Transpl Int (1999) 12: 250–253


ORIGINAL ARTICLE

**Didier Ducloux**
**Pierre-Louis Carron**
**Evelyne Racadot**
**Jean-Michel Rebibou**
**Catherine Bresson-Vautrin**
**Yves Saint Hillier**
**Jean-Marc Chalopin**

# T-cell immune defect and B-cell activation in renal transplant recipients with monoclonal gammopathies

Received: 10 June 1998
Received after revision: 1 February 1999
Accepted: 19 April 1999

D. Ducloux (✉) · P. L. Carron · J. M. Rebibou · C. Bresson-Vautrin · Y. Saint Hillier · J.-M. Chalopin
Department of Nephrology and Renal Transplantation, Hopital Saint-Jacques, 2, Place Saint-Jacques, F-25030 Besancon, France
Fax: + 33-3-8121-8781

E. Racadot
Blood Transfusion Center of Franche-Comté, 2, Boulevard Fleming, F-25030 Besancon, France

**Abstract** Monoclonal immunoglobulins (molg) have repeatedly been described in organ and bone marrow transplantation. Although their exact significance is not known, their occurence is often associated with intensive immunosuppression. We investigated whether molg reflect T-cell immune defect and B-cell activation in renal transplant recipients. Immunofixations and lymphocyte subset analysis (CD4, CD8, CD19) were performed in 182 renal transplant recipients. Soluble CD23 concentrations were measured in patients with molg and in control transplant patients without molg. Monoclonal immunoglobulins were identified in 54 patients (29.6%). Transplant endurance was shorter (62 +/– 53 months vs 81 +/– 47 months; $P < 0.02$) and age was older (53 +/– 13 years vs 46 +/– 13 years; $P < 0.005$) in patients with molg. Maintenance immunosuppression did not differ between patients with and without molg. Mean CD4-cell count was significantly lower in patients with molg (387 +/– 286/mm$^3$ vs 538 +/– 341/mm$^3$; $P < 0.005$). Both CD8- and CD19-cell counts were similar for the 2 groups. Soluble CD23 concentrations were higher in patients with abnormal immunoglobulin values than in patients with normal immunofixation (12.8 +/– 8 vs 1.9 +/– 1.8 μg/l; $P < 0.005$). Our study provides new evidence that molg reflect T-cell immune defect in renal transplant recipients. Further studies are required to determine whether CD4-cell count and sCD23 may help to predict the risk of lymphoma in transplant patients with molg.

**Key words** Renal transplantation · Monoclonal gammopathy · CD4 lymphocyte · Soluble CD23

## Introduction

Transplant patients are prone to develop B-cell lymphomas with a frequency increased by "high-dose" immunosuppressive regimens [11]. Indeed, with the advent of increasingly specific immunosuppressive drugs targeting the T-lymphocyte, uncontrolled expansion of B-lymphocyte clones may occur as a result of T-lymphocytes depletion [13]. Monoclonal immunoglobulins (molg) have frequently been described in organ and bone marrow transplantation [2, 10, 16, 17, 20]. Although their exact significance is not known, their occurence is often associated with intensive immunosuppression. There is some evidence to support the hypothesis that posttransplant molg are the result of an imbalance between T-lymphocyte depletion and B-cell activation. In bone-marrow transplantation, molg are more frequently encountered in patients with T-cell deficiencies (SCID, functional T-cell deficiencies, CID, and Wiskott-Aldrich syndrome) [5]. More recently, the occurence of molg has been associated with more aggressive immunosuppressive therapy in renal transplant recipients [14].

CD23 is a B-cell differentiation and activation marker expressed on B-cells. Soluble CD23 (sCD23) also is

a B-cell-stimulatory factor. Over the past few years, interest has been focused on sCD23 as a disease marker with prognostic significance for B-cell chronic lymphocytic leukemia (B-CCL). It has been shown that sera from B-CCL patients contain 3–500 times more sCD23 than sera from healthy individuals [19]. It has also been suggested that sCD23 levels may serve as a clinical tool for early detection of PTLD [7, 9] and lymphoma in HIV patients [25]. To our knowledge, no study has focused on sCD23 in posttransplant monoclonal gammopathies.

In this study, we investigated whether molg reflect a T-cell immune defect and/or B-cell activation in renal transplant recipients.

## Patients and methods

At the time of the study, 255 patients were followed in our unit. We have recently been able to demonstrate that all lymphocyte subpopulations markedly decrease on the first day after transplantation, and then progressively increase, reaching pretransplant values one year after transplantation (not published data). Because of the great variation in lymphocyte sub-population in the first year after transplantation, we chose transplant that had been operated longer than 1 year before January 1995 for our study. Of the 255 patients, 50 had undergone transplantation less than 1 year before the beginning of the study, and had to be excluded, further 23 could not be included because of the lack of one or more biological tests, leaving 182 renal transplant recipients for our study. Blood and serum samples were collected from January 1995 to June 1997. All patients were seen regularly in the outpatient nephrology clinic.

Immunofixations were performed on a hydrasis apparatus (SEBIA-France) Lymphocyte subset analysis (CD4, CD8, CD19) was simultaneously performed by flow-cytometry. Total blood samples were incubated with anti-CD4, anti-CD8 and anti-CD19 monoclonal antibodies labelled with FITC (DIACLONE-France). After red cell lysis, sample were analyzed on a facs calibur flow-cytometer (Becton-Dickinson). Clinical correlations of age, gender, hemodialysis duration before transplantation, transplant duration, immunosuppressive regimen, and biological correlations, serum creatinine concentration were also studied. Patients with monoclonal immunoglobulins were placed in group 1, patients with normal immunofixation were placed in group 2. Soluble CD23 was measured by ELISA technique (sCD23 kit-Binding site) in renal transplant recipients with molg. Transplant patients without molg and matched for age, gender, transplant duration, exposure to EBV and immunosuppressive therapy were selected, and sCD23 was measured in each control. Student's *t*-test was used for comparing differences between groups, and the Spearman rank correlation test for estimating relationship between variables. The ordinal data were analyzed using a chi-square test. Results are expressed as means +/– standard deviations.

## Results

Monoclonal immunoglobulins were identified in 54 patients (group 1). In most sera (74.1 %) molg were multiple and secreted by distinct clones. One hundred twenty-eight patients had normal serum immunofixation (70.4 %, group 2). Most molg were not identified on serum electrophoresis. Differences between patients of groups 1 and 2 are summarized in table 1. Transplant endurance was shorter in patients of group 1 (62 +/– 53 months vs 81 +/– 47 months; $P < 0.02$). Patients of group 1 were significantly older (53 +/– 13 years vs 46 +/– 13 years; $P < 0.005$). Hemodialysis duration was similar in both groups (27 +/– 37 months vs 24 +/– 33 months; $P$ = NS). The sex ratio was identical between the 2 groups.

**Table 1** Differences between renal transplant patients with (molg(+)) and without (molg(–))

| | molg (–) | molg (+) | *P* |
|---|---|---|---|
| Age in years | 46 +/– 13 | 53 +/– 13 | < 0.005 |
| Hemodialysis duration in months | 24 +/– 33 | 27 +/– 37 | NS |
| Transplant endurance in months | 81 +/– 47 | 62 +/– 53 | < 0.02 |
| CD4 cell count in $mm^3$ | 538 +/– 341 | 387 +/– 286 | < 0.005 |

Maintenance immunosuppression did not differ between patients with and without abnormal serum immunofixation. All the patients received low-dose steroids and azathioprine (1.5–2.5 mg/kg per day). Forty-three patients in group 1 and 102 patients in group 2 received Cyclosporin A (CsA) (2–4 mg/kg per day) (81 % vs 80 %; $P$ = NS). CsA trough levels were comparable in both groups (102 +/– 29 vs 98 +/– 32 ng/ml; $P$ = NS). The mean dosage of azathioprine was similar in the two groups (79 +/– 23 mg/d vs 84 +/– 25 mg/d; $P$ = NS). All the patients had received polyclonal antilymphocyte globulins as immunosuppressive induction.

Mean CD4 cell count was significantly lower in patients of group 1 (387 +/– 286/$mm^3$ vs 538 +/– 341/$mm^3$; $P < 0.005$). Both CD8- and CD19-cell counts were similar for the 2 groups.

Soluble CD23 concentrations were significantly higher in patients with posttransplant molg than in patients with normal immunofixation (12.9 +/– 8 vs 1.8 +/– 1.7 µg/l; $P < 0.005$). Soluble CD23 concentrations did not differ between EBV seronegative and EBV seropositive patients. Mean CD19 cell counts were similar in both groups (38 +/– 34/$mm^3$ vs 34 +/– 41/$mm^3$; $P$ = NS). We found no correlation between sCD23 concentration and CD19 cell count (r-sq = 0.02; $P$ = NS).

## Discussion

Our study demonstrates a high occurance of serum monoclonal immunoglobulins, as detected by a sensitive immunofixation method [18]. This prevalence is similar to previous studies using the same technique in a com-

parable population [3]. The significance of molg in transplant patients remains controversial. It has been suggested that their great incidence hamper their interest in predicting the subsequent development of a B-cell posttransplant lymphoproliferative disorder.

Our study demonstrates that the occurence of serum monoclonal immunoglobulins is associated with a more pronounced T-cell immunodeficiency, and is consistent with the increased incidence of monoclonal immunoglobulins in transplant patients who have received more aggressive immunosuppressive therapy [14, 15, 17, 18]. The immunosuppressive agents modify T-cell lymphokine production and T-cell function [23]. The same mediators are intimately involved with the regulation of B-cell function and immunoglobulin production [4]. The diminution of T-cell mediated immune surveillance might allow B-cell proliferation to get out of control. If control systems remain suppressed for a prolonged period, progression to lymphoma might ensue.

Soluble CD23 may be a B-cell growth factor, and recent studies have provided evidence that sCD23 is a highly sensitive and specific parameter for B-cell leukemia, lymphoma and PTLD [7, 9, 19, 25]. A major finding of this study is that sCD23 is markedly increased in renal transplant recipients with monoclonal gammopathies. It is conceivable that the high sCD23 concentrations found in the patients with gammopathies are a reflection of high sCD23 production by B-lymphocytes that escaped T-cell control because of immunosuppression. Thus, the secreted form of CD23 might also contribute to B-cell clone expansion by autocrine stimulation of B-cells in renal transplant recipients with monoclonal gammopathies. If control systems remained suppressed for a prolonged period, progression to lymphoma might ensue.

It has been suggested that molg might progress to myeloma. However, few studies support this hypothesis [25]. Moreover, high levels of sCD23 have not been found in patients with multiple myeloma [8], and CD23 is not expressed on B- and preB-cells of patients with multiple myeloma. However, the increased level of sCD23 in transplant patients with molg and such with PTLD might suggest a link between the two disorders.

Although there have been reports of altered amounts of B-cells in patients with molg [6], several other studies have failed to find any difference in either the absolute number or the percentage of B cells in patients in molg [1, 12]. Contrary to previous reports, we did not find any correlation between CD19-cell count and sCD23 concentration.

Older patients had a higher occurence of molg. An increase in the incidence of molg has previously been associated with age in both the normal- and transplant population [2].

With renal transplant recipients, molg are most often found in the two first years after transplantation [2, 3, 14]. We also observed a shorter transplant endurance in patients with molg. This may be due to more intense immunosuppression in the early posttransplant period. Alternatively, viral infections and alloantigenic stimulation by the allograft may also contribute. While the precise etiology of PTLD is unknown, EBV has been implicated by the demonstration of the EBV genome or its products in the majority of PTLD cases [21]. Moreover it has been shown that EBV nuclear antigens 2 and 3c transactivate CD23 [22]. The secreted form of CD23 might be a B-cell growth factor, but the full-length molecule may be a receptor thus providing autocrine stimulation of EBV-infected B-cells [24].

There are some limitations to our study due to the crossectional design and the lack of longitudinal observation. It would be of interest to know whether the appearance and disappearance of monoclonal gammopathies are correlated with the variations in both CD4 cell count and sCD23 concentrations.

To conclude, our study provides new evidence that molg reflect a T-cell immune defect and B-cell activation in renal transplant recipients. Further studies are required to determine whether kinetics of CD4-cell count and sCD23 concentrations may help to predict the risk of lymphoma in transplant patients with molg.

## References


1. Bataille R, Duperray C, Zhang XG, Brochier J, Klein B (1992) CD5 B lymphocyte antigen in monoclonal gammopathy. Am J Hematol 41: 102
2. Chakalarovski C, Lang Ph, Buisson CI, Weil B (1991) Immunoglobulines monoclonales en transplantation rénale. Caractérisation, évolution et factuers de risque. Presse Med 20: 2033–2035
3. Deteix P, Chapuis-cellier C, Ghais Z, Lefrançois N, Brusa M, Touraine JL, Traeger J (1985) Systemic survey of immunoglobulin abnormalities: frequency and evolution in organ transplant recipients. Transplant Proc 17: 2651–2653
4. Dinarello CA, Mier JW (1987) Lymphokines. N Engl J Med 317: 940–948
5. Fischer AM, Simon F, Le Deist F, Blanche S, Griscelli C, Fischer A (1990) Prospective study of the occurence of monoclonal gammapathies following bone marrow transplantation in young children. Transplantation 49: 731–735

6. Jensen GS, Belch AR, Kherani F, Mant MJ, Ruether BA, Pilarski LM (1992) Restricted expression of immunoglobulin light chain mRNA and of the adhesion molecule CDIIb on circulating monoclonal B lineage cells in peripheral blood of myeloma patients. Scand J Immunol 36: 843
7. Kato H, Inamoto T, Nakamura H, Furuke K, Uemoto S, Tanaka K, Ozawa K, Hori T, Yodoi J (1993) Soluble CD23 as a sensitive marker for Epstein-Barr virus-related disorders after liver transplantation. Transplantation 56: 1109–1113
8. Klouche M, Wilhelm D, Kirchner H (1994) Zytokine, Immunoglobuline und IgG-Subklassen bei Patienten mit IgG-Plasmozitomen. Immun Infekt 22: 149–151
9. Martinez OM, Villanueva JC, Lawrence-Miyasaki L, Quinn MB, Cox K, Krams SM (1995) Viral and immunologic aspects of Epstein-Barr virus infection in pediatric liver transplant recipients. Transplantation 59: 519–524
10. Modesto A, Rostaing L, Abbal M, Niquet L, Tkaczuk J, Durand D, Ohayon E (1994) Long term follow-up of de novo monoclonal gammapathies. Transplant Proc 26: 282–283
11. Nalesnik MA, Starzl TE (1994) Epstein-Barr virus, infectious mononucleosis, and posttransplant lymphoproliferative disorders. Transplant Science 4: 61–79
12. Omede P, Boccadorao M, Gallone G (1990) Multiple myeloma: increased circulating lymphocytes carrying plasma cell-associated antigens as an indicator of poor survival. Blood 76: 1375
13. Opelz G, Henderson R (1993) For the collaborative transplant study. Incidence of non-Hodgkin's lymphoma in kidney and heart transplant recipients. Lancet 342: 1514–1519
14. Passweg J, Thiel G, Bock HA (1996) Monoclonal gammopathy after intense induction immunosuppression in renal transplant patients. Nephrol Dial Transplant 11: 2461–2465
15. Peest D, Schaper B, Nashan B (1988) High incidence of monoclonal immunoglobulins in patients after liver or heart transplantation. Transplantation 46: 389–395
16. Pollock CA, Mahony JF, Ibels LS, Caterson RJ, Waugh DA, Wells JV, Ross Sheil AG (1989) Immunoglobulin abnormalities in renal transplant recipients. Transplantation 47: 952–956
17. Radl J, Valentijn RM, Haaijman JJ, Paul LC (1985) Monoclonal gammapathies in patients undergoing immunosuppressive treatment after renal transplantation. Clin Immunol Immunopathol 37: 98–102
18. Ritchie RF, Smith R (1976) Immunofixation: II. Application to the typing of monoclonal proteins. Clin Chem 22: 1982–1987
19. Sarfati M, Bron D, Lagneaux L (1988) Elevation of IgE-binding factors in serum of patients with B cell-derived chronic lymphocytic leukemia. Blood 71: 94–98
20. Stanko CK, Jeffery JR, Rush DIN (1989) Monoclonal and multiclonal gammapathies after renal transplantation. Transplant Proc 21: 3330–3332
21. Stephanian E, Gruber SA, Dunn DL, Matas AJ (1991) Posttransplant lymphoproliferative disorders. Transplant Rev 5: 120
22. Strauss SE (1993) Epstein-Barr virus infections: biology, pathogenesis, and management. Ann Intern Med 118: 45–58
23. Strom TB, Tilney NL (1986) Renal transplantation: clinical aspects. In: Brenner BM, Rector FC (eds) The kidney. Saunders, Philadelphia, p 1944
24. Swendeman S, Thorley-Lawson DA (1987) The activation antigen BLAST-2, when shed, is an autocrine BCGF for normal and transformed B cells. EMBO J 6: 1637–1642
25. Yawetz S, Cumberland WG, Van der Meyden M, Martinez-Maza O (1995) Elevated serum levels of soluble CD23 (sCD23) precede the appearance of acquired immunodeficiency syndrome-associated non-Hodgkin's lymphoma. Blood 85: 1843–1849