ANNALES
UNIVERSITATIS MARIAE CURIE-SKŁODOWSKA
LUBLIN-POLONIA
VOL. LXI, N1, 53 SECTIO D 2006

Chair and Department of Infectious Diseases, Medical University of Lublin

HANNA FOTA-MARKOWSKA, ROMA MODRZEWSKA, MONIKA WÓJTOWICZ

Serum levels of interleukin 6 (IL-6), IL-6 receptor (IL-6R) in patients with acute symptomatic mononucleosis (IM)

The Epstein-Barr virus (EBV) is a ubiquitous gamma herpesvirus that infects epithelial cells and B lymphocytes (13). In the developed world, 40% to 65% of individuals with EBV become infected during childhood, and the remainder suffer primary infection during adolescence or adulthood (4, 7). About half of the infected individuals suffer from acute infectious mononucleosis (IM). IM patients usually present with mild symptoms. However, serious complications and eve deaths have been reported, particularly in immunosuppressed persons (5). EBV has also been associated with several malignant diseases such as Burkitt's lymphoma, Hodgkin's disease, non-Hodgkin's lymphoma, immunoblastic B-cell lymphoma, and nasopharyngeal carcinoma (10. 11).

Several lines of evidence suggest that cytokines play a role in the pathogenesis of IM (1, 3, 5, 6, 8, 11, 12, 14, 15). The aim of the work was to analyse the level of interleukin 6 (IL-6) and IL-6 receptor (IL-6R) in acute symptomatic IM.

MATERIAL AND METHODS

We examined 24 patients, 11 women at the age of 18–26 and 13 men at the age 16–24 in acute symptomatic phase of mononucleosis (IM), who were hospitalised at the Department of Infectious Diseases, Medical University of Lublin during the period 2003–2004. The diagnosis was confirmed by the presence of high titers of IgM anti-VCA antibodies higher than 20AU/ml, immunoenzymatic method by Vironostica ® Bio-Merieux. The control group included 18 healthy persons, 13 women at the age of 16–28 and 5 men at the age of 17–25. Once in all the examined people we assessed the level of IL-6 and sIL-6R in blood serum by ELISA immunoenzymatic method by Bender MedSystems. The obtained data were analysed with the use of U Mann-Whitney statistical test.

RESULTS

Comparison of serum level IL-6 and s IL-6R is shown in Table 1. In control group IL-6 the serum level was 0.00–1.21±0.36 SD pg/ml. In IM patients 0.71-32.94±6.60 SD pg/ml. We observed statistically important difference between IM patients and control group in the levels of IL-6 ($p<0.001$). In the control group sIL-6R was 118.75-204.63±52.29 pg/ml. In IM patients it was 152.00-383.50±64.76 SD pg/ml. We observed statistically important difference between IM patients and control group in the levels of s IL-6R ($p<0.004$).

Table 1. Comparison of IL-6 and sIL-6R in infectious mononucleosis (IM) and control group (C)

		IL-6 (pg/ml)	sIL-6R (pg/ml)
Minimum	IM	0.71	152.00
	C	0.00	118.75
Maximum	IM	32.94	383.50
	C	1.21	297.50
Median	IM	3.03	290.25
	C	0.15	204.63
SD	IM	6.60	64.76
	C	0.36	52.29

DISCUSSION

EBV is an etiologic agent of infectious mononucleosis (IM) which is a self-limiting lymphoproliferative disorder caused by primary EBV infection (13). The life cycle of EBV appears to depend upon a mechanism to replicate and maintain the viral genome in an autonomous state in expanding B-cell populations during the initial phase of latency. T-cells immune activation plays an important role in IM and EBV associated diseases (9). It was interesting to identify IL-6 an IL-6R serum level, because this cytokine was involved in the induction of B-cell differentiation and proliferation and differentiation of T-cells.

IL-6 is a multi-functional cytokine that regulates immune responses, acute phase reactions and hematopoiesis and may play a central role in host defense mechanisms (6, 8, 11, 12, 14, 15).

The gene for human IL-6 has been localized in chromosome 7p21. The genomic sequence has been determined. IL-6 is usually not produced constitutively by normal cells, but its expression is readily induced by a variety of cytokines, lipopolysaccharide or viral infections. The IL-6 gene product is a single chain protein with a molecular mass ranging from 21 to 28 kDa, depending on the cellular source. Extensive posttranslational modifications like N- and O-linked glycosylation as well as phosphorylation seem to account for this heterogeneity. The cDNA for IL-6 predicts a precursor protein of 212 amino acids (6).

IL-6 is a pleiotropic cytokine produced by a variety of cells. It acts on a wide range of tissues, exerting growth-induction, growth-inhibition, and differentiation respectively, depending on the nature of the target cells. IL-6 is involved in the induction of acute phase proteins in liver cells, growth promotion of myeloma/plasmacytoma/hybridoma cells and induction of IL-2 and IL-2 receptor expression. It is very important that IL-6 inhibits cell growth of certain myeloid leukemic cell lines and inducts their differentiation to macrophages. A lot of evidence suggested enhancement of IL-3-induced multipotential colony cell formation in hematopoietic stem cells and induction of maturation of megakaryocytes as thrombopoietic factor.

Several investigators have used various techniques to identify cytokines that are overexpressed in IM. We used the IL-6 ELISA as an enzyme-linked immunosorbent assay for quantitive detection of human Interleukin-6 in human serum. We observed statistically important difference between IM patients and control group in levels of IL-6 ($p<0.001$). Hornef et al. detected high levels of IL-6 in stimulated IM peripheral blood cells but not in patients' sera (5). In contrast, Linde et al. reported decreased concentrations of IL-6 (8). Schuster et al. found high concentration of IL-6 in EBV associated diseases (10). Other investigators studied single cells from both tonsillar tissue and peripheral blood obtained from IM patients. Foss et al. used a double *in situ* hybridization procedure (3). This technique enabled them both to detect cells bearing nuclear EBV-encoded trancripts and determine their cytokine expression. They demonstrated that EBV-infected cells expressed predominantly LT, and to a smaller extent, TNF-$_{\alpha}$ In addition, specific signals for IL-6 were found only in a few EBV-infected cells. However, the transcripts of these cytokines were spotted in other non-EBV tonsillar cells. Anderson et al. used indirect immuno-

fluorescence and immunocytochemical methods to detect Il-6 protein in single cells obtained from IM patients (1).

In most of the cytokine-related autocrine-growth stimulatory loops reported thus far, the released extracellular cytokine interacted with its corresponding receptor to stimulate cellular growth. Therefore, we chose to measure serum Il-6 level using ELISAs specifically designed to detect low Il-6 concentration.

The abnormal production of IL-6 was first suggested to be related to polyclonal B-cell activation with autoantibody production in patients with cardiac myxoma. Since then, IL-6 has been suggested to be involved in the pathogenesis of a variety of diseases. Measurement of IL-6 levels in serum and other body fluids thus provides more detailed insights into various pathological situations. In infections body fluids of patients with acute local bacterial or viral infections and serum of patients with gram-negative or positive bacteriemia contain elevated levels of biologically active IL-6. In obstetric infections IL-6 has emerged as a reporter cytokine for intraamniotic infection. IL-6 plays an important role in diseases associated with an altered immune system (polyclonal B-cell abnormalities or autoimmune diseases). Elevated levels of circulating IL-6 have been detected in patients with cardiac myxoma, Castleman's disease, rheumatoid arthritis, IgM gammapathy and in those with aquired immunodeficiency syndrome as well as in alcoholic liver cirrhosis. Elevated plasma levels of IL-6 are observed in patients with psoriasis and mesangial glomerulonephritis. Increased systemic levels of IL-6 have been detected in patients with multiple myeloma, other B-cell discrasias, Lennert's T lymhoma, Castleman's disease, renal cell carcinoma and various other solid tumors. IL-6 is involved in the induction of acute phase proteins and induction of fever. Elevated serum levels of IL-6 are also found in patients with severe burns, in serum and plasma as a marker for predicting postoperative complications, in serum and urine of recipients of kidney transplantations before rejection, in the serum of septic shock patients and in patients with inflammatory arthritis and traumatic arthritis.

IL-6 exerts its action via a cell surface receptor which consists of two subunits, an 80 kDa ligand binding subunit (gp80) of 468 amino acids and a 130 kDa signal transducing protein (gp 130) of 896 amino acids residues (14). The cDNA of both proteins have been cloned (13). Both subunits belong to the recently recognized hematopoietic receptor superfamily which includes many cytokines receptors. Characterisation of the extracellular portion of the 80 kDa IL-6 receptor revealed the existence of a single immunoglobulin-like domain in the NH_2-terminal of the extracellular region, which does not contribute to ligand binding. The remainder of the extracellular domain, however, is essential for low affinity ligand binding, which consecutively triggers the association of the receptor and gp130 thus forming a high affinity binding site for IL-6 (6).

For many cytokine receptors soluble forms have been demonstrated (2). These soluble molecules have been observed to retain ligand binding capacity and therefore compete with the membrane receptors, thus acting as antagonists (2). A soluble form of the human gp80 protein has been detected in serum and urine samples. This 55 kDa protein representing the extracellular portion of gp 80 is generated by shedding, a process that seems to be controlled by protein kinase C. It is still functional, indicating that soluble gp 80 plays a biological role in promoting IL-6 activity (6). So far, the soluble IL-6 receptor is unique in acting as an agonist together with its ligand (6).

We used the sIL-6R ELISA as an enzyme-linked immunosorbent assay for quantitive detection of soluble human interleukin-6 receptor levels in human serum. We observed statistically important difference between IM patients and the control group in levels of s IL-6R ($p<0.004$).

The role of soluble Il-6R as a marker has been demonstrated in HIV infection, in multiple myeloma (MM), monoclonal gammapathy of undetermined significance (MGUS). Serum soluble IL-6R levels are significantly increased in individuals with MGUS and in patients with MM as compared to age-related healthy individuals. These levels are independent of previously recognized prognostic factors in MM, especially serum IL-6 levels and myeloma cell mass. Elevated levels of IL-6 receptor expression in the mixed cellularity of Hodgkin's Disease (HD) have been demonstrated.

In immunocompetence conditions EBV infection takes place under the control of the immunological system and the key mediators in the process of cooperation and communication of cells participating in immune response are cytokines. The measurement serum levels of IL-6 and IL-6R are useful markers of immunologic response during acute symptomatic phase IM.

REFERENCES

1. Andersson J., Andersson U.: Characterization of cytokine production in infectious mononucleosis studied at a single-cell level in tonsil and peripheral blood. Clin. Exp. Immunol., 92, 7, 1993.
2. Fernandez-Botran R.: Soluble cytokine receptors: their role in immunoregulation. FASEB J. 5, 2567, 1991.
3. Foss H. et al.: Patterns of cytokine gene expression in infectious mononucleosis. Blood, 83, 707, 1994.
4. Hesse J. et al.: Prevalescence of antibodies to Epstein-Barr virus (EBV) in childhood and adolescence in Denmark. Scand. J. Infect. Dis., 15, 335, 1998.
5. Hornef M. et al.: Cytokine production in whole blood assay after Epstein-Barr infection *in vivo*. Clin. Diagn. Lab. Immunol., 2, 209, 1995.
6. Kishimoto T. et al.: Interleukin-6 and its receptor: a paradigm for cytokines. Science, 258, 593, 1992.
7. Lai P. et al.: Epidemiological studies of Epstein-Barr herpesvirus infection in Western Australia. J. Hyg. Lond., 74, 329, 1975.
8. Linde A. et al.: Serum levels of lymphokines and soluble cellular receptors in primary Epstein-Barr infection and in patients with chronic fatigue syndrome. J. Infect. Dis., 165, 994, 1992.
9. Masucci M., Ernberg I.: Epstein-Barr virus: adaptation to a life within the immune system. Trends Microbiol., 2, 125, 1994.
10. Niedobitek G., Young L.: Epstein-Barr virus persistence and virus associated tumors. Lancet, 343, 333, 1994.
11. Schuster V. et al.: Serum concentration of interferon gamma, interleukin-6, and neopterin in patients with infectious mononucleosis and other Epstein-Barr virus-related lymphoproliferative diseases. Infection, 21, 210, 1993.
12. Sehgal P. et al.: Regulation of the acute phase and immune responses: Interleukin-6. Ann. NY Acad. Sci., 557, 1, 1989.
13. Strauss S.: Acute progressive Epstein-Barr virus infections. Ann. Rev. Med., 43, 437, 1992.
14. Taga T. et al.: Interleukin-6 triggers the association of its receptor with a possible signal transducer, gp130. Cell, 58, 573, 1989.
15. Wright-Browe V. et al.: Serum cytokine levels in infectious mononucleosis at diagnosis and convalescence. Leukemia and Lymphoma, 30, 583, 1998.

SUMMARY

The Epstein–Barr virus (EBV) is an etiologic agent of infectious mononucleosis (IM) which is a self-limiting lymphoproliferative disorder caused by primary EBV infection. EBV is a ubiquitous gamma herpesvirus that infects epithelial cells and B lymphocytes. T-cells immune activation plays an important role in IM and EBV associated diseases. Several lines of evidence suggest that cytokines play a role in the pathogenesis of IM. IL-6 is a multi-functional cytokine that regulates immune responses, acute phase reactions and hematopoiesis and may play a central role in host defense mechanisms. IL-6 is involved in the induction of B-cell differentiation and proliferation and differentiation of T-cells. The aim of the work was to analyze to level of interleukin 6 (IL-6) and IL-6 receptor (IL-6R) in acute symptomatic IM. We examined 24 patients, who were

hospitalized at the Department of Infectious Diseases, Medical University of Lublin. The diagnosis was confirmed by the presence of high titers of IgM anti-VCA antibodies. The control group included 18 healthy persons. Once in all the examined people there was assessed the level of IL-6 and sIL-6R in blood serum by ELISA immunoenzymatic method. The obtained data were analyzed with the use of U Mann-Whitney statistical test. We observed statistically important difference between IM patients and control group in levels of IL-6 ($p<0.001$). We observed the statistically important difference between IM patients and the control group in levels of sIL-6R ($p<0.004$).

Poziom IL-6 i IL-6R w surowicy krwi chorych w ostrej objawowej fazie mononukleozy zakaźnej

Wirus Epstein-Barr (EBV) jest czynnikiem etiologicznym mononukleozy zakaźnej (MZ) samoograniczającego procesu limfoproliferacyjnego, związanego z pierwotną infekcją EBV. Wirus ten należy do gamma-herpeswirusów, które zakażają komórki epitelialne i limfocyty B. W MZ i w innych chorobach związanych z infekcją EBV ważna jest także aktywacja limfocytów T. Liczne dane wskazują na to, że w mechanizmach patogenetycznych MZ istotną rolę odgrywa aktywacja wybranych cytokin. IL-6 wykazuje wielokierunkową aktywność w regulacji odpowiedzi immunologicznej. Indukuje różnicowanie limfocytów B oraz dodatkowo proliferacje limfocytów T. Celem pracy była ocena poziomu IL-6 i IL-6R w surowicy krwi chorych w ostrej objawowej fazie MZ. Badaniami objęto 24 chorych hospitalizowanych w Klinice Chorób Zakaźnych AM w Lublinie. Potwierdzeniem diagnozy była obecności anty-VCA IgM w surowicy krwi. Grupę kontrolną stanowiło 18 zdrowych osób. U wszystkich badanych jednorazowo oznaczano poziom IL-6 i IL-6R w surowicy metodą ELISA. Uzyskane dane liczbowe poddano analizie statystycznej z wykorzystaniem testu U Mann-Whitneya. W przeprowadzonych badaniach stwierdzono statystycznie istotny wzrost poziomu IL-6 oraz IL-6R w surowicy krwi chorych w porównaniu z grupą osób zdrowych.