ICAM-1, Soluble-CD23, and Interleukin-10 Concentrations in Serum in Renal-Transplant Recipients with Epstein-Barr Virus Reactivation

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Primary and reactivated Epstein-Barr virus (EBV) infections after organ transplantation are associated with the development of posttransplant lymphoproliferative malignancies. Since viral reactivation frequently stays asymptomatic, early diagnosis and treatment are challenges during posttransplant patient monitoring. Both soluble-CD23 (sCD23) and intercellular adhesion molecule 1 (ICAM-1) cell surface expression as well as interleukin-10 (IL-10) production are closely associated with viral gene expression. Therefore, immunoglobulin M (IgM), IgG, IgA, sCD23, ICAM-1, and IL-10 concentrations were measured in serum samples from patients during EBV reactivation (n = 14) and were compared with those in samples from patients without EBV reactivation (n = 10) following renal transplantation. In addition, serum sCD23, ICAM-1, and IL-10 concentrations were measured longitudinally in weekly to biweekly samples from 10 patients with EBV reactivation for at least 20 weeks following transplantation. A significant elevation of sCD23 was found during viral reactivation (P < 0.05), whereas ICAM-1 levels showed a nonsignificant increase. The finding of a highly significant elevation of the serum IL-10 concentration during EBV reactivation (P < 0.001) may support speculations about its role in EBV-induced lymphoproliferation and in the development of opportunistic infections and secondary malignancies. Maximum serum IL-10 levels at the time of EBV reactivation were found in 7 of 10 patients. Well-defined ICAM-1 and sCD23 concentration peaks were found in 9 of 10 and 8 of 10 patients, respectively. Although both markers are not specific for EBV reactivation and therefore may not be useful for primary diagnosis, sCD23 and ICAM-1 might be potent tools for the clinical monitoring of EBV activity and virus-induced lymphoproliferation.

Epstein-Barr virus (EBV) is the causative agent of infectious mononucleosis and has been implicated in the pathogenesis of a broad spectrum of lymphoid malignancies, such as the endemic Burkitt’s lymphoma, the nasopharynx carcinoma, some subtypes of Hodgkin’s lymphoma and T-cell lymphomas, human immunodeficiency virus-associated lymphomas, and posttransplant lymphoproliferative disorder (PTLD) (6, 22). Following primary infection, EBV persists in healthy individuals for life. Immunosuppression after organ transplantation disrupts the established virus-host balance, and viral reactivation occurs in 25 to 35% of organ recipients. The incidence of PTLD ranges from 1.0 to 4.6% depending on the kind of organ transplanted, the immunosuppressive regimen, and the age of the organ recipient. PTLD is associated with an overall survival rate of 31% (4, 11, 15, 16, 21).

While there have been intense efforts to determine the pathogenic mechanisms in primary EBV infection and immunosurveillance during viral latent persistence, little is known about pathogenesis and host immune response during viral reactivation. Therefore, a better understanding of the process of viral reactivation as well as a noninvasive and sensitive assay to determine the development of malignant lymphoid proliferation would help to monitor immunocompromised individuals and thus aid in the prevention and treatment of EBV-associated disease.

Cell surface expression of both soluble CD23 (sCD23) and intercellular adhesion molecule 1 (ICAM-1) is closely correlated with the expression of viral latent membrane protein 1 (LMP1) and EBV nuclear antigen 2 (EBNA2), upregulation of bcl-2, and subsequent prevention of apoptosis (9, 13). Interleukin-10 (IL-10) of human as well as viral origin was shown to stimulate B-cell proliferation and immunoglobulin secretion while inhibiting T-cell function (20). These three molecule might therefore be useful markers of virus-induced lymphoproliferation. In this study we compared the concentrations of sCD23, ICAM-1, IL-10, and immunoglobulins in sera from patients during EBV reactivation with the concentrations in sera from patients without any evidence of viral activity following transplantation. Posttransplant serum samples from all renal-organ recipients were also examined for monoclonal immunoglobulins. In addition, we included patients with primary EBV infection as well as healthy blood donors to investigate differences between primary and reactivated infections. In order to determine the time course of sCD23, ICAM-1, and IL-10 production with regard to EBV reactivation, cytomegalovirus (CMV) infection, or rejection crises, we further examined weekly to biweekly serum samples from 10 patients with viral reactivation for at least 20 weeks following transplantation.

MATERIALS AND METHODS

Patients. A group of 79 patients who had undergone transplantation with renal organs from cadavers (16) constituted the patient population. Fourteen patients showing clear serological evidence of EBV reactivation were selected. As a control, another 10 patients without any serological sign of viral activity were
included (transplant controls). All patients underwent surgery between 1990 and 1993 at our institution. The observation course was 20 to 39 weeks postoperatively. Serum samples were examined weekly to biweekly for specific anti-EBV immunoglobulin G (IgG) and IgM antibodies to determine whether EBV had reactedivate (see below). EBV reactivation occurred 9 weeks (median) 3 to 18 weeks after transplantation, and all 14 patients remained asymptomatic. None of the patients developed PTLD during the examined period. Additionally, serum samples from 10 patients with acute primary EBV infection (duration of clinical symptoms, 3 to 28 days) as well as samples from 10 healthy blood donors (healthy controls) were included.

Furthermore, weekly and biweekly serum samples from 10 patients with EBV reactivation after renal transplantation were examined longitudinally for 25 weeks (median, 20 to 36 weeks) after transplantation. Six of these patients also showed evidence of CMV infection during the examined time course. CMV infection occurred during the sixth week (median, range, 5 to 9 weeks). Clinical symptomatic CMV infection was found in two of six patients; all six patients showed positive CMV pp65 antigenemia. Signs of organ rejection were seen in 6 of the 10 patients during the examined time course.

Posttransplant patient care. The immunosuppressive regimen after transplantation consisted of prednisolone (25 mg/day, decreasing dosage), azathioprine (initially at 2 mg/kg of body weight), and cyclosporine (3 to 5 mg/kg) (triple regimen). Second- or third-transplant recipients received antithymocyte immunoglobulin (3 mg/kg) or antilymphocyte immunoglobulin (30 mg/kg) in addition to the triple-drug therapy (quadruple regimen). Organ rejection was treated with high-dose steroids (500 mg of prednisolone on three consecutive days), antithymocyte immunoglobulin, or the mouse monoclonal antibody OKT-3 (5 mg). The patient groups were matched for the immunosuppressive regimen. Prophylactic antiviral therapy (aciclovir per os, 800 mg/day) was given to all patients receiving an organ from a CMV-positive donor.

EBV serology. Weekly to biweekly serum samples were collected and stored at −80°C. Levels of EBV-specific IgG and IgM antibodies were determined by an enzyme-linked immunosorbent assay (ELISA) employing the recombinant antigen p54 and p138 (Biotest, Dreieich, Germany). In order to absorb rheumatoid factor, the samples were absorbed with anti-human γ-chain antibody (Behring, Marburg, Germany) prior to determination of the IgM level. The assays were performed according to the manufacturer’s instructions. The following serological parameters were used as criteria for EBV reactivation: (i) a more than fourfold rise in the anti-early antigen (EA) IgG optical density value above the preoperative value and (ii) seroconversion of the anti-EA IgM (11, 21).

Determination of ICAM-1, sCD23, and IL-10 concentrations. Serum ICAM-1 and sCD23 concentrations were measured by ELISA (T Cell Diagnostics, Cambridge, Mass.). The IL-10 ELISA was obtained from PerSeptive Diagnostics, Cambridge, Mass. The ELISAs were performed according to the manufacturers’ recommended protocols.

Serum immunoglobulin concentration and immunofixation electrophoresis. The test equipment was provided by Behring (quantitative immunoglobulin determination) and Beckman Instruments Inc., Brea, Calif. (immunofixation electrophoresis). Examination of serum samples and interpretation of results were performed according to the manufacturers’ protocols.

Statistical analysis. Statistical evaluation for independent samples was performed by using the nonparametric Mann-Whitney U test.

RESULTS

Serum IgG, IgM, and IgA concentrations. Serum samples taken during EBV reactivation showed a significant elevation of the IgM and IgA concentrations compared with samples taken from patients without any evidence of viral activity after renal transplantation (P < 0.01 and P < 0.05, respectively). Serum IgG concentrations were found not to differ significantly for the two groups (Table 1). The majority of the values were within the normal range (640 to 1,400 mg/dl for IgG, 35 to 240 mg/dl for IgM, and 70 to 410 mg/dl for IgA). The serum IgA concentration during EBV reactivation was significantly higher than in patients with acute infectious mononucleosis (P < 0.05).

Serum sCD23 and ICAM-1 concentrations. The data in Fig. 1 show a significant difference in sCD23 concentration for the group of patients with EBV reactivation and those without viral reactivation (P < 0.05). The difference in serum ICAM-1 levels between the two groups was not significant. Regarding only the group of patients with EBV reactivation, a significant increase was found for sCD23 and ICAM-1 during EBV reactivation compared with an early posttransplantation value (P < 0.05 and P < 0.001, respectively). However, only the ICAM-1 concentration decreased significantly during the following weeks after reactivation, whereas sCD23 showed a nonsignificant decrease (Table 2).

Compared with healthy blood donors, patients with EBV reactivation had significantly elevated levels of both sCD23 and ICAM-1 during EBV reactivation (P < 0.01 and P < 0.05, respectively). In contrast, there was no significant difference for either marker between the concentrations in serum during EBV reactivation and acute primary EBV infection (Fig. 1).

Serum IL-10 concentration. IL-10 levels during EBV reactivation showed a significant elevation compared to levels in patients without EBV reactivation (P < 0.001) (Fig. 2), although the IL-10 concentrations varied widely, which resulted in a high standard deviation. Weeks after viral reactivation, the IL-10 concentration decreased to the preoperative level (P < 0.001) (Table 2).

A highly significant elevation was also found during EBV reactivation compared to the level in healthy blood donors (P < 0.002). Patients with primary EBV infection showed an insignificant tendency towards increased serum IL-10 levels (Fig. 2).

Longitudinal measurement of markers in serum. With respect to the longitudinal course of the serum sCD23 concentration, 8 of 10 patients showed a well-defined concentration peak at the time of serological evidence of viral reactivation. However, in two patients only minor concentration changes could be found; these could not be correlated with EBV reactivation. Concentration peaks for ICAM-1 were found in 9 of the 10 patients. The maximum concentration occurred 3 weeks after serological evidence of EBV activity (median), range, 1 week before to 5 weeks after reactivation. The longitudinal measurement of the serum IL-10 concentration revealed a well-defined maximum in 7 of the 10 patients at the time of serological evidence of EBV reactivation (data not shown).

Figure 3 demonstrates an example of the longitudinal course of serum ICAM-1, sCD23, and IL-10 concentrations after transplantation. EBV serology revealed increasing anti-EA IgG and IgM seroconversion during the 10th week after transplantation, with maximum titers during the 12th week. Accordingly, IL-10, ICAM-1, and sCD23 concentration peaks were found during the 12th and 13th weeks. However, an early IL-10 peak during the second week and an sCD23 increase during the sixth week after transplantation could not be correlated with EBV reactivation but may have been caused by initial immunosuppression and antirejection treatment due to a rejection episode during the fourth week.

Immunofixation electrophoresis. A monoclonal gammapathy (kappa type) was found in a serum sample from one patient 27 weeks after renal transplantation. A preoperative sample from the same patient was found to be negative for paraproteins. This patient showed serological evidence of EBV reac-

<table>
<thead>
<tr>
<th>Antibody</th>
<th>With EBV reactivation (n = 14)</th>
<th>Without EBV reactivation (n = 10)</th>
<th>With acute primary EBV infection (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG</td>
<td>912.98 ± 271.25</td>
<td>837.70 ± 237.75</td>
<td>1110.22 ± 267.11</td>
</tr>
<tr>
<td>IgM</td>
<td>349.68 ± 163.34 A</td>
<td>178.99 ± 82.52 A</td>
<td>193.86 ± 138.69 A</td>
</tr>
<tr>
<td>IgA</td>
<td>320.80 ± 158.11 B,C</td>
<td>214.41 ± 108.84 B</td>
<td>209.95 ± 84.58 C</td>
</tr>
</tbody>
</table>

* Values are means ± standard deviations. Capital letters indicate significant differences as follows: A, P < 0.01; B, P < 0.05. Normal ranges are 640 to 1,400 mg/dl for IgG, 35 to 240 mg/dl for IgM, and 70 to 410 mg/dl for IgA.

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TABLE 1. Serum immunoglobulin concentrations in renal-transplant recipient with and without EBV reactivation as well as patients with acute primary EBV infection.
tivation during the 10th week after transplantation. An up-to-date control 5 years after transplantation showed normal values for total serum IgG, IgA, and IgM concentrations (IgG, 1,308.2 mg/dl; IgA, 290.4 mg/dl; and IgM, 63.8 mg/dl).

DISCUSSION

An association of EBV with lymphoproliferative disease in transplant recipients was first reported in 1980 by Crawford et al. (5). Further experiments with the heterogenous group of lymphoid malignancies revealed a cellular phenotype similar to that of B cells transformed in vitro with EBV, with high expression of the viral antigens LMP1 and EBNA2 (8, 10, 30). However, a number of recent reports have shown a more heterogeneous picture with respect to viral gene expression on cells proliferating during PTLD (3, 23). EBNA2 and LMP1 are both thought to be important viral mediators of B-lymphocyte growth. LMP1 was shown to protect B cells from apoptosis at least in part by induction of bcl-2, whereas EBNA2 itself up-regulates the promoter of LMP1 (13, 32). Furthermore, expression of the viral gene products LMP1 and EBNA2 is closely associated with upregulation of the cell surface proteins CD23 and ICAM-1, and high levels of these surface markers have been found on cells proliferating during PTLD (30). Therefore, ICAM-1 and sCD23 may be useful markers for EBV-induced B-cell transformation and proliferation.

Consistent with this, sCD23 was recently described as a sensitive marker for EBV-related disorders after liver transplantation (18, 33). Moreover, a good correlation between sCD23 concentration in culture supernatant and LMP1-induced cell surface CD23 expression has been reported (24, 25). Binding of ICAM-1 to leukocyte functional antigen 1 molecules on the surfaces of APCs might therefore influence APC–T-cell interaction and modulate T-cell-mediated immune response.

The observed elevation of the serum sCD23 and ICAM-1 concentrations in most patients during EBV reactivation might reflect virus-induced upregulation on EBV-infected cells and subsequent shedding of the molecule. However, some upregulation is not EBV specific, as ICAM-1 and CD23 are synthesized by a broad range of different cell types and elevated levels in serum have been found in transplant recipients during rejection episodes and other inflammatory, neoplastic, and immune disorders (12, 26). Nine of 14 patients included in this study showed CMV infection; in 5 patients CMV infection occurred at about the time of EBV reactivation. Furthermore, seven patients underwent rejection episodes and four of these occurred at about the time of EBV reactivation. The increase of the measured markers in serum in these patients might at least partly be due to inflammatory disorders. Nevertheless, we believe that the values may provide useful information about the course and activity of viral reactivation since expression of all three markers is closely linked to viral activity and pathogenesis. Therefore, although sCD23 and ICAM-1 might not be useful for primary diagnosis of EBV reactivation, they may prove useful in the posttherapeutic follow-up of patients. Approaches for primary diagnosis use more specific tools such as PCR or NASBA (nucleic acid sequence-based assay) techniques as well as the immunocytochemical detection of EBV-associated proteins (17). Interestingly, the concentrations of

![FIG. 1. Serum sCD23 and ICAM-1 concentrations (conc.) in renal-organ recipients with (n = 14) and without (n = 10) EBV reactivation after transplantation. Serum samples taken at the time of serological evidence of EBV reactivation as well as serum samples from the 9th week following transplantation (transplant controls) were examined. Serum sCD23 and ICAM-1 concentrations for 10 healthy blood donors (healthy control) as well as 10 patients with acute primary EBV infection were included (values are means ± standard deviations).](http://cvi.asm.org/)

### TABLE 2. Serum marker concentrations before, during, and after EBV reactivation

<table>
<thead>
<tr>
<th>Time of sampling</th>
<th>ICAM-1 concn (ng/ml)</th>
<th>sCD23 concn (U/ml)</th>
<th>IL-10 concn (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before EBV reactivation</td>
<td>383.9 ± 98.8 A</td>
<td>71.45 ± 32.98 C</td>
<td>4.85 ± 5.29 B</td>
</tr>
<tr>
<td>During EBV reactivation</td>
<td>648.3 ± 371.7 A, D</td>
<td>134.19 ± 75.44 C</td>
<td>33.17 ± 45.03 B</td>
</tr>
<tr>
<td>After EBV reactivation</td>
<td>401.1 ± 126.0 D</td>
<td>118.36 ± 117.43</td>
<td>3.93 ± 4.42 D</td>
</tr>
</tbody>
</table>

*Values are means ± standard deviations. Capital letters indicate significant differences as follows: A and B, *P* < 0.01; C and D, *P* < 0.05.

*The samples were taken during the second to third week postoperatively, at the time of EBV reactivation, and 10 to 17 weeks after viral reactivation.
both proteins in serum almost reached the same levels measured during acute primary EBV infection, whereas all patients with EBV reactivation stayed asymptomatic. Taking into account that the clinical symptoms of primary EBV infection, such as hypersplenism, fever, and angina, are mainly caused by the immune response, patients after transplantation with a mainly T-cell-deficient immune system might not be able to mount the same level of response and therefore may not show the same level of symptoms.

Virus-induced activation of B lymphocytes and stimulation of polyclonal immunoglobulin synthesis were demonstrated by a significant increase of the serum IgM and IgA concentrations during EBV reactivation (1). However, no increase of the serum IgG levels was found during primary and reactivated EBV infections. Interestingly, immunofixation electrophoresis of a serum sample from one patient which was taken 27 weeks after transplantation showed a monoclonal gammopathy (kappa type), whereas a preoperative control was negative for para-proteins. This 48-year-old patient showed clear evidence of EBV reactivation during the 10th week after transplantation. However, the patient also showed moderate positive CMV antigenemia during the 10th and 11th weeks after transplantation. The further posttransplant course was excellent; an up-to-date control in September 1996 showed serum IgG, IgA, and IgM concentrations within the normal range and good renal function and clinical performance. The coincidence of EBV and CMV infection in this case poses interesting questions about their potential role in the development of this apparently benign monoclonal gammopathy.

IL-10 stimulates B-cell proliferation and differentiation but inhibits gamma interferon and monokine synthesis, weakening the acute antiviral T-cell immune response (20). Furthermore, IL-10 was shown to prevent apoptosis in responding T cells, which may favor the establishment of immunologic memory (29). Interestingly, the EBV genome encodes an 84% homolog of human IL-10, and both human IL-10 and viral IL-10 were shown to be expressed by EBV-transformed B lymphocytes (2, 19). A highly significant increase in IL-10 concentration was found during EBV reactivation, attaining even the quantity found during primary EBV infection. IL-10-induced inhibition of T-cell-mediated immune regulation during EBV reactivation could therefore play a role in the reported coincidence of EBV reactivation and symptomatic CMV infection after renal transplantation (16). Viral IL-10 was further shown to induce local anergy to allogeneic and syngeneic tumors in mice and to enhance transformation of human B cells (27, 28). Therefore, local elevation of IL-10 levels might favor the development of lymphoproliferative malignancies (14). Successful treatment of PTLD by inhibition of the T\textsubscript{H-2} cells with alpha interferon was recently reported (7).
In summary, elevated serum IL-10, sCD23, and ICAM-1 levels were found in patients with asymptomatic EBV reactivation after renal transplantation. Both sCD23 and ICAM-1 might therefore be markers for EBV-induced lymphoproliferation and viral activity. Longitudinal follow-up and correlation with clinical performance as well as outcome are now required to estimate the value of potential markers in postoperative patient monitoring. Elevated serum IL-10 levels may play a role in the observed elevated susceptibility to other opportunistic infections during EBV reactivation and the development of secondary malignancies following organ transplantation. IL-10 might therefore present a target for therapeutic strategies in infectious and neoplastic posttransplant complications.

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REFERENCES