High prevalence and usual persistence of serum monoclonal immunoglobulins evidenced by sensitive methods in renal transplant recipients

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Abstract

Background. The occurrence of serum monoclonal immunoglobulins in kidney transplant recipients is well known but their significance and predictive value for the occurrence of lymphoma are a matter of debate. We therefore conducted a study of monoclonal immunoglobulins by a sensitive method during the long-term follow up of grafted patients.

Methods. Monoclonal immunoglobulins were characterized by high-resolution electrophoresis, conventional immunoelectrophoretic analysis, and a sensitive Western blotting procedure in the serum from 84 renal transplant recipients prior to grafting and subsequently, with a 1–8-year follow-up and excluding the patients who developed post-transplant lymphoma.

Results. Low abundance monoclonal immunoglobulins were detectable prior to transplantation in 56 cases (66.6%) and after graft in 72 cases (85.5%) (and in 1 case (1.2%) and 18 cases (21.4%) of cases respectively, by immunoelectrophoresis). These abnormalities were often multiple in individual sera. Monoclonal components detected by immunoblotting were transient in 23.8% of patients only (whereas those evidenced by immunoelectrophoresis usually became undetectable by this method) and their pattern was remarkably stable in the majority of cases. The frequency of post-transplant monoclonal immunoglobulins was higher in patients of more than 50 years of age than in younger patients. The appearance of monoclonal components after grafting and their transient character correlated with CMV infections. No correlation was found with various other parameters. The isotropic distribution of monoclonal immunoglobulins with an IgM, IgG3, and IgG1 predominance and an abnormally low κ/λ ratio was the same as that observed in various immunodeficiency states. The monoclonal immunoglobulin pattern in three further patients who developed post-transplant lymphoma was unremarkable.

Conclusion. Monoclonal immunoglobulins hence are not discriminant for lymphoma and their characterization does not appear to be necessary in the evaluation of followed up grafted patients, at least for a prediction of post-transplant lymphoma.

Key words: cytomegalovirus; immunoblotting; immunoelectrophoresis; kidney transplantation; monoclonal immunoglobulins; serum

Introduction

Serum monoclonal immunoglobulins (moIg) are observed in a variety of conditions, including immunoproliferative disorders and numerous non-malignant processes such as inherited and acquired immunodeficiency states and ageing [1–5]. Their frequency greatly varies according to the detection procedures. The occurrence of serum moIg in renal transplant recipients was long known, with a low frequency when sera were studied by conventional immunoochemical analysis (i.e. immunoelectrophoresis) [6]. With the use of immunofixation, moIg were found in 3% [7], 7% [8], 8% [9], 12% [10], 13% [11] or 30% [12] of cases. A key question is whether the occurrence of serum moIg could be indicative of the risk of lymphoma and should lead to carefully follow up those patients [14], as also suggested after bone marrow transplantation [13], or to reduce immunosuppressive therapy [7,9]. Indeed, transplanted patients are prone to develop B cell lymphomas, or more rarely myeloma, with a frequency increased by high-dose immunosuppressive regimens [15–20]. Most renal transplant patients who developed immunoproliferative diseases (and asymptomatic patients also) had serum moIg, in relatively large amounts in some cases [21], but the significance of small amounts of moIg detected by immunofixation...
remained unclear in the above-mentioned studies, partly because of insufficient follow-up in certain studies.

Immunofixation underestimates the actual incidence of moIg. In a study [9], serum moIg were found in 8% of cases by immunofixation and 21% by isoelectric focusing. Some laboratories, including Radl’s and our laboratory, independently developed a Western blotting procedure which is more sensitive than immunofixation. The threshold level of moIg detection was evaluated to be 0.5 μg/ml by Radl et al. [22] and 14 μg/ml in our study [5,23–25], which is lower than the detection threshold by immunofixation (50 μg/ml).

Characterization of serum monoclonal immunoglobulins

Standard immuno-electrophoresis was performed in 1.2% agarose in 50 mM barbital buffer, pH 8.2, using antisera polyclonal for human serum proteins and for IgG, IgA and IgM (Sebia, Issy-les-Moulineaux, France) and antisera specific for γ, α, κ and λ chains (Silenus, Hawthorn, Australia).

Serum moIg were searched for by HRE in all serum samples. One μl of serum was deposited on thin-layer (0.4 mm) agarose (Paragon®, Beckman, Brea, CA) and migrated during 25 mn at 100 V in 50 mM barbital buffer, pH 8.6. Electrophoresis were fixed with a methanolacetic acid solution, desiccated and stained by amido black.

A single pressure Western blot technique [5,23–25] was used for moIg isotype characterization in 72 patients. Brieﬂy, sera diluted 1:50 to 1:500 were fractionated by HRE as above; proteins were then transferred onto nitrocellulose sheets (HAHY, Millipore, Bedford, MA) under fat milk, the blots were incubated with appropriate isotype-specific anti-IgG antibodies. Alkaline phosphatase-conjugated poly-coupled anti-murine IgG antibodies (Immunotech).

Serum moIg were defined on the basis of electric charge (narrow mobilities that are not identified by immunofixation) and light and heavy chain isotype restriction.

Subjects and methods

Patients

Serum samples were collected in 84 consecutive patients at the occasion of normal routine investigations just before grafting, then 6 months and 1 year after grafting and every year subsequently. The patients under study were followed up for 12–101 months (mean 47.6 ± 24.9 months). Twenty-three patients were followed up for 6 years or more. The patients were aged 15–69 years at time of transplantations (mean 40.7 ± 13.6 years), with a sex ratio of 0.6. The patients were unselected except that the three patients who developed a post-transplant lymphoma during the same period were excluded from the study. Indeed, lymphoma was diagnosed upon clinical and pathological grounds early in these patients, thus preventing long-term follow up (transplantectomy was anti-pated upon clinical and pathological grounds early in these patients, (Marseille, France) respectively, and conjugated antibodies excluded from the study. Indeed, lymphoma was diagnosed when possible, corticosteroids and anti-CD3 antibodies in the last patient, which is not different from antirejection therapy in patients without CMV infection.

Statistical analysis

Percentages were compared using the χ2 test and mean values were compared using the Student’s t test.
Results

Conventional immunochemical analysis allowed us to detect a single mIg before graft and 20 mIg (13 IgM and 7 IgG with a $\kappa/\lambda$ ratio of 2) in the serum from 18 kidney recipients only (21.4%). In 13 of the latter patients, the mIg present in sufficiently large amounts to be detected by immunoelectrophoresis were transient, whereas in five cases the immunoelectrophoretic pattern was similar at subsequent examinations, the last one being performed after 1 year (1 case), 3 year (1 case) and 5 year (3 cases) follow-up respectively. Only three of the patients with such relatively large amounts mIg had documented CMV infection, and there was no correlation between the occurrence of these mIg and CMV infection (neither with the patients’ age, therapy, or occurrence of rejection episodes).

In contrast, mIg most often present in very small amounts were detectable by HRE and IB in the serum from 56 patients (66%) just before transplantation. In these 56 patients, serum mIg became undetectable after grafting in six cases and remained unchanged in 19 cases, whereas their number per serum increased in 31 cases. As for the 28 patients without detectable serum mIg before graft, mIg were found in 22 cases after transplantation. In addition to the increase in the number of mIg after transplantation, those mIg, which were already present before, usually became more easily detectable due to moderate increases in amount (in keeping with some of them being then detectable by immunoelectrophoresis) and/or decrease of the polyclonal immunoglobulin background. Altogether, HRE and IB evidenced mIg in 72 patients (85.5%) 6 months and/or 1 year after graft, an incidence statistically different from that observed before transplantation ($P<0.01$). These mIg were generally multiple (Table 1), most sera showing a pattern sometimes named oligoclonal. Altogether 224 mIg were characterized, with a $\kappa/\lambda$ ratio of 1.1, including 141 IgG ($\kappa/\lambda$ ratio 0.8), 82 IgM ($\kappa/\lambda$ ratio 1.5) and a single IgA (Table 2). Study of the subclass distribution of monoclonal IgG showed an IgG3 and IgG1 predominance (Table 2).

During subsequent follow-up, the disappearance of one or more mIg was observed in 20 patients (which does not mean that their sera did not contain any mIg any more since most of them contained several mIg early after transplantation). Apart from these cases, the major finding was the striking stability of the serum mIg. In most cases, the HRE and IB patterns observed at repeated yearly studies were virtually superimposable. This was observed in the whole series as well as in the 23 patients in whom follow-up was 6 years or more: 19 of them had serum mIg which persisted in all but four cases. In the latter patients, mIg became undetectable 3 or 4 years (1 and 3 cases respectively) after transplantation.

Search for clinical correlations was rather disappointing except for two findings. The incidence of mIg was higher in patients of more than 50 years of age than in younger patients after transplantation (96.8% of the older patients had mIg versus 78.8% of the younger ones, $P=0.025$) but not prior to grafting (85.5% versus 66.5%, $P=0.18$), which related to a more frequent occurrence after graft of mIg that were undetectable before transplant in the older patients (88% of them instead of 62% of younger patients, $P=0.006$). Although the overall frequency of mIg did not correlate with CMV infections, the incidence of the appearance of previously unrecognized abnormalities was higher in patients with (90% of cases) than without (64%) CMV infection ($P=0.027$). Transient mIg also tended to be more frequent after CMV infection and the difference almost reached statistical significance ($P=0.053$).

The incidence of mIg prior to and after graft did not correlate with sex ratio (0.6 in every group), or type and duration of dialysis (27±10.6 months versus 18±10.2 months for patients with and without mIg respectively, $P=0.27$). There was no correlation between the incidence of mIg after transplantation and the number of HLA class I and class II incompatibilities, the occurrence of rejection episodes and their therapy, or the maintenance immunosuppressive regimens (Table 3). Neither did the appearance and disappearance of mIg correlate with the latter parameters.

Discussion

The present study in 84 kidney transplant patients without post-transplant lymphoma confirms the very high incidence of mIg detectable by IB reported in a previous study [22]. We further provide data on long-term follow up, a careful search for clinical

| Table 1. Distribution of the serum numbers (and percentages) of mIg evidenced by immunoblotting in 72 patients in the first post-transplant year |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| 0 mIg | 1 mIg | 2 mIg | 3 or 4 mIg | 5 or 6 mIg | 7 mIg or more |
| 12 (16.7%) | 14 (19.4%) | 7 (9.7%) | 21 (29.2%) | 9 (12.5%) | 9 (12.5%) |

<p>| Table 2. Isotypic distribution (percentages) of mIg characterized by immunoblotting in the first post-transplant year |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th>Immunoglobulin classes</th>
<th>IgG</th>
<th>IgA</th>
<th>IgM</th>
<th>IgG1</th>
<th>IgG2</th>
<th>IgG3</th>
<th>IgG4</th>
</tr>
</thead>
<tbody>
<tr>
<td>62.9</td>
<td>0.45</td>
<td>36.6</td>
<td>38.3</td>
<td>10</td>
<td>45.8</td>
<td>5.8</td>
<td></td>
</tr>
</tbody>
</table>
correlations, and a study of the IgG subclass distribution of molg. The high frequency (85.5%) of molg detected using a sensitive method in the present series of patients without lymphoma, the fact that numerous molg were already present before transplantation, and the remarkable stability of the molg pattern in the majority of cases which was already mentioned in some previous studies [6,8] clearly show that serum molg have no predictive value for the occurrence of post-transplant lymphoma. This is also true for molg present in sufficient amount to be evidenced by immunoelectrophoresis, since such molg were found in 21% of cases. Three patients (excluded from the analysis) developed an EBV-positive lymphoma during the time course of the study. Their serum molg patterns were similar to those of the other grafted patients. They all had several molg detected by IB and one of them also had a more abundant monoclonal IgG that had already been evidenced by immunoelectrophoretic studies performed prior to grafting. Hence, these lymphoma patients could not be distinguished from the other patients on the basis of serum molg.

Molg detected by immunoelectrophoresis in the present study were often transient, as already observed for molg evidenced by immunofixation [8–11], a method which is less sensitive and less accurate than IB. Some of these previous studies reported a correlation between molg and CMV infection [8,9,11], which was expected, since this virus was long known to be able to induce transient serum molg [26]. In our study CMV infection correlated with the appearance of de novo molg after graft and probably also with molg disappearance. This infection is often associated with relatively abundant molg that are detectable by immunofixation and even immunoelectrophoresis. There is hence no basic discrepancy between previous studies and ours. That we could not establish a correlation between molg detectable by immunoelectrophoresis and CMV infection probably relates to the small numbers of patients with known infection or with such molg and the possibility that CMV infection may have remained unrecognized in certain patients. In Radl et al.’s first study, immunofixation revealed molg in 3% of patients with chronic renal failure treated by dialysis as compared with 30% of grafted patients [12]. This is not really contradictory to the present data collected just before transplantation in spite of the high incidence of molg observed, since these molg were present in very discrete amounts. The same study showed a correlation between the occurrence of molg after graft and the patients’ age, which is confirmed in the present study. This is not surprising since ageing is known to be associated with a high incidence of molg, reflecting the immunodeficiency inherent to ageing [4,5].

Chronic renal failure induces an acquired immunodeficiency state. It might play a role in the high incidence of serum molg before transplantation, together with an overproduction of IL-6, a major cytokine in lymphocyte differentiation, plasma cell proliferation, and Ig secretion. Indeed, serum IL-6 levels are high in non-dialysed patients with chronic renal failure [33]. Furthermore, IL-6 synthesis by peripheral blood mononuclear cells is higher in patients haemodialysed using cuprophane membranes, which is known to cause a prolonged activation of monocytes, than in those dialysed using more biocompatible membranes such as polymethyl-methacylate [34]. In the present work the high incidence of molg prior graft may be related to the use of a cuprophane membrane in 66% of cases (71% of the haemodialysed patients). The absence of correlation between the incidence of molg and the type of dialysis (peritoneal dialysis or haemodialysis) on the one hand, and the dialysis membranes used, on the other, probably relates to the small number of patients under peritoneal dialysis or haemodialysed using biocompatible membranes.

The immunodeficiency state is considerably increased after transplantation by the immunosuppressive regimen and complications such as viral infections. This and the correlation observed previously between molg and CsA therapy [10,11] suggested that molg were merely the result of the post-transplant immunodeficiency state and antigen stimulation [4,12,22]. The isotypic distribution of molg in the present study, with an IgM, IgG3, and IgG1 predominance and an abnormally low κ/λ ratio strongly argue for the same conclusion, since the same distribution was previously observed in various immunodeficiency states [3–6, 22–25, 27–29], in contrast to the isotypic distribution of normal plasma cells [30] and myeloma proteins [3,4,28,31]. The decreasing order of frequency of heavy-chain classes of molg observed in the present study and in other immunodeficiency states nearly follows the 5'-3' order of the heavy chain constant region genes and is probably the reflect of the

Table 3. Lack of correlation between the occurrence of molg and various clinical parameters (univariate analysis)

<table>
<thead>
<tr>
<th>Patients (n)</th>
<th>With molg</th>
<th>Without molg</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>43</td>
<td>7</td>
<td>0.99</td>
</tr>
<tr>
<td>F</td>
<td>29</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>CMV infection</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>19</td>
<td>1</td>
<td>0.27</td>
</tr>
<tr>
<td>No</td>
<td>53</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>Maintenance therapy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CSA monotherapy</td>
<td>29</td>
<td>6</td>
<td>0.79</td>
</tr>
<tr>
<td>Bi- and Tri-therapy</td>
<td>43</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Rejection episodes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>27</td>
<td>4</td>
<td>0.80</td>
</tr>
<tr>
<td>No</td>
<td>45</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Rejection therapy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corticosteroids</td>
<td>20</td>
<td>2</td>
<td>0.55</td>
</tr>
<tr>
<td>Anti-CD3 antibodies</td>
<td>7</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>HLA incompatibilities</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥3</td>
<td>41</td>
<td>8</td>
<td>0.51</td>
</tr>
<tr>
<td>&lt;2</td>
<td>30</td>
<td>4</td>
<td></td>
</tr>
</tbody>
</table>
increasing requirement for T cell help for the expression of 3'-located genes [32]. The striking stability of the molg pattern in a majority of patients might be due to the necessarily continuous immunosuppressive therapy. In any case, it led us to remove the study of serum molg from the list of examinations performed in the currently followed-up patients.

References


Received for publication: 21.10.96
Accepted in revised form: 15.1.97