrate with scattered blastoid B cells positive for EBV-encoded RNA by in situ hybridization and latent membrane protein 1. She
proceeded to have an aggressive clinical course, including fever, pancytopenia, splenomegaly, and disseminated intravascular coagulation. Bone marrow biopsy revealed hemophagocytosis in the bone marrow of erythroid, leukocyte, and platelet precursors (with normal cytogenetics). Symptoms were responsive to high-dose steroid therapy and hemoasotic support.

Between the ages of 17 and 20 years, she presented with multiple episodes of severe bacterial pneumonia, including pneumonia due to *Pseudomonas aeruginosa* and methicillin-resistant *S. aureus*. These were complicated by hepatosplenomegaly, elevated liver enzymes, trilineage cytopenias and atypical T cell lymphocytosis (normal NK cell numbers). She had 1 episode of meningoencephalitis with detectable EBV DNA in cerebral spinal fluid samples, requiring intensive care admission, and recurrent EBV viremia (up to 6400 viral copies/mL) [2]. Informed consent was provided for further laboratory investigation. Blood samples obtained 27 and 17 months before transplantation (during EBV viremia and while the patient was not receiving tacrolimus) showed ex vivo EBV peptide-specific CD8+ cytotoxic T lymphocyte activity (by chromium release assay; data not shown), typical of that observed during a primary T cell response [3]. Episodes were successfully managed with antibiotics, antivirals, and discontinuation of immunosuppression. Because the patient now had absent circulating B cells and profound hypogammaglobulinemia, she received monthly intravenous immunoglobulin.

Real-time reverse-transcription polymerase chain reaction (RT-PCR) revealed a striking reduction in the basal levels of CD25 (the IL-2 receptor α chain) and IL-2 transcripts in patient peripheral blood mononuclear cells (Figure 1A) that were not associated with IL-2 or CD25 gene sequence abnormalities. Lack of CD25 was confirmed by flow cytometry (Figure 1B). Addition of modest doses of exogenous IL-2 was able to restore CD25 (but not IL-2) gene expression and CD25 surface expression in CD4+ T cells cultured in vitro (Figure 1B), confirming the signalling pathway downstream of IL-2 was intact. NFAT-1 (a regulator of IL-2 transcription) gene expression was
not reduced. CD4+FOXP3+ regulatory T cells (Tregs) were 2.97% (ie, modestly less than the normal range of 3.63%–7.45% [mean, 5.1%]), and the coding and proximal promoter regions of the FOXP3 were normal.

IL-2 and CD25 are expressed as a consequence of T cell receptor activation. We therefore investigated T cell receptor signalling in this patient. First, EBV-specific CD8+ cytotoxic T lymphocytes were expanded in culture (supplemented with IL-2) in response to cognate peptide [4]. Pharmacological stimulation with ionomycin/phorbol 12-myristate 13-acetate of the patient’s and a healthy control subject’s CD8+ cytotoxic T lymphocyte lines resulted in up-regulation of CD25 and IL-2 gene expression, as noted by real-time PCR (Figure 1C). However, we found that, when the same CD8+ cytotoxic T lymphocyte lines were stimulated with relevant human leukocyte antigen (HLA) class I–restricted peptides and then quantified by real-time RT-PCR, levels of CD25, IL-2, and interferon-γ were not up-regulated, indicating impairment in cytokine synthesis in response to T cell receptor signalling via the cognate peptide (Figure 1D).

At the age of 20 years, the patient underwent an HLA-matched sibling, fludarabine/melphalan-reduced intensity HSCT [5]. As of 20 months after transplantation and her clinical phenotype has resolved. Chimeric studies indicate 100% donor engraftment. Blood specimens obtained 14 months after HSCT revealed that CD4+FOXP3+ T cell percentages (4.35%) and graft rejection. Blood specimens obtained 14 months after HSCT [5]. As of 20 months after transplantation and her clinical phenotype is sufficiently distinctive to be considered unique. XLPS presents in male infants with various defects including inability to control EBV infection, severe HPS, and the development of progressive hypogammaglobulinemia [6]. XLPS is due to mutations in the SH2D1A gene which encodes the adaptor protein signalling lymphocyte activation molecule [SLAM]–associated protein (SAP) through SLAM–family receptors expressed on T cells. Although T cell receptor stimulation leads to normal production of IL-2 and interferon-γ, SAP-deficient CD8+ cytotoxic T lymphocytes from patients with XLPS display a specific lytic defect against autologous EBV-infected B cells [7]. In contrast we found peptide-specific CD8+ cytotoxic T lymphocyte lines cultured in vitro showed high levels of lysis of EBV-sensitized targets, and sequence analysis revealed a normal SAP gene coding region and promoter, and normal gene expression of SAP, SLAM, and 2B-4 (a SLAM family member) by real-time RT-PCR.

The FIM episode followed contact with a new partner and would be epidemiologically consistent with a primary EBV infection. Initial EBV infection was not associated with detectable anti-EBV antibodies. This likely contributed to her life-threatening presentation. The patient went on to have HPS, which is characterized by inappropriately activated effector T cells. We speculate that the lack of baseline CD25 expression compromised the ability of IL-2 to drive Tregs to proliferate and limit the excessive EBV-specific CD8+ cytotoxic T lymphocyte response observed. Although such a scenario has been shown in infectious mononucleosis [8], the alternative explanation is that the ex vivo EBV-specific CD8+ cytotoxic T lymphocyte activity was driven by the viremia irrespective of the reduced CD25 expression.

Familial HPS typically occurs within the first year of life. Rare adolescent cases have been described [9], although not in the context of autoimmune enteritis. Typical findings include family history, elevated soluble CD25 levels, and impaired lysis of autologous EBV-infected B cells [7, 10].

Two cases of CD25 deficiency are described. Both presented in infancy with autoimmunity (with slightly reduced CD4+FOXP3+Tregs), and in addition, there was an increased susceptibility to recurrent herpes virus infections [11, 12]. One patient had EBV-positive lymphoid hyperplasia but the clinical picture was otherwise distinct from ours and, specifically, was not associated with life-threatening EBV pathology or recurrent EBV-reactivation. In CD25 deficiency, the markedly reduced CD4+T cell proliferative responses could be restored with high-dose (1000 IU/mL) exogenous IL-2, most likely by signalling via the IL-2 receptor γ and β chains. Similarly, we observed that exogenous IL-2 enabled total CD4+ T cells to proliferate in vitro. However, this required only 20 IU/mL to induce proliferation, most likely as a result of IL-2 signalling via the up-regulated α chain (CD25). SCID is a severe form of heritable immunodeficiency presenting in infancy. Most cases are X-linked recessive due to mutations in the IL-2 receptor γ-chain gene (IL-2Rγ). Multiple cytokine, cellular, and humoral defects occur. Recurrent candidiasis, ear infections, and Pneumocystis jirovecii pneumonia are common. FIM may be a presenting feature. In contrast to our observations, in IL-2Rγ–mutated SCID, T cell proliferation remains impaired despite high-doses of exogenous IL-2. In 1 infant, defective NFAT-1 binding complex resulted in a SCID-like clinical picture including generalized zoster and hypogammaglobulinemia but not FIM [13]. That case was characterized by defective cytokine production which was not restored by ionomycin/phorbol 12-myristate 13-acetate.

Our investigations revealed a T cell receptor signalling defect that resulted in impaired cytokine secretion and CD25 ex-
pression. The defect underlying these observations was not able to be elucidated. The signalling events evoked following cognate peptide–T cell receptor interactions are complex and numerous, and the restricted patient sample availability limited our ability to precisely delineate the causal defect. Following recognition of viral peptide by the T cell receptor, a signalling cascade is initiated by the CD3-receptor complex. Although defects in the CD3 chain have been associated with T cell abnormalities [14], these rare cases are associated with reduced CD3 T cell receptor levels, elevated B cells and immunoglobulins and reduced αβ–T cell receptor complexes, none of which were observed in this patient. A more probable scenario would place the defect downstream of the CD3 chain. It is quite possible that a common defect underlay the lack of antibody responses and the T cell receptor abnormality. Although mutations in CD40 and its ligand have been associated with elevated IgM and abnormal T cell interactions, the normal levels of IgG1 and IgA at diagnosis make this particular defect less likely.

Our patient had immune dysregulation with clinical features of both immunodeficiency and autoimmunity, complicated by life-threatening infections. We outline how functional assays of EBV-specific T cell immunity can be performed to diagnose patients with symptoms reminiscent of this case. HSCT resulted in abolition of the clinical and immunological phenotype and is a valid therapeutic option.

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References