IL-18 Serum Concentration Is Markedly Elevated in Acute EBV Infection and Can Serve as a Marker for Disease Severity

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Epstein Barr virus (EBV)–related diseases encompass both acute infections that result in acute infectious mononucleosis and chronic infections that result in lymphoproliferative malignant diseases. While classical inflammatory parameters such as C-reactive protein (CRP) have proven their usefulness during bacterial and fungal infections, they are often low and nondiscriminatory in viral infections. Here, we show that IL-18 is markedly elevated during acute EBV infections and EBV-associated diseases, while ferritin concentrations are also elevated during acute EBV infection and correlate with IL-18. Therefore, IL-18 and ferritin may represent infection markers for viral infections such as EBV, similar to CRP for bacterial infections.

Epstein Barr virus (EBV) infection in adolescents and young adults frequently results in acute infectious mononucleosis (IM). Rarely, acute EBV infection converts into a protracted chronic or recurrent course. EBV has a pathogenic role in some cases of Burkitt’s lymphoma and nasopharyngeal carcinoma, and the virus can be detected in cases of Hodgkin’s disease and T-cell lymphoma. This wide range of EBV-related diseases underlines the need to further elucidate the immune control mechanisms to the virus.

EBV viral load correlates with severity of primary EBV disease and might be used to monitor the immune response to the virus [1]. Elevated levels of interferon-γ (IFNγ) and soluble interleukin-2 (IL-2) receptor are found in EBV-induced IM. However, because production of these cytokines is mainly a local feature, circulating concentrations of these factors are low and often undetectable. Interleukin-18 (IL-18), formerly called IFNγ-inducing factor, is a cytokine mainly produced by cells of the macrophage lineage [2]. In contrast to other cytokines that have very low circulating levels, elevated serum IL-18 concentrations have been found in viral infections, specifically HIV-type 1, rotavirus, human papillomavirus, and dengue virus infections [3, 4]. Significant amounts of IL-18 can be detected in lymphoid tissues during EBV-induced IM [5], and IL-18 expression has been associated with regression of EBV-associated Burkitt’s lymphoma [6]. These observations suggest an important role for IL-18 in EBV infection. In the present study, we investigated the IL-18 response in acute EBV infection and EBV-related disease.

MATERIALS AND METHODS

Study Design
Patients ≥18 years with a clinical diagnosis of IM, displaying immunoglobulin M (IgM) antibodies to EBV viral capsid antigen (VCA) and who presented within 8 weeks after onset of symptoms were eligible to participate. Patients were excluded if they were immunocompromised or used immunosuppressive therapy. Three evaluations were performed (first visit, 2 weeks later, and 6 weeks after the first visit), and each included a medical history, physical examination, and grading of the severity of illness on a scale of 0 to 6 by a validated score [7]. Ten patients were enrolled in a time period of 11 months. The study was approved by the local Medical Ethical Committee and informed consent was obtained before enrollment.

Serum Samples
At each visit, a serum sample was collected for laboratory evaluation, serology and quantitative polymerase chain reaction (PCR). Next to the 30 samples from patients with acute IM, we collected 56 serum samples from 31 healthy volunteers; 17 EBV seropositive chronic fatigue syndrome (CFS) patients with 5 matched healthy controls, and 3 patients with EBV-related disease.
RESULTS

Ferritin levels were determined in stored serum samples (−20°C) using the commercially available VIDAS (bioMérieux, Inc., Durham, NC) test. CRP levels and alanine aminotransferase (ALT) levels were measured using an Aeroset 2.0 analyzer (Abbott Diagnostics, Santa Clara, CA).

EBV Antibodies

Enzyme-linked immunosorbent assay (ELISA) for IgM and immunoglobulin G (IgG) antibodies to EBV VCA (Panbio Limited, Brisbane, Australia) was performed according to the manufacturer’s instructions.

DNA Isolation

Two hundred microliters serum and 10 μL phocine herpes virus (PhHV; as internal control) were added to 2 mL lysis buffer. DNA was extracted using the NucliSens EasyMAG extraction system (bioMérieux, Boxtel, The Netherlands) according to the manufacturer’s instructions.

EBV Quantitative PCR

Real-time PCR detection of EBV was based on amplification of a 74 base pair (bp) region of the nonglycosylated membrane protein BNRFI p143 as described [8]. Detection of PhHV, the internal control, was based on detection of an 89 bp fragment of the gB polymerase gene [9]. ABI Prism sequence detection system 7000 (Applied Biosystems, Nieuwerkerk a/d IJssel, The Netherlands) was used for amplification and detection.

Cytokine Assays

IL-18 concentration was measured using a commercial ELISA kit (MBL, Japan) according to the manufacturer’s instructions.

Statistical Analysis

The Mann–Whitney U test and Wilcoxon signed rank test were used to determine the differences between groups. Relationships between variables were examined using the Spearman test. P < .05 was considered statistically significant.

RESULTS

Study Population With Acute EBV Infection

Ten subjects (6 men and 4 women) were included in the study. The median age of the study subjects was 31 years (range, 18–43 years). Subjects were enrolled at a median of 27 days (range, 15–42 days) after onset of illness. The median follow-up period was 40 days (range, 34–52 days). Clinical diagnosis of acute EBV infection was confirmed by the presence of IgM antibodies to EBV VCA. The median time point of first-obtained serum was 11 days [range, 4–26 days] after onset of disease. All patients developed IgG antibodies to EBV VCA. Retrospectively, the onset of disease was associated with a median severity score of 5.6 (9 patients had a maximal score of 6 and 1 patient had a score of 2). Fatigue and myalgia were the most common clinical symptoms together with sweating, pharyngitis, and weight loss as the most common clinical signs. Fever was noticed in 6 of the 10 subjects. On enrollment, the median severity score was 4.2 [range, 2–6]. The only symptom that persisted throughout follow-up was abnormal fatigue, and this was still present in 4 of the 10 subjects at their last visit. As expected, the severity of disease correlated with time after onset of disease \((r = .8160, P < .05)\) (Figure 1A). The viral load did not correlate with severity of disease (Figure 1B).

IL-18 Serum and Ferritin Concentrations Are a Prominent Feature in Acute EBV Disease

IL-18 concentration on enrollment was clearly elevated in all subjects with a median of 1018 pg/mL (range, 425–3125 pg/mL), compared to plasma concentration of the healthy volunteers with a median of 200 pg/mL (range, 80–405 pg/mL) (Figure 1C). Serum IL-18 in acute EBV disease was associated with days after onset of illness (Figure 1D), severity of disease (Figure 1E), and ALT (data not shown), but not with EBV viral load (Figure 1F). IL-18 was not correlated with other markers of inflammation, most notably CRP concentrations and lymphocyte counts. CRP concentrations were low and very often under the detection limit. On the other hand, all subjects had higher ferritin levels on enrollment compared to their levels at the last visit (Figure 2A). The median ferritin level at enrollment was 431 mg/L (range, 56–824 mg/L) versus 115 mg/L (range, 34–150 mg/L) at the last visit for males and 87 mg/L (range 51–112 mg/L) versus 57 mg/L (range 11–90 mg/L) for females. Ferritin levels correlated ALT (data not shown), and IL-18 concentrations (Figure 2C). Additionally, we tested IL-18 concentration in 3 patients with well-known EBV-related disease (EBV-related lymphoma, EBV-related hemolytic anemia, and EBV-related hemophagocytic syndrome), IL-18 was elevated (2595 pg/mL, 19300 pg/mL, and 5915 pg/mL, respectively).

IL-18 Concentration Is Not Associated with Chronic Fatigue Syndrome

We noted that serum IL-18 remained elevated during EBV infection until the moment when fatigue was the only symptom present (Figure 2D). Although it is generally accepted that EBV is not causing CFS, some patients report the start of their CFS symptoms after an episode of infectious mononucleosis and thus IL-18 might act as a potential perpetuating factor. Therefore, we compared the IL-18 concentration in 17 EBV seropositive patients with CFS and 5 EBV seropositive healthy controls of similar age, sex, and geographical area. There was no significant difference in serum IL-18 between patients with chronic fatigue syndrome and matched healthy controls (Figure 2E).
DISCUSSION

In the present study, we demonstrate that IL-18 circulating plasma concentrations are markedly elevated in acute EBV infection. The level of IL-18 correlates with the severity of disease and of the day of illness when the IL-18 concentration was measured. Additional remarkable findings were that the viral load neither correlates with IL-18 concentrations, nor with severity of disease. Notably, in acute EBV infection, ferritin levels are elevated compared to baseline and correlate with IL-18 production.

In patients with acute EBV infection, serum IL-18 concentrations were markedly elevated in conjunction with severity of disease, and slowly decreased during recovery. The rise of IL-18 concentrations in serum during acute viral infections is remarkable because this cytokine does not modulate the classic acute phase response [10]. The high amounts of IL-18 that have been found in lymphoid tissues of IM patients [5] and the elevated concentrations of IL-18 in serum during acute EBV infection shown here suggest that IL-18 plays an important role in regulating the immune response against

Figure 1.  A. The severity of EBV infection inversely correlated with time after onset of disease ($r^2 = -0.82$, $P < .05$). B. Correlation plot between viral loads and severity of illness (not significant). C. IL-18 concentrations in healthy controls and patients with EBV infection. Correlations of IL-18 concentrations with (D) days after the onset of disease ($r^2 = -0.49$, $P < .05$), (E) severity of illness ($r^2 = 0.46$, $P < .05$), and (F) viral loads (not significant). Abbreviations: EBV, Epstein–Barr virus; IL-18, interleukin-18.
EBV. This is in line with the observations that IL-18 was elevated in children with infectious mononucleosis [11]. Although IL-18 may play a role in the immune response against EBV infection, we cannot exclude that IL-18 is only a factor contributing to symptoms and signs of disease, or even only an epiphenomenon. Nevertheless, given the strong correlation between IL-18 and severity of illness during acute EBV infection, IL-18 could serve as a severity marker. Furthermore, the biological activity of IL-18 is regulated by the amount of IL-18 binding protein (IL-18BP) present in the serum [12].

Figure 2. A. Ferritin concentrations are increased in the acute phase of EBV infection in both males and females. Correlation between ferritin concentrations and (B) ALT ($r^2 = 0.76$, $P < .05$) and IL-18 (C) ($r^2 = 0.76$, $P < .05$) are shown. Correlation data are given as fold over increase to ferritin concentrations measured at the last visit when disease activity was lowest and liver tests had returned to normal, because of wide interpersonal variation in ferritin levels and differences between the range of normal values for ferritin in males and females. D. IL-18 concentrations in patients that completely recovered from EBV infection (no symptoms) and patients that still experienced post-EBV infection fatigue (fatigue) ($P < .05$). E. IL-18 concentrations in healthy patients and patients with CFS (not significant). Abbreviations: ALT, alanine aminotransferase; CFS, chronic fatigue syndrome; EBV, Epstein Barr virus; IL-18, interleukin-18.
IL-18BP binds to IL-18 and lowers its biological activity [13]. Although we did not measure IL-18BP in our study due to the lack of commercially available validated test kits, we speculate that the ratio of free IL-18:IL-18BP is elevated because IFNγ has been described to be elevated in infectious mononucleosis.

Given the striking IL-18 elevation in acute EBV infection, we also analyzed IL-18 concentrations in other EBV-related diseases. First, we determined whether IL-18 could also serve as a disease marker for CFS. This was of particular interest because during convalescence of IM, patients that were still fatigued exhibited the highest IL-18 concentrations. In a well-established cohort of CFS patients with positive serology for EBV [14], we found no differences in IL-18 circulating concentrations between CFS patients and healthy controls. When we assessed IL-18 concentration in patients that had a severe EBV-related disease, IL-18 was clearly elevated.

Another interesting finding is the elevated ferritin concentration in patients with EBV infection. Increased ferritin concentrations in infectious diseases have been associated with the acute phase responses in which CRP concentrations are also elevated. In acute EBV infection, all samples exhibited CRP levels below the detection limit, a feature often found in some (although not all) viral infections. This strongly suggests that the inflammatory pathway resulting in the induction of ferritin production is different from the pathway leading to CRP production. This hypothesis could be the explanation why many studies failed to demonstrate a correlation between CRP and ferritin in inflammatory diseases like systemic lupus erythematosus, rheumatoid arthritis, or adult-onset Still's disease (AOSD).

In the present study, ferritin correlated with IL-18 concentration. This association has also been noted in AOSD, but not in viral infections. It is intriguing to speculate that increased IL-18 and ferritin levels could be the reflection of a specific inflammatory route that is shared by acute EBV infection and AOSD. Several mechanisms can account for the elevated serum ferritin levels. First, ferritin can be elevated because of release from hepatocytes surrounded by infiltrated mononuclear cells, which is in line with our finding of a correlation between ferritin levels and ALT levels. A second explanation is an increased production of ferritin by activated macrophages, supported by the observation that the highest concentration of IL-18 was measured in a macrophage-activation syndrome [15].

In this patient cohort with acute EBV infection, we did not detect a correlation with viral load and severity of disease. This was an unexpected finding because it has been reported that viral load correlates with clinical disease in acute EBV infection [7]. This could be explained by the fact that the first measurement of the viral load in our cohort was performed at a median of 27 days, while the study that showed a correlation measured samples that were taken in the first week of EBV infection.

In conclusion, we have shown that IL-18 concentrations are markedly elevated during acute EBV infection. In addition, ferritin concentrations have also been elevated during acute EBV infection and correlate with serum IL-18 levels. Therefore, IL-18 and ferritin may represent infection markers for certain viral infections such as EBV, similar to the role played by CRP and procalcitonin for bacterial infections.

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