PROLIFERATION OF CYTOTOXIC AND ACTIVATED T CELLS DURING ACUTE EPSTEIN-BARR VIRUS INDUCED INFECTIOUS MONONUCLEOSIS

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Abstract: ***The immune responses that develop following Epstien–Barr Virus (EBV) infection are complex and involve both humoral and to a greater extent cell-mediated immune mechanisms. To evaluate the immune response, flow cytometric analysis of the peripheral blood of six patients during the acute phase of EBV infection was performed. This analysis revealed a significant increase in the percentages and the absolute number of CD8+ cytotoxic and activated (HLA-DR+) T lymphocytes and in some cases with a concomitant decrease in the percentages of B (CD19+) lymphocytes and T helper (CD4+) lymphocytes. These patients invariably had inverted CD4/CD8 ratio. All changes reversed to normal level during the recovery phase of infection. It is therefore concluded that EBV specific cytotoxic and activated T lymphocytes are essential in controlling acute EBV infection presented by the infected B cells.***
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INTRODUCTION

Epstien-Barr virus belongs to herpes virus family and is the primary agent of infectious mononucleosis. More that 90% of the human population acquire EBV in infancy and retain a lifelong infection without any clinical consequences. Nevertheless, EBV has been associated with several human tumors including endemic Burkitts lymphoma and B cell lymphomas

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(1). EBV infection is a major concern in immuno-suppressed patients. Infection of humans with EBV results in both humoral and cellular immunity to the virus. Although the finding of antibodies produced against viral antigens is important for the diagnosis of infection, the cellular immune response is more important for the control of EBV infection (2). Natural killer cells (NK), CD4+ helper and CD8+ cytotoxic T lymphocytes control proliferating EBV–infected B lymphocytes during primary infection (3). In infectious mononucleosis, up to 40% of CD8+ T lymphocytes are targeted to one replicative EBV protein, whereas 2% are targeted to one latent EBV protien (4). After recovery from acute infection, HLA- restricted cytotoxic T lymphocytes are important in controlling EBV, and CD8+ T cells are targeted to similar percentages of replicative and latent antigens (5).

The ability of EBV to persist and become latent, despite potent immune effector responses against it, indicates that the virus has evolved mechanisms to evade the immune system. One mechanism that EBV uses to elude the immune system is interference with cytokine activity. EBV encodes a cytokine that has more than 80% homology with human interlukine-10 (IL-10) (6). EBV IL-10 inhibits the synthesis of IFN-γ by lymphocytes and NK cells. This cytokine also inhibits the synthesis of IL-1, IL-12 and TNF by macrophages as well as macrophage-dependent proliferation of T lymphocytes (7-8). Another mechanism that EBV can use for immune evasion is interference with cytolytic T lymphocytes (CTLs) activity that would otherwise limit virus spread. Killing of EBV-infected B cells requires CTLs recognize viral peptides on the surface of B cells in the context of HLA class I and also participation of the adhesion molecules such as intercellular adhesion molecule-l (ICAM-1) on the surface of the infected cell. Thus, reduced expression of HLA class I,

transporter-associated proteins and adhesion molecules may protect infected B cells from CTLs (9).

The vast majority of individuals with primary EBV infection recover uneventfully and develop a high degree of long lasting immunity. However, in certain hosts EBV infection has been associated with a number of complications such as rash, splenic rupture (a potentially life-threatening complication), airway obstruction, neurologic disease and malignancies (1). EBV is also associated with lymphoproliferative disease in patients with congenital or acquired immunodeficiency. These include patients with severe combined immunodeficiency, recipient of organ or bone marrow transplants, and patients with AIDS (2). Such patients have impaired T cell immunity and are unable to control the proliferation of EBV–infected B cell. Perturbation of celluar immune responses may result in poorly controlled EBV infection. Due to these complications that might arise, assessment of the cellular componet of immune system deems important for the management of the disease.

MATERIALS AND METHODS

Six patients with typical infectious mononucleosis due to EBV-infection were studied between June-October 2000. The age range was 14 to 39 and consisted of two males and four females. Clinical findings consisted of fever, pharyngitis, generalized lymphoadenopathy and splenomegaly with marked lymphocytosis and more than 10% atypical lymphocytes in peripheral blood. The diagnosis was confirmed by the measurement of anti-EBV capsid specific IgM and IgG, using an ELISA method where an antibody titer of greater than l.l IU was considered positive. Throat culture for group A beta hemolytic streptococci and serologic tests for CMV, HIV, toxoplasma, HBV and HCV were negative. There was no history of drug consumption.

Antibodies

Becton-Dickinson (CA, USA) Simultest two-color direct immunofluorescence reagents were used from the vials with no dilution as recommended by the manufacturer. The antibodies used were fluorescin isothiocyanate (FITC) and phycoerythrin (PE) conjugated. The following antibodies were used: Leukogate (CD45-FITC/CD14-PE), Control (IgG_1-FITC/IgG_2a-PE), CD3-FITC/CD19-PE, CD3-FITC/CD4-PE, CD3-FITC /CD8-PE, CD3-FITC/ HLA-DR-PE, CD3-FITC/CD16+56-PE.

Sample preparation and flow Cytometric analysis

Whole blood samples (2 ml) were obtained by venipuncture into a sterile blood collection tube. Seven dual color tubes containing 20µl of an appropriate monoclonal antibody were prepared for each sample. One hundred µL of blood sample was placed in each tube and mixed thoroughly and incubated at room temperature for 20 min before lysing with FACS (BS, USA) lysing solution. Samples were then washed twice and 15000 cells were analyzed immediately using FACSCalibur (BD, USA) flow cytometry. Mean while total IgG, IgM, IgA were determined employing SRID during acute phase of infection for four subject.

Statistical Analysis

All results are expressed as the mean±SD. To assess the differences between two phases of infection the t-paried test was employed.

RESULTS

The distribution of peripheral blood T lymphocyte subsets, CD4/CD8 ratio, B lymphocytes and NK cells of the patients during the acute phase of EBV infection are summarized in table l. As shown in table l, the percentages and absolute count of CD8+ and HLA-DR+ T lymphocytes were markedly increased in all patients during the acute phase of infection ($p < 0.001$) as compared to normal range. The results also show that the percentages of CD4+ T lymphocytes (in four cases) are below the normal range (Table 1). Table 1 also shows that the percentages of NK cells are within normal values during the recovery phase of infection. Significant differences were observed for the CD4/CD8 ratio during the acute phase of infection when compared to the normal values (Table 1). During the recovery phase all changes had resolved (Table 2). Further, during the course of analysis, it was noticed that the number of B cells in patients was below normal range, although no changes in the amount of serum immunoglobulins were noticed (Table 3). This may be explained by the fact that immunoglobulins have long half-lives and due to the rapid recovery of B cells in our patients, it was not deemed necessary to measure antibody levels in convalescent phase.

DISCUSSION

Whereas most EBV infection of infants and children are asymptomatic, infection of adolescents and adults frequently result in infectious

mononucleosis (10-11). Most patients with infectious mononucleosis have leukocytosis with an absolute increase in the number of pripheral blood mononuclear cells and atypical lymphocytes (12). The atypical lymphocytes are primarily T cells, many of which are responding to the EBV- infected cells (13-14).

In this report attempts were made to evaluate whether or not the cellular component of the immune system of patients suffering from infectious mononucleosis due to EBV infection responded appropriately to infection. We therefore analyzed the cellular component of the immune system of patients by flow cytometry. The values found in the blood counts of EBV-infected patients during the acute phase of infection as compared to normal values and the convalescent phase of infection revealed significant lymphocytosis for CD8+ and HLA-DR+ T lymphocytes ($p < 0.001$), indicating expansion (activation and proliferation) of these cells in response to EBV-specific antigen(s).

The atypical lymphocytosis that appeared in the peripheral blood of patients with acute infectious mononucleosis between one and three weeks after the onset of symptoms were primarily activated (HLA-DR+) and CD8+ T cells. These findings are consistent and support earlier works performed by others. Activated CD8+ T cells play a central role in anti-viral immunity. Activated CD8+ T-cell clone reactive against the viral antigens exhibit cytolytic activities and produce IFN-γ. The results presented in this study also indicated that the number of B cells were below normal range without having any reduction in the level of immunoglobulins. It therefore seems that B lymphocytopenia observed in this study can be explained by the killing of virally infected B cells by CTLs and/or lysis of EBV-infected B cells following viral progeny synthesis. With recovery from illness, the atypical lymphocytosis gradually resolved. During the convalescent phase of infection, EBV-specific CTLs are thought to be the predominant cells controlling virus infection. It was also demonstrated that there was statistically no significant difference in the percentages of NK cells between the two phases of illness, although reports indicate that NK cells play an important role in controlling the proliferating EBV-infected cells during primary infection (15).

Although no complications were observed in our patients, but EBV infection has reportedly been associated with a number of complications and in certain hosts delayed recovery. Therefore peripheral blood cell analysis provides a useful correlation between immunological status and clinical course of the disease. No specific therapy is required for most patients suffering from infectious mononucleosis, since it is generally a self-limited illness. Corticosteroid therapy should be considered for patients with severe complications of infectious mononucleosis such as impending airway obstruction, acute hemolytic anemia, severe cardiac involvement, or neurologic disease (16-17). Corticosteroids treatment shorten the duration of fever and oropharyngeal symptoms associated with infectious mononucleosis and is believed to suppress/inhibit cytotoxic T lymphocyte activity and their recruitment to affected organs (18). The findings of this study provide insights into the role of the immune system in controlling viral infection and it is also believed that most of the clinical symptoms of infectious mononucleosis are attributed to the activation and proliferation of T cells particularly CD8+ and HLA-DR+, in response to infection.

Table 1. Percentages and absolute count of T lymphocytes, subsets, B cells, NK cells and activated T cells in peripheral blood of the patients during the acute phase of EBV- infection.

patient	CD3% (Absolute Count)	CD4% (Absolute Count	CD8% (Absolute Count	CD4/CD8	CD19% (Absolute Count	CD16% (Absolute Count	CD3%/HLA-DR$^+$ (Absolute Count)
1	**84 (1561)**	**46 (855)**	**48 (892)**	**0.96**	**11 (204)**	**17 (315)**	36 (664)
2	**78 (1561)**	**29 (950)**	**53 (1736)**	**0.55**	**7 (224)**	**15 (491)**	46 (1375)
3	**88 (3344)**	**36 (1368)**	**58 (2204)**	**0.62**	**4 (152)**	**11 (418)**	73 (2771)
4	**73 (5410)**	**9 (667)**	**59 (4377)**	**0.15**	**1.3 (96)**	**12 (889)**	65 (2817)
5	**90 (10469)**	**35 (4082**	**62 (7231)**	**0.56**	**2 (233)**	**7 (816)**	76 (8863)
6	**88 (8697)**	**9.4 (595)**	**77 (4878)**	**0.12**	**4.7 (297)**	**14 (1383)**	68 (6720)
Normal Range%	59.4-84.6	29-59	19-48	1.3-2	6.4-22.6	5.6-30.9	9-22

Table 2. Percentages and absolute count of T lymphocytes, subsets, B cells, NK cells and activated T cells in peripheral blood of the patients during the recovery phase EBV- infection.

patient	CD3% (Absolute Count)	CD4% (Absolute Count	CD8% (Absolute Count	CD4/CD8	CD19% (Absolute Count	CD16% (Absolute Count	CD3%/HLA-DR$^+$ (Absolute Count)
1	**80 (1179)**	**63 (926)**	**21 (310)**	**3**	**17 (250)**	**14 (206)**	10 (147)
2	**82 (2709)**	**45 (1486)**	**32 (1057)**	**1.4**	**15 (697)**	**9 (297)**	11 (363)
3	**78 (3198)**	**40 (1640)**	**32 (1312)**	**1.25**	**17 (697)**	**12 (492)**	12 (492)
4	**70 (3694)**	**32 (1689)**	**36 (1900)**	**0.9**	**14 (739)**	**18 (632)**	40 (2111)
5	**86 (6096)**	**61 (4821)**	**44 (3477)**	**1.4**	**5 (395)**	**8 (632)**	12 (948)
6	**63 (2045)**	**26 (844)**	**35 (1136)**	**0.75**	**20 (649)**	**16 (519)**	32 (649)
Normal Range%	59.4-84.6	29-59	19-48	1.3-2	6.4-22.9	5.6-30.9	9-22

Table 3. Serum immunoglobulin levels of four patients during the acute phase of EBV- infection.

Patients (No)	IgG	IgA	IgM
2	**900**	**60**	80
4	**1400**	**230**	220
5	**1700**	**310**	317
6	**1050**	**450**	400
Normal Range	800-1800	90- 450	60- 250

REFERENCES

1. Aronson MD, et al. Heterophile antibody in adults with sore throat: Frequency and clinical presentation. Ann Intern Med 1982; 96:505-10.

2. Cohen JI. Epstein-Barr virus infection. New Engl J Med 2000; 7:481-92.

3. Rickinson AB, Moss DJ. Human cytotoxic T lymphocyte responses to Epstein-Barr virus infection. Annu Rev Immunol 1997; 15:405 –31.

4. Callan MFC, et al. Direct visualization of antigen-specific CD8+ T cells during the primary immune response to Epstien-Barr virus in vivo. J Exp Med 1998; 187:1395-402.

5. Tan LC, et al. A re-evaluation of the frequency of CD8+ T cells specific for EBV in healthy virus carriers. J Immunol 1999; 162:1827-35.

6. Moore KW, et al. Homology of cytotoxic synthesis inhibitory factor (IL-10) to the Epstein-Barr virus gene BCFRl. Science.1990; [Erratum 1990 250:494] 284: 1230-4.

7. Hsu DH, et al. Expression of interleukine-10 activity by Epstein-Barr virus protein BCRF1. Science1990; 250:830-2.

8. Swaminathan S, Kieff E. The role of BCRFl/vIL-10 in the life cycle of Epstein-Barr Virus. In: McFadden G, ed. Viroreceptors, Virokines and Related Immune Modulators Encoded by DNA Viruses. Austin, Te:RG Landes Co: 1994; 111-125.

9. Cohen JI. Epstein-Barr virus and the immune system. JAMA 1997; 287:508-13.

10. Henke CE, Kurland LT, Elveback LR. Infectious mononucleosis in Rochester, Minnesota, 1950 through 1969. Am J Epidemiol 1973; 98:483-90.

11. Straus SE, Cohen JI, Tosato G, Mejir J. NIH conference: Epstein-Barr virus infection: biology, pathogenesis, and management. Ann Intern Med 1974; 118:1-11.

12. Papamichail M, Sheldon PJ, Holborow EJ. T- and B-cell subpopulations in infectious mononucleosis. Clin Exp Immunol 1974; 18:1-11.

13. Engberg RN, Eberle BJ, Williams RC. T-and B-cells in peripheral blood during infectious mononucleosis. J Infect Dis 1974; 130:104-11.

14. Pattengaie PK, Smith RW, Perlin E. Atypical lymphocytes in acute infectious mononucleosis. Identification by multiple T and B markers. N Engl J Med 1974; 292:1145-8.

15. Biazar B, et al. Increased sensitivity of human lymphoid lines to natural killer cells after induction of the Epstein-Barr virus cycle by superinfection or sodium butyrate. J Exp Med. 1980; 151:614-27.

16. Bender CE. The value of corticosteroids in the treatment of infection mononucleosis. JAMA 1967 15:529-31.

17. Klein EM, Cochran JF, Buck RL. The effects of short-term corticostroid therapy on the symptoms of infectious mononucleosis pharyngotonsilitis: A double blind study. J Am Coll Health Assoc 1969; 17: 446-52.

18. Collins M, Fleischer G, Kresberg J, Fager S. Role of steroid in the treatment of infectious mononucleosis im the ambulatory college student. J Am Coll Health Assoc 1984; 33: 101-5.