HIGH TH1-TYPE CYTOKINE SERUM LEVELS IN PATIENTS WITH INFECTIOUS MONONUCLEOSIS

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Received April 14, 2004; accepted November 4, 2004

Summary. – Most adults are asymptomatically infected with Epstein-Barr virus (EBV). Primary EBV infection is commonly associated with acute infectious mononucleosis (IM). T cell immune activation plays an important role in EBV-associated diseases. IM shows a mainly Th1-type profile, so Th1-type cytokines such as interleukin-2 (IL-2), interferon-γ (IFN-γ), and lymphotoxin (LT) are moderately enhanced. We measured IL-2 and IFN-γ in serum during acute phase of the disease and during convalescence. Sera were collected from 23 IM patients, 13 patients with similar clinical manifestations but without IM, and 10 healthy donors. The levels of IL-2, IFN-γ and IL-12 were significantly higher in patients with acute IM than in healthy individuals. IL-2, IFN-γ and IL-12 decreased during convalescence. These three cytokines may be useful as sensitive markers of IM during severe illness and its later phases.

Key words: infectious mononucleosis; interferon-γ; interleukin-2; interleukin-12

Introduction

Symptomatic acute EBV infection in IM causes fever, hepatosplenomegaly, lymphadenopathy, and an increase in the number of activated $CD8^+$ T lymphocytes in peripheral blood (Callan *et al*., 1996). These manifestations are due to the cytotoxic T lymphocyte (CTL) response to polyclonal proliferation of EBV-infected B cells (Rickinson *et al*., 1996). Subpopulations of EBV-specific $CD8^+$ T cells from patients with IM express IFN-γ and macrophage inflammatory protein 1β (MIP-1β) after stimulation with antigen (Ohshima *et al*., 2003).

During acute phase of the disease, patients suffer from lymphadenopathy, sore throat and fatigue, and the absolute lymphocyte count usually exceeds 4,000 with 10% of atypical forms. These patients have only a little increased number of CD4-positive T cells, but a markedly elevated number of CD8-positive cells. Usually patients have high serum levels of IgM and IgG antibodies against viral capsid antigen (VCA), and IgG antibodies to Epstein-Barr nuclear antigens (EBNA). IM patients present a predominantly Th1-type profile, which is consistent with Th1-type cytokine secretion.

IL-12 is an important factor in controlling the differentiation of Th cells (Hsieh *et al*., 1993), favoring the expansion of Th1 cells (Seder *et al*., 1993). Since it is largely accepted that cytokines are involved in the pathogenesis of IM, the present study was focused on the expression of the Th1-type cytokines, namely IL-2, IFN-γ and IL-12 in the sera of 23 patients with IM during the acute phase and convalescence.

Materials and Methods

Patients. Blood samples were taken from 23 patients with clinical symptoms of acute IM either during the acute phase or between days 10 and 30 of convalescence (median day 20). Blood sam-

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Abbreviations: CTL = cytotoxic T lymphocyte; EBV = Epstein-Barr virus; EBNA = Epstein-Barr nuclear antigen; IM = infectious mononucleosis; IFN = interferon; IL = interleukin; LT = lymphotoxin; MP1-1β = macrophage inflammatory protein 1β; VCA = viral capsid antigen

Table 1. Serum IFN-γ, IL-2 and IL-12 levels in healthy individuals, patients with IM, convalescing IM patients and positive control patients

Cytokine	Healthy individuals	IM acute phase patients	Convalescing IM patients	Positive control patients
IFN-γ (IU/ml)	1.2 ± 0.7	6.3 ± 2.7	1.4 ± 0.7	6.35 ± 2.7
IL-2 (pg/ml)	9.5 ± 2.6	55.9 ± 6.7	15.8 ± 2.3	35.87 ± 4.7
IL-12 (pg/ml)	7.8 ± 2.2	73.9 ± 3.5	23.3 ± 3.5	45.7 ± 3.8

The results represent means ± SD.

ples from 13 clinically symptomatic patients with negative or slightly positive serological analysis were used as positive controls. Samples from 10 young healthy adult donors were used as negative controls. Sera aliquots were stored at -20°C for laboratory assays. This study was approved by the Ethical Committees of the institutions involved and informed consent was obtained from patients.

ELISA. Human cytokine ELISA systems (R&D Systems, USA) were used to measure serum cytokine levels. Briefly, 100 μl of serum and standard dilutions of the tested cytokine were added to the test wells in duplicate. Each assay was carried out according to the manufacturer's instructions. The absorbance was read within 15 mins at 405 nm for IL-2 and at 450 nm for IFN-γ and IL-12 using a microplate autoreader (EL-800, Bioteck, USA). Results are expressed in pg/ml for IL-2 and IL-12 and in IU/ml for IFN-γ. The intra-assay coefficient of variation was 8.5%.

Statistical analysis. Differences between cytokine levels in patients during the early phase of disease or in convalescence, healthy donors and positive controls were analyzed by the ANOVA and Tukey-Kramer multiple comparison test. Differences with P below 0.05 were considered significant. Results are expressed as mean ± SD (GraphPad, InStat, USA).

Results

All IM patients recovered fully, with no specific therapy. The ELISA was standardized to measure the lowest serum level, i.e. 0.5 IU/ml of IFN-γ and 1 pg/ml of IL-2 and IL-12. The results are summarized in Table 1.

The levels of IFN-g were significantly higher in the patients with acute IM than in healthy individuals or in the patients during convalescence from IM. IFN-γ returned to normal level during convalescence. IFN-γ levels were almost the same in the patients with acute IM and in those with similar symptoms but no IM (positive controls). These patients all had significantly higher levels of IFN-γ than healthy individuals.

The IL-2 levels were significantly higher in the patients with acute IM than in healthy individuals or patients convalescing after IM. IL-2 dropped gradually during convalescence to a level similar to healthy individuals. IL-2 levels in the patients with acute IM or in remission were significantly different from positive controls with similar symptomatology.

The IL-12 levels were significantly higher in the patients with acute IM than in healthy individuals or patients convalescing from IM. IL-12 levels in patients with acute IM or in remission were significantly different from positive controls with similar symptomatology. IL-12 dropped during convalescence but was still higher than in healthy individuals.

Discussion

There is ample evidence that the synthesis of various T cell-derived cytokines such as IFN-g, IL-2 and IL-6 influences the inflammatory response to EBV infection (Attarbashi *et al*., 2003). In the present study, we investigated the cytokine profile of the Th1-type subset in the patients with symptomatic acute EBV infection. There was a clear increase in IFN-γ and IL-2 levels.

Serum cytokine levels may rise as a result of production by EBV-infected cells (Andersson and Andersson, 1993), monocytes (Hornef *et al*., 1995) or activated T lymphocytes (Tosato *et al*., 1990). There are various points in favor of overproduction of cytokines in IM. High levels of TNF-α, IL-1β, IL-6, IFN-γ, and soluble IL-2 receptors (sIL-2R) (Hornef *et al*., 1995) may be produced by stimulated peripheral blood cells in IM, but they are not rescued in patients' sera. Elevated concentrations of IL-2, IFN-γ and the monocyte activation factor neopterin have been reported in EBV infection and in chronic fatigue syndrome (Linde *et al*., 1992). High concentrations of IL-6 have been found in several EBV-related diseases (Shuster *et al*., 1993). Biglino *et al*. (1996) have detected high serum levels of IL-2, IL-4, sIL-2R, and TNF-α in IM patients. Wright-Brown *et al*. (1998) have also found significantly increased levels of TNF-α and IL-6 in most IM patients. Research on Th2-type cytokines such as IL-10 points to elevated serum levels of this cytokine, which may inhibit apoptotic cell death in IM (Taga *et al*., 1994, 1995).

In this study, we analyzed sera from IM patients with a serologically and cytomorphologically confirmed diagnosis. A VCA-IgM-positive and EBNA-negative serological pattern is considered a marker of acute infection; antibodies to EBNA are classically regarded as indicating resolution of the infection because they are not initially present but appear weeks or months later (Rea *et al*., 2002).

As negative controls we used sera from healthy donors and patients who did not have IM but presented similar symptoms. IFN-γ, IL-2 and IL-12 levels increased significantly in IM. They dropped during convalescence, becoming similar to those in healthy individuals.

It has been shown *in vitro* that IFN-γ is important for the blockade of EBV transformation (Gosselin *et al*., 1989); thus it has probably an essential role in mechanisms of immune response to EBV infection but may simultaneously contribute to the appearance and maintenance of the symptoms of IM. Cytokines such as IL-2 and IFN-γ appearing in the cascade of cellular activation induced by antigens can by themselves induce many of the symptoms of infectious disease. It is not clear, however, which cytokines are active *in vivo* in IM (Browne *et al*., 1998).

The patients with IM showed a mixed pattern of up-regulated expression of genes such as MIP-1α and MIP-1β. These are chemokine genes expressed by reactive T cells, cytotoxic cells and Th1-type, but not Th2-type T cells. IM therefore appears to have mainly the Th1-type profile (Ohshima *et al*., 2003). Attarbashi *et al*. (2003) have reported a striking expansion of IFN-γ-producing CD8+ T cells as a key factor in clinically overt disease. In addition, CD8 T cell clones may depend on cytokine stimulation for survival (Macallan *et al*., 2003). Because CD8 T cell differentiation needs Th1 cells, whose development is driven by IL-12, our data appear to suggest a Th1-type profile in acute EBV infection with increased IL-12 serum levels. The role of IL-12 as a key cytokine in the development of Th1 responses has been elucidated in several models. IL-12 promotes Th1 differentiation through activation of the signal transducer and activator of transcription 4 (STAT4) and triggers a cascade of events including IFN-γ production, potentially leading to Th1 differentiation (Durali *et al*., 2003). Only a few studies deal with its effects in IM (Villacres-Eriksson *et al*., 1997). Nevertheless IL-12 is known to induce IFN-γ production by T cells (Pulendran, 2004). An IL-12 pretreatment may result in endogenous IFN-γ production (Lesinski *et al*., 2004).

To the best of our knowledge, there are no previous reports of plasma IL-12 levels in IM, except for a group that has investigated serum levels of IL-12 and IL-10 in patients with bacterial infections, mononucleosis and anaphylactoid purpura (Katayama *et al*., 2000).

In conclusion, our findings suggest that IFN-γ, IL-2 and IL-12 have probably a pathophysiological role in IM. It is therefore possible that molecules that suppress the biological activity of these cytokines or inhibit the interaction with their receptors will prove beneficial in the treatment of IM.

Acknowledgements. This work was supported by a grant from Ministero dell' Università della Ricerca Scientifica e Technologica. We are grateful to Ms. J. Baggott for revising the manuscript.

References

Andersson J, Andersson U (1993): Characterization of cytokine production in infectious mononucleosis studied at a single-cell level in tonsil and peripheral blood. *Clin. Exp. Immunol.* **92**, 7–13.

Attarbashi T, Willheim M, Ramharter M, Hofmann A, Wahl K, Winkler H, Graninger W, Winkler S (2003): T-cell cytokine profile during primary Epstein-Barr virus infection (infectious mononucleosis. *Eur. Cytokine Netw.* **14**, 34–39.

Biglino A, Sinicco A, Forno B (1996): Serum cytokine profiles in acute primary HLV-1 infection and infectious mononucleosis. *Clin. Immunol. Immunopathol.* **78**, 61–69.

Browne VW, Schnee AM, Jenkins MA, Thall PF, Aggarwal BB,Talpaz M, Estrov Z (1998): Serum cytokine levels in infectious mononucleosis at diagnosis and convalescence. *Leuk. Lymphoma* **30**, 583–589.

Callan MFC, Steven N, Krausa MG, Wilson JD, Moss PA, Gillespie GM, Bell JI, Rickinson AB, McMichael AJ (1996): Large clonal expansion of CD8+ T cells in acute infectious mononucleosis. *Nat. Med.* **2**, 906–911.

Durali D, de Goer de Herve MG, Giron-Michel J, Azzarone B, Delfraissy JF, Taoufik Y (2003): In human B cells, IL-12 triggers a cascade of molecular events similar to Th_1 commitment. *Blood* **102**, 4084–4089.

Gosselin J, Menezes J, Mercier G, Lamoureux G, Oth D (1989): Differential interleukin-2 and interferon-γ production by human lymphocyte cultures exceptionally resistent to Epstein-Barr virus immortalization. *Cell Immunol.* **122**, 440–449.

Hornef MW, Wagner HJ, Kruse A, Kirchner H (1995): Cytokine production in whole blood assay after Epstein-Barr virus infection in vivo. *Clin. Diagn. Lab. Immunol.* **2**, 209–213.

Hsieh CS, Macatonia SE, Tripp CS, Wolf SF, Ogarra A, Murphy KM (1993): Development of TH1 CD4+ T cells through IL-12 produced by Lysteria-induced macrophages. *Science* **260**, 547–549.

Katayama K, Matsubara T, Fujiwara M, Koga M, Furukawa S (2000): CD14+CD16+ monocyte subpopulation in Kawasaki disease. *Clin. Exp. Immunol.* **121**, 566–570.

Lesinski GB, Badgwell B, Zimmerer J, Crespin T, Hu Y, Abood G, Carson WE (2004): IL-12 pretreatments enhance IFN-alpha-induced Janus Kinase-STAT signaling and potentiate the antitumor effects of IFN-alpha in a murine model of malignant melanoma. *J. Immunol.* **172**, 7368–7376.

Linde A, Andersson B, Svenson BS, Ahrne H, Carlsson M, Forsberg I, Hugo H, Karstorp A, Lenkei R, Lindwall A, Loftenius A,Sall C, Andersson J (1992): Serum levels of lymphokines and soluble cellular receptors in primary Epstein-Barr virus infection and in patients with chronic fatigue syndrome. *J. Infect. Dis.* **165**, 994–1000.

Macallan DC, Wallace DL, Irvine AJ, Asquuith B, Worth A, Ghattas H, Zhang YGE, Tough DF, Beverley PC (2003): Rapid turnover of T cells in acute infectious mononucleosis. *Eur. J. Immunol.* **33**, 2655–2665.

Ohshima K, Karube K, Hamasaki M, Tutiya T, Yamaguchi T, Suefui H, Suzuki K, Suzumiya J, Ohga S,Kikuchi M (2003): Differential chemokine, chemokine receptor and cytokine expression in Epstein-Barr virus-associated lymphoproliferative diseases. *Leuk. Lymphoma* **44**, 1367–1378.

Pulendran B (2004): Modulating Th_1/Th_2 responses with microbes, dendritic cells, and pathogen recognition receptors. *Immunol. Res.* **29**, 187–196.

Rea TD, Ashley RL, Russo JE,Buchwald DS (20029: A systematic study of Epstein-Barr virus serologic assays following acute infection. *Am. J. Clin. Pathol.* **117**, 156–161.

Rickinson EC, Lee SP, Steven NM (1996): Cytotoxic T-lymphocyte responses to Epstein-Barr virus. *Curr. Opin. Immunol.* **8**, 492–497.

Seder RA, Gazzinelli R, Sher A, Paul WE (1993): Interleukin 12 acts directly on CD4+ T cells to enhance priming for interferon gamma production and diminishes interleukin 4 inhibition of such priming. *Proc. Natl. Acad. Sci. USA* **90**, 10188–10192.

Shuster V, Herold M, Wachter H, Reinegger G (1993): Serum concentrations of interferon-γ, interleukin-6, and neopterin with infectious mononucleosis and other Epstein-Barr virus-related lymphoproliferative diseases. *Infection* **21**, 210–213.

Taga K, Chretien J, Cherney B, Diaz L, Brown M, Tosato G (1994): Interleukin-10 inhibits apoptotic cell death in infectious mononucleosis T cells. *J. Clin. Invest.* **94**, 251–260.

Taga H, Taga K, Wang F, Chretien J, Tosato G (1995): Human and viral interleukin-10 in acute Epstein-Barr-induced infectious mononucleosis. *J. Infect. Dis.* **171**, 1347–1350.

Tosato G, Tanner J, Jones KD, Revel M,Pike SE (1990): Identification of interleukin-6 as an autocrine growth factor for Epstein-Barr virus-immortalized B-cells. *J. Virol.* **64**, 3033–3041.

Villacres-Eriksson M, Behboudi S, Morgan AJ, Trinchieri G, Morein B (1997): Immunomodulation by Quillaja saponaria adjuvant formulations: in vivo stimulation of interleukin 12 and its effects on the antibody response. *Cytokine* **9**, 73–82.