Original Article

Soluble CD-4 and CD-8 as markers of immunological activation in renal transplant recipients

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Abstract

Background. T lymphocytes are activated following kidney transplantation in cases of acute graft rejection and viral infections. In plasma, elevated levels of T-cell markers can be measured in soluble form. The reason for this shedding is still not entirely understood.

Methods. Plasma concentrations of soluble CD-4 and CD-8 (sCD-4, sCD-8) were determined in 78 patients following kidney transplantation by commercially available enzyme-linked immunosorbent assay (ELISA) test kits.

Results. The concentrations of both soluble T-cell markers increased significantly in the course of acute allograft rejection and cytomegalovirus (CMV) infections. Frequently, the parameters increased shortly before clinical diagnosis and decreased under successful therapy. Additionally, sCD-8 showed significant higher plasma concentrations in cases of CMV infection as compared with acute allograft rejections. Accordingly, the sCD-4/sCD-8 ratio increased in cases of acute allograft rejection and decreased during CMV infections. Cyclosporin A nephrotoxicity caused no significant changes in the sCD-4 and sCD-8 levels in plasma.

Conclusion. The present study demonstrates that sCD-4 and sCD-8 are markers of immunological activation and may enable a further differentiation of T-cell activation if serial measurements are performed. However, further prospective investigations are necessary to elucidate the diagnostic potential of sCD-4 and sCD-8 for monitoring acute rejection and viral infection in kidney graft recipients.

Key words: CMV infection; cyclosporin A nephrotoxicity; immune monitoring; renal allograft rejection; soluble CD-4; soluble CD-8

Introduction

T lymphocytes are involved in acute allograft rejection [1,2]. T-cell receptor (TCR), CD-3, CD-4 or CD-8 receptors play an important role in antigen recognition and T-cell activation. TCR/CD-3 and CD-4 recognize antigen in combination with major histocompatibility complex (MHC) class II, TCR/CD-3 and CD-8 recognize antigen in combination with MHC class I molecules [3–5]. Both, CD-4 and CD-8 co-receptors are transmembrane proteins [6]. The cytoplasmic tails of CD-4 and CD-8 are associated with tyrosine kinases which transmit signals for the regulation of T-cell growth and play an important role in T-cell activation [7,8]. T-cell activation and proliferation are also modulated by soluble lymphokines which bind to lymphokine receptors, such as interleukin-2 receptor (IL-2R) [9]. For optimal T-cell activation, cooperation of these receptors is necessary.

Under certain not entirely understood circumstances, CD-4 and CD-8 receptors are released from the cell surface and circulate in the plasma as soluble CD-4 and CD-8 (sCD-4, sCD-8). Abnormally high levels of sCD-8 or sCD4 can be detected in the plasma of patients with infectious diseases (e.g. Kawasaki disease, infectious mononucleosis, chronic viral hepatitis, AIDS) [10–15], haematological malignancies (e.g. hairy cell leukaemia, childhood acute lymphoblastic leukaemia), lymphomas (e.g. non-Hodgkin's lymphoma, Hodgkin's disease) [16–18] and autoimmune conditions (e.g. rheumatoid arthritis, polymyalgia rheumatica, autoimmune hepatitis, systemic lupus erythematosus, insulin-dependent diabetes mellitus) [19–24]. Increased sCD-8 levels were found in the cerebrospinal fluid of patients with multiple sclerosis and other inflammatory neurologic diseases [25]. High sCD-8 levels were correlated with advanced disease and poor prognosis [14,17].

In the present study, we measured sCD-4 and sCD-8 concentrations in plasma of renal transplant recipients to obtain further information about T-cell subset activation in the course of immunological processes.
following kidney transplantation. Additionally, we investigated whether soluble T-cell markers can contribute to a better monitoring of allograft rejection and viral infection in kidney transplant recipients.

**Subjects and methods**

**Patients**

Plasma samples from 19 healthy individuals without impairment of renal function (10 females and nine males) and a mean age of 34 years (range 24–40 years) were examined (controls). Seventy-eight kidney transplant recipients, 25 females and 53 males with an age range of 23–67 years, were studied. Seventy-six patients had received their first transplant and two patients the second transplant. The mean follow-up was 43 days post-transplant (range 25–72 days). Patients were immunosuppressed with a combination of prednisolone and cyclosporin A (CsA). In 32 patients, azathioprine was added to a ‘triple-therapy’ regime because of unstable graft function. Anti-rejection therapy commonly consisted of methylprednisolone bolus therapy (500 mg i.v. for three consecutive days).

The patients could be divided into four groups:

- **Stable graft function:** Stable graft function was defined when serum creatinine, sIL-2R, urine excretion and ultrasonography was not pathological and an infection could be excluded. Serum creatinine should not exceed 150 µmol/l. Volume of urine excretion should be > 1.8 l/day under constant body weight.
- **Acute rejection:** All cases of acute rejection were documented with allograft histology. Acute rejection was clinically suspected when the serum creatinine was increased above 250 µmol/l or showed an increasing trend of 20 µmol/l per day, and urine excretion declined. Additionally, plasma levels of sIL-2R increased [26,27]. Allograft ultrasonography (renal enlargement in all dimensions, prominent hypoechoic pyramids or effacement of renal sinuses) and Doppler ultrasonography (resistance index of Pourcelot (RI) > 0.8 with an increment of 0.1–0.2/day) showed pathological changes [28]. A renal biopsy was performed in all patients when an acute allograft rejection was clinically suspected.
- **CMV infection:** The diagnosis of cytomegalovirus (CMV) infection was made by the detection of CMV-pp65-EA or CMV-PCR. Serologic detection of specific CMV immunoglobulin M by enzyme immunoassay assisted in confirming a CMV infection.
- **Cyclosporin A nephrotoxicity:** CsA-induced renal dysfunction was differentiated from acute rejection by renal allograft biopsy. For statistical analysis, the measured values of soluble T-cell markers on the day with the highest CsA level and the following 3 days were chosen.

**Samples**

Plasma samples were obtained three times a week. Heparinized blood was collected and stored frozen at – 20°C after centrifugation.

**Creatinine measurement**

Creatinine was measured with a Beckman creatinine analyser according to Jaffe’s test. A 25 µl aliquot of plasma was added to a modified Jaffe solution. The increase in absorption was measured during 25.6 s at a wavelength of 520 nm.

**Soluble T-cell marker assays**

sCD-4 and sCD-8 were determined in triplicate by commercially available enzyme-linked immunosorbent assay (ELISA)-test kits ‘cell-free CD-4 test kit’ and ‘T-8 test kit’ (T Cell Science, Cambridge, MA) according to the manufacturer’s instructions with slight modification in terms of the length of the incubation periods. The first monoclonal mouse antibody was coated onto polystyrene microtitre plate wells. For sCD-4, 50 μl and for sCD-8 10 μl of standards or plasma samples were added to the wells, washed and incubated with a mouse anti-mouse antibody conjugated with horseradish peroxidase for the same length of time. After incubation, the wells were washed again and a substrate solution of o-phenylenediamine was applied. The reaction was stopped with 2 M H₂SO₄ following incubation for 45 min, and absorbance was measured at 490 nm. The ELISA reader Milenia Kinetic EIA System and Kinetic Analyser (DPC, Los Angeles, CA) were used to determine the standard curve and the sample values.

The sIL-2R levels in plasma were measured with a commercially available ELISA (ImmuneTech S.A., Marseilles, France). Briefly, 50 μl of plasma sample were incubated with 100 μl of anti sIL-2R monoclonal antibody conjugated with alkaline phosphatase for 120 min at room temperature. Soluble IL-2R present in the plasma sample bound to antibody on the well and, after washing of the unreacted materials, 200 μl of vial substrate buffer (pNPP) were added for 60 min. Thereafter, the reaction was stopped with 50 μl of 1 M NaOH and the absorbance was determined at 405 nm.

**Statistical analyses**

The median (minimum – maximum) values were calculated for each parameter in each group. The significance of differences between groups was calculated using the Mann–Whitney U test for unpaired data or multivariate analysis (ANOVA). The sCD-4/sCD-8 ratio of every patient was calculated. The median (minimum – maximum) of all sCD-4/sCD-8 ratios are shown in Table 3.

**Results**

Two typical courses are demonstrated as follows: the first patient (Figure 1) had a normal graft function during the first 3 weeks following kidney transplantation. From the 24th day after transplantation, serum creatinine slowly increased. The increment of the soluble T-cell markers in plasma appeared shortly before the increase in serum creatinine. On day 28 after transplantation, an acute interstitial rejection could be differentiated from acute rejection by renal allograft biopsy. An anti-rejection therapy commonly consisted of methylprednisolone conjugated with horseradish peroxidase for the same length of time. After incubation, the wells were washed again and a substrate solution of o-phenylenediamine was applied. The reaction was stopped with 2 M H₂SO₄ following incubation for 45 min, and absorbance was measured at 490 nm. The ELISA reader Milenia Kinetic EIA System and Kinetic Analyser (DPC, Los Angeles, CA) were used to determine the standard curve and the sample values.

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**Results**

The diagnosis of cytomegalovirus (CMV) infection was made by the detection of CMV-pp65-EA or CMV-PCR. Serologic detection of specific CMV immunoglobulin M by enzyme immunoassay assisted in confirming a CMV infection.

Cyclosporin A nephrotoxicity. CsA-induced renal dysfunction was differentiated from acute rejection by renal allograft biopsy. For statistical analysis, the measured values of soluble T-cell markers on the day with the highest CsA level and the following 3 days were chosen.

**Samples**

Plasma samples were obtained three times a week. Heparinized blood was collected and stored frozen at – 20°C after centrifugation.

**Creatinine measurement**

Creatinine was measured with a Beckman creatinine analyser according to Jaffe’s test. A 25 µl aliquot of plasma was added to a modified Jaffe solution. The increase in absorption was measured during 25.6 s at a wavelength of 520 nm.
Fig. 1. Patient with acute allograft rejection. Serial serum levels of creatinine (▲), plasma levels of sCD-4 (■) and sCD-8 (□) of a patient with acute interstitial rejection are shown. On the 24th day after kidney transplantation, serum creatinine began to increase. Four days later, an allograft rejection could be verified by means of biopsy. An anti-rejection therapy with methylprednisolone i.v. for 3 days was started, and 6 days later the serum creatinine began to decline. Characteristically, the plasma levels of sCD-4 and sCD-8 increased shortly before serum creatinine and decreased immediately after successful anti-rejection therapy.

allograft biopsy was performed and an acute rejection or CsA nephrotoxicity could be excluded. The patient developed fever (40 °C) and the laboratory tests revealed a leukopenia (2.1 × 10⁹/l), anaemia (haemoglobin 8.5 g/dl) and an increase in AST (90 U/l) and ALT (140 U/l). The diagnosis of CMV infection was made by the detection of specific CMV-pp65-EA in blood specimens. The immunosuppressive regimen was reduced and ganciclovir (adjusted for renal dysfunction) was administered for 15 days. The clinical symptoms regressed, and sCD-4 and sCD-8 decreased followed by a delayed reduction of serum creatinine.

Patients with stable allograft function showed plasma sCD-4 and sCD-8 levels in the range of those of healthy persons with normal kidney function. In cases of acute allograft rejection, sIL-2R (data not shown), sCD-4 and sCD-8 levels increased significantly compared with cases with stable allograft function (Tables 1 and 2). Soluble CD-4 showed a more pronounced increase in cases of acute rejection than did sCD-8. CMV infections also caused a significant increment of sCD-4 and sCD-8 plasma levels compared with cases with stable graft function. Additionally, sCD-8 showed significant higher plasma concentrations in cases of CMV infection compared with cases of acute rejection. Soluble T-cell markers of healthy individuals showed no significant differences compared with transplant recipients with stable graft function.

During acute allograft rejections, the sCD-4/sCD-8 ratio in plasma increased, during CMV infections the sCD-4/sCD-8 ratio decreased (Table 3). The sensitivity, specificity and predictive values of sCD-4 and sCD-8 are shown in Table 4.

Discussion

As a sign of early activation of T lymphocytes during episodes of acute rejection and viral infection following renal transplantation, significantly elevated plasma levels of sCD-4 and sCD-8 were found. Soluble CD-4 showed a 3.2-fold increase during acute allograft rejection with no further significant difference from episodes of CMV infections. Soluble CD-8, however, was remarkably elevated in plasma during CMV infections (3.2-fold) compared with acute rejection episodes.
Fig. 2. Patient with CMV infection. Serial serum levels of creatinine (▲), plasma levels of sCD-4 (■) and sCD-8 (□) of a patient with CMV infection following kidney transplantation are shown. About 5 weeks after kidney transplantation, plasma levels of sCD-4 and sCD-8 began to increase. The sCD-8 plasma levels showed a more pronounced increment compared with sCD-4. A delayed increment of serum creatinine followed. By means of allograft biopsy, an acute allograft rejection or CsA nephrotoxicity could be excluded. A CMV infection, however, could be diagnosed by the detection of specific CMV-pp65EA in blood specimens. In the course of ganciclovir therapy, sCD-4 and sCD-8 decreased followed by a delayed reduction of serum creatinine.

Table 1. Soluble CD-4 concentrations (U/ml) in plasma

<table>
<thead>
<tr>
<th>Groups</th>
<th>sCD-4 in plasma (U/ml)</th>
<th>n</th>
<th>Median (minimum – maximum)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Post-transplant</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stable graft function</td>
<td>52</td>
<td>14</td>
<td>(9–26)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Acute rejection</td>
<td>37</td>
<td>45</td>
<td>(13–88)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>CMV infection</td>
<td>10</td>
<td>51</td>
<td>(21–111)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>CsA toxicity</td>
<td>6</td>
<td>25</td>
<td>(0–46)</td>
<td>NS</td>
</tr>
<tr>
<td>Controls</td>
<td>19</td>
<td>7</td>
<td>(0–23)</td>
<td></td>
</tr>
</tbody>
</table>

The sCD-4 plasma levels were significantly elevated during acute rejection and infection compared with cases with stable graft function (P<0.001*). There was no significant difference between acute rejection and infection. CsA nephrotoxicity induced no significant sCD-4 changes in plasma.

Table 2. Soluble CD-8 concentrations (U/ml) in plasma

<table>
<thead>
<tr>
<th>Groups</th>
<th>sCD-8 in plasma (U/ml)</th>
<th>n</th>
<th>Median (minimum – maximum)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Post-transplant</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stable graft function</td>
<td>52</td>
<td>362</td>
<td>(185–532)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Acute rejection</td>
<td>37</td>
<td>582</td>
<td>(278–896)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>CMV infection</td>
<td>10</td>
<td>1141</td>
<td>(477–2327)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>CsA toxicity</td>
<td>6</td>
<td>375</td>
<td>(205–530)</td>
<td>NS</td>
</tr>
<tr>
<td>Controls</td>
<td>19</td>
<td>393</td>
<td>(257–550)</td>
<td></td>
</tr>
</tbody>
</table>

The sCD-8 plasma levels were significantly elevated during acute rejection and infection compared with cases with stable graft function (P<0.001*). Additionally, there was a significant difference between acute rejection and viral infection (P<0.005**). CsA nephrotoxicity induced no significant sCD-4 changes in plasma.

(1.5-fold). Thus, the sCD-4/sCD-8 ratio in plasma increased in cases of acute allograft rejections and decreased during CMV infections as a sign of activation of different T-cell subsets.

These data underline the importance of CD4+ cells for initiating graft rejection [29,30]. Biopsy studies revealed that both CD4+ and CD8+ T cells infiltrate allograft tissue with a different pattern in the course of acute rejection. However, the relative proportions
Table 3. Soluble CD-5/soluble CD-8 ratio in plasma

<table>
<thead>
<tr>
<th></th>
<th>sCD-5/sCD-8 ratio of medians (minimum–maximum) in plasma x 1000</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stable graft function (n=52)</td>
<td>49 (9–76)</td>
</tr>
<tr>
<td>Acute rejection (n=37)</td>
<td>85 (19–204)</td>
</tr>
<tr>
<td>CMV infection (n=10)</td>
<td>35 (8–160)</td>
</tr>
</tbody>
</table>

The sCD-4/sCD-8 ratio increased significantly (P<0.05) during acute allograft rejection. Additionally, there was a significant difference (P<0.05) between acute allograft rejection and CMV infections.

Table 4. Sensitivity, specificity and predictive values of sCD-4 and sCD-8 for the diagnosis of acute allograft rejection and CMV infection

<table>
<thead>
<tr>
<th>Soluble receptors</th>
<th>Acute rejection–stable</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>Predictive value (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>sCD-4</td>
<td></td>
<td>78</td>
<td>39</td>
<td>63</td>
</tr>
<tr>
<td>CMV</td>
<td></td>
<td>85</td>
<td>78</td>
<td>81</td>
</tr>
<tr>
<td>sCD-8</td>
<td>Acute rejection–stable</td>
<td>58</td>
<td>23</td>
<td>40</td>
</tr>
<tr>
<td>CMV</td>
<td>Acute rejection–stable</td>
<td>92</td>
<td>86</td>
<td>88</td>
</tr>
</tbody>
</table>

Stable = stable graft function; CMV = CMV infection.
tion of polymorphonuclear neutrophils (PMN) in vivo and in vitro. Thus, it is speculated that sCD-4 could be another chemotactic factor and, as such, constitutes a link within the immune system between specific and non-specific inflammatory responses [48]. The functional significance of soluble T-cell markers still awaits clarification.

In conclusion, the present study demonstrates that sCD-4 and sCD-8 are markers of immunological activation and may enable a further differentiation of T-cell activation. Therefore, a serial measurement of these soluble T-cell markers in a transplant recipient may contribute to more precise characterization of the immunological processes following kidney transplantation. However, further prospective investigations are necessary to elucidate the diagnostic potential of sCD-4 and sCD-8 for monitoring acute rejection and viral infection in kidney graft recipients.

References

36. Belles-Isles M, Houde I, Lachance JG, Noel R, Roy R. Monitoring of cytomegalovirus infections by the detection of polymorphonuclear neutrophils (PMN) in vivo and in vitro. Thus, it is speculated that sCD-4 could be another chemotactic factor and, as such, constitutes a link within the immune system between specific and non-specific inflammatory responses [48]. The functional significance of soluble T-cell markers still awaits clarification.

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