



**Universiteit
Leiden**
The Netherlands

Renal transplant patients monitored by the Cell-Mediated-Lympholysis assay: evaluation of its clinical value

Goulmy, E.A.J.M.; Stijnen, T.; Groenewoud, A.F.; Persijn, G.G.; Blokland, E.; Pool, J.; ... ; Rood, J.J. van

Citation

Goulmy, E. A. J. M., Stijnen, T., Groenewoud, A. F., Persijn, G. G., Blokland, E., Pool, J., ... Rood, J. J. van. (1989). Renal transplant patients monitored by the Cell-Mediated-Lympholysis assay: evaluation of its clinical value. *Transplantation*, 48, 559-563.
Retrieved from <https://hdl.handle.net/1887/2948>

Version: Not Applicable (or Unknown)

License:

Downloaded from: <https://hdl.handle.net/1887/2948>

Note: To cite this publication please use the final published version (if applicable).

RENAL TRANSPLANT PATIENTS MONITORED BY THE CELL-MEDIATED LYMPHOLYSIS ASSAY

EVALUATION OF ITS CLINICAL VALUE¹

ELS GOULMY, THEO STIJNEN,² ALBERT F GROENEWOUD,³ GUIDO G PERSIJN, ELS BLOKLAND,
JOS POOL, LEEN C PAUL,⁴ AND JON J VAN ROOD

Department of Immunohaematology and Eurotransplant Foundation University Hospital, Leiden, Department of Biostatistics, Erasmus University, Rotterdam the Netherlands, and Klinikum Rechts der ISAR, Ismaningerstrasse 22, 8000 Munchen 80, West Germany

Donor-specific cytotoxic T cell activity was measured over a period of 5 years after transplantation using the cell-mediated lympholysis (CML) test in 124 recipients of unrelated kidney allografts who received conventional immunosuppressive therapy consisting of azathioprine and prednisone. Since patients with a functioning transplant frequently display donor-specific CML non-responsiveness *in vitro*, we addressed the question of whether the CML status has a predictive value regarding the graft prognosis at any time interval until 5 years posttransplantation. From log-rank type analyses we conclude that the estimated relative risk calculated over the whole follow-up period of a CML-responder in the category of transplant rejectors is 1.25 with 95% confidence bounds between 0.94 and 1.65. Measurements of CML responder status during follow-up seem to have only limited prognostic value, although the relative risk is borderline significant when the analysis is restricted to the period between 2 weeks and 6 months posttransplantation.

The long-term immunologic acceptance of a histoincompatible organ graft, which is often assumed to reflect a tolerant state, could be measured *in vitro* by the use of the cell-mediated lympholysis (CML)* assay. Peripheral blood lymphocytes from renal transplant patients with a well-functioning graft have often been shown to exhibit donor-specific CML-nonresponsiveness (CML-NR) while the CML reactivity against randomly chosen stimulator cells usually remains intact (1-7).

The exact mechanism(s) that underlie the development of such donor specific CML-NR after transplantation are not known, but evidence has been presented that the donor HLA system may regulate this phenomenon (8). Mechanisms that may be involved include the involvement of suppressor cells (9-14), antidiotypic antibodies (15, 16), Fc receptor blocking

¹This work was supported in part by the Dutch Foundation for Medical and Health Research (Medigon), the J. A. Cohen Institute for Radiopathology and Radiation Protection (IRS), the Dutch Kidney Foundation (NSN), the Eurotransplant Foundation, and the Kuratorium für Hemodialyse (KfH).

²Department of Biostatistics, Erasmus University, Rotterdam

³Klinikum Rechts der ISAR, Ismaningerstrasse 22, 8000 Munchen 80, West Germany

⁴Present address: Foothills Hospital, 1403 29th Street N.W., Calgary Alberta Canada

* Abbreviations: CML, cell mediated lympholysis; CML NR, cell mediated lympholysis nonresponsiveness; CML R, cell mediated lympholysis responsiveness

antibodies (17), antidiotypic T cells (18), veto cells (19), or clonal deletion (13, 20, 21). Since the observations of CML-NR are essentially retrospective evaluations in selective groups of patients, we decided to investigate the predictive value of the CML status for graft survival. Here we present the results of 372 donor-specific CML studies in 124 renal transplant recipients in relation to allograft survival.

MATERIALS AND METHODS

Patients PBLs from 124 renal patients who had received a first cadaveric renal transplant through the services of Eurotransplant were investigated for their cytolytic potential against donor splenocytes as well as against control cells. From 69 patients additional pretransplant PBLs were available for study. All patients had received one or more blood transfusion(s) before transplantation. Immunosuppression consisted of prednisone and azathioprine. Transplantation was considered successful if the recipient remained alive without (re)institution of dialysis. Patients who rejected their grafts were studied till graft nephrectomy.

In vitro studies Serial samples of recipients' PBLs were collected at several intervals up to 5 years after transplantation. The time intervals of blood sample collection and the total number of samples (average 4, maximum 13) available for study varied for each patient. The PBLs (i.e. 10⁶ responder cells) were sensitized *in vitro* for 6 days against 10⁶ irradiated splenocytes from the specific kidney donor as well as against 10⁶ control cells from healthy unrelated individuals. Depending on the amount of lymphocytes available, which was limited in most of the cases, either tissue culture flasks or 2 ml cluster wells were used, the ratio responder/stimulator cell however is identical in both culture conditions. After the culture period, the effector cells were harvested and tested in the standard CML assay against their specific stimulator cells (i.e. splenocytes of the specific kidney donor and control cells of healthy unrelated individuals) as target cells.

Donor lymphocytes were obtained from the spleen. All patients' blood samples, the donor spleen cells, and the control cells were frozen and stored in liquid nitrogen until used.

CML NR or CML R These terms are used to describe the CML nonresponsiveness or CML responsiveness, respectively, exhibited by the recipients' PBLs against the specific kidney donor splenocytes. Almost all the recipients showed a normal cytolytic response to HLA incompatible control cells. The few cases in which the response to the control cells remained low in repeated experiments were excluded from the analyses.

The CML assay has been described in detail (22). The percentages of lysis were determined using phytohemagglutinin stimulated blast cells in a 4 hr ⁵¹Cr assay. Cytotoxicity (i.e. the amount of isotope released from ⁵¹Cr labeled target cells) was determined and calculated according to the described method (22). Standard errors of the mean of triplicate determinations were less than 5%. Positive and negative

assignments were made on the basis of a 10% specific ⁵¹Cr release value and on a positive slope, i.e. the various effector to target cell ratios are plotted and must give an S shaped curve (or in the case of transforming the percentage of lysis to a log scale a straight line). All experiments were repeated at least twice at different effector to target cell ratios.

Statistical analyses In order to assess the association between CML responder status during follow up and subsequent graft failure, a log rank type analysis was performed following the method outlined by Mantel and Byar (23). At each time point *t* (days posttransplantation) on which one or more failures occurred, the group of patients at risk was divided into two groups, CML NR or CML R, according to their last CML test done before time *t* (Patients without CML test before time *t* were considered to be not at risk). Next, for each group the expected numbers of failures (under the hypothesis that the probability of failure does not depend on the CML status) were computed in the usual way. Then expected and observed numbers of failures were compared analogously to the procedure of the standard log rank test.

Two analyses were carried out. In the first one the endpoint was graft failures from all causes including death of the patient. In the second analysis the endpoint was acute rejection. Observations of graft failures with another cause and observations of patients who died with a functioning graft were treated as censored in this analysis.

RESULTS

Longitudinal CML studies up to 5 years after transplantation were performed with the lymphocytes from 124 patients who had received a kidney transplant from an unrelated donor. Three examples of serial investigations are shown in Figure 1. The period in which the CML responder status may convert from positive to negative differs between individuals, as exemplified in Figure 1.

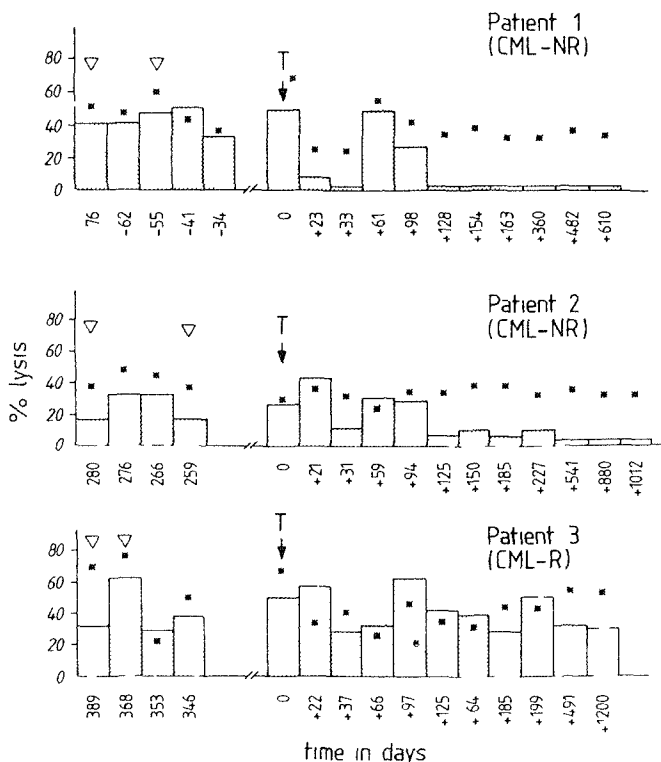


FIGURE 1 Examples of CTL reactivity patterns in 3 patients (∇) blood transfusion and (T) day of renal transplantation. Bars indicate the percentage of lysis against the specific kidney donor splenocytes (*): percentage of anticontrol cell lysis (CML NR) CML Non Responder (CML R) CML Responder

TABLE 1 Observed and expected numbers of graft failures according to CML status and time posttransplantation

Time (days posttransplant)	CML status NR ^a			CML status R ^b		
	N at risk	N obs failures	N exp failures	N at risk	N obs failures	N exp failures
2	19	0	0.24	60	1	0.76
6	24	0	0.30	55	1	0.70
7	25	0	0.32	53	1	0.68
12	30	1	0.37	52	0	0.63
13	31	1	0.38	50	0	0.62
14	31	0	0.39	49	1	0.61
15	31	0	0.39	48	1	0.61
19	32	0	0.41	46	1	0.59
20	31	0	0.40	46	1	0.60
21	32	0	0.42	44	1	0.58
34	33	0	0.44	42	1	0.56
36	33	1	0.45	41	0	0.55
37	32	0	0.44	41	1	0.56
40	32	0	0.44	40	1	0.56
42	32	0	0.45	39	1	0.55
80	30	0	0.43	40	1	0.57
89	30	0	0.43	39	1	0.57
95	30	1	0.45	37	0	0.55
104	30	1	0.45	36	0	0.55
110	29	0	0.45	36	1	0.55
133	35	1	0.52	32	0	0.48
153	35	0	0.53	31	1	0.47
177	36	0	0.55	30	1	0.45
376	49	0	0.66	25	1	0.34
438	53	1	0.71	22	0	0.29
562	59	1	0.76	19	0	0.24
627	60	1	0.75	20	0	0.25
739	67	0	0.78	19	1	0.22
748	67	0	0.79	18	1	0.21
810	69	1	0.79	18	0	0.21
830	69	1	0.79	18	0	0.21
871	69	1	0.78	19	0	0.22
912	68	1	0.78	19	0	0.22
1214	68	1	0.78	19	0	0.22
1243	64	1	0.78	18	0	0.22
1758	47	1	0.47	19	0	0.29
2007	44	0	0.71	18	1	0.29
2073	44	0	0.72	17	1	0.28
2205	43	1	0.74	15	0	0.26
2241	42	1	0.74	15	0	0.26
2421	40	1	0.74	14	0	0.26
Total ^a	19	22	92	23	17	84

^a Log rank analysis calculated over the whole follow up period yield *P* = 0.13

^b CML status according to the last previous measurement

In order to assess the relationship between CML status during follow up and the risk of graft failure, the log rank analysis was carried out. Table 1 shows the observed and expected numbers of failures according to CML status at each time that a failure occurred. Although a trend is visible (on average more rejections observed than expected in CML R patients), we could not accept the hypothesis that CML R patients have more graft failures than CML NR (*P* = 0.13). The Mantel Haenszel estimate of the relative risk (CML R versus CML-NR) was 1.25 with 95% confidence interval of 0.94-1.65. Since most renal transplants are lost because of acute rejection in the first 6 months after transplantation (24),

we carried out the same analysis for this time trajectory. The results shown in Table 2 demonstrate that the number of graft rejections in the CML-R group is higher than expected on the basis of random distribution of rejection cases between CML-NR and CML-R patients. The effect is most prominent in the period between 2 weeks to 6 months posttransplantation, which just reached statistical significance ($P = 0.052$).

The statistical analysis as shown above was done based on the information that the graft was either "functioning" or "nonfunctioning." Therefore "nonfunctioning" grafts included acute rejections, which were about two thirds of all failures, recurrent original disease, chronic rejection, death of the patient, or other not further-identified causes of failures. Consequently, the same statistical analysis was carried out on functioning renal allografts versus acute rejections only. This analysis as shown in Table 3 demonstrates that although a trend is observed similar to the results of the analysis comprising all "nonfunctioning" grafts (Table 2), the data fail to reach statistical significance ($P = 0.085$, posttransplant period 15-153 days).

We also investigated whether the pretransplant CML status has predictive capability for the likelihood of posttransplant development of CML-NR. The availability of pretransplant blood samples of 69 recipients provided the information concerning the donor-directed CTL reactivity prior to grafting. Fifty one recipients showed CML reactivity against the splenocytes of the kidney donor. Twenty eight of 51 patients who exhibited pretransplant CML reactivity developed donor-specific CML nonreactivity at different times posttransplant, whereas 23 patients persistently demonstrated donor-directed CTL activity, 15 pretransplant CML-NR patients remained

CML-NR posttransplantation. Three patients with acute irreversible rejection showed "reversed" CML conversion: absence of pretransplant donor directed CTL activity but high levels of cytotoxic activity shortly after grafting prior to graft nephrectomy. Acute irreversible graft rejection was observed within 7, 12, and 15 days, respectively, this "reversed conversion" of CML-NR pretransplant to CML-R posttransplant might appear to be an indication for poor graft survival.

DISCUSSION

In vitro donor specific cell-mediated lympholysis nonresponsiveness exhibited by cells from recipients with well-functioning kidney allografts is a generally observed association (1-7). All studies except one (6) were carried out with a short-time follow-up. Notwithstanding these observations, we assessed to what extent this apparent in vitro reflection of acquired tolerance can be explained by selection for patients who get the chance to develop CML-NR. The availability of over 350 CML studies in 124 renal transplant patients with a follow-up period of approximately 5 years enabled us to investigate whether the CML-NR predicts graft survival during follow-up.

By assessing the correlation of the outcome of the CML tests and graft survival for different time intervals, it appeared that only the posttransplant period between 2 weeks and 6 months showed a (marginally) significant correlation ($P = 0.05$) between CML-NR and graft survival on one hand, and CML-R and graft lost on the other hand; the data failed however to reach statistical significance when analyzing only the acute rejections ($P = 0.085$, Table 3). Nevertheless, these trends could be of interest in view of the clinical observation that most acute rejection episodes occur within this time period (24). The results obtained with the 69 patients serially monitored from pre- to posttransplantation demonstrated that pretransplant donor-directed CTL activity is not a contraindication for transplantation and does not preclude the development of CML-NR posttransplantation. Conversely, pretransplant CML-NR converting shortly after transplant to CML-R was in 3 out of 3 cases associated with acute rejection and graft loss.

It also appeared from our studies that several patients had a well-functioning graft while demonstrating in vitro donor-specific CML activity, likewise a number of patients rejected their graft despite their CML-NR status. The situation in which patients with a well-functioning graft show in vitro donor-specific CML activity is compatible with the experimental animal studies in which specific cytotoxic T cells were found in nonrejected rat kidneys (25). The cellular mechanism of graft destruction is probably composed of phenotypically and functionally different T cell subsets directed against MHC class I and class II transplantation antigens, as was recently reported by Miceli et al (26) and by Bonneville et al (27). These observations are consistent with the findings that cytotoxic T effector cells are not the sole mediators of graft rejection as was first observed by Loveland et al (28). Although these and numerous experimental animal studies that followed (29), which were designed to unravel the effector mechanisms of allograft rejection, concern skin grafting in nonimmune suppressed animals, they provide important insights into the complexity of immunologic responses in human allografting. Effector mechanisms operating in vascularized human allograft rejection are also dependent on the genetic constitution of the recipient (30), and antigen expression status of the graft (31),

TABLE 2 Results of log rank analyses for the relationship between CML status and graft survival^a in some different time trajectories

Period in days	CML NR ^b		CML R		P value	Estimated relative risk (95% confidence bounds)
	N obs	N exp	N obs	N exp		
0-2421	19	22.92	23	17.84	0.13	1.25 (0.94-1.65)
0-183	6	10.08	18	13.91	0.09	1.69 (0.92-3.09)
15-183	4	8.08	14	9.92	0.05	2.04 (0.99-4.17)
34-183	4	6.46	10	7.54	0.19	1.62 (0.79-3.33)
365-2421	13	13.5	5	4.48	0.78	1.04 (0.79-1.37)

^a Endpoint is graft failure from all causes (death of a patient always being considered as graft failure).

^b See legends Table 1
Whole period

TABLE 3 Results of log rank analyses for the relationship between CML status and graft survival^a in some different time trajectories

Period in days	CML NR ^b		CML R		P value	Estimated relative risk (95% confidence bounds)
	N obs	N exp	N obs	N exp		
0-2073	12	15.40	17	13.60	0.18	1.29 (0.89-1.86)
0-183	5	7.95	14	11.05	0.17	1.60 (0.82-3.11)
15-183	3	6.19	11	7.81	0.085	2.08 (0.90-4.73)
31-183	3	4.57	7	5.43	0.32	1.53 (0.66-3.53)
365-2073	7	7.45	3	2.55	0.75	1.06 (0.73-1.55)

^a Endpoint is acute rejection (observations of patients who die with functioning grafts are censored).

See legends Table 1
Whole period

32). Furthermore, pretransplant blood transfusions are known to have a beneficial effect on kidney graft prognosis (33, 34). We reported earlier that CML-NR occurs with the same frequency in both single and multitransfused patients (4). Recently, we designed a prospective study to analyze blood-transfusion-induced changes in cellular and humoral immunity (35). Preliminary results of this ongoing study show that if the transfusion donor and recipient are mismatched for both HLA-DR antigens, the recipient is immunized resulting in a transient increase of cytotoxic activity, after an HLA DR shared transfusion, the *in vitro* test remained unchanged or slightly decreased. However in none of cases studied so far, has it led to CML-NR.

A method of monitoring the graft certainly is to perform graft biopsies (36) or fine-needle aspirates (37). The *in vitro* monitoring by measurement of the donor-directed cytotoxic T cell activity as described herein may provide further information regarding acute rejection. Although predictions regarding the likelihood of the occurrence of posttransplant CML-NR must be made with great caution, posttransplant monitoring using the CML assay may at least for the first 6 months after transplantation be helpful for the interpretation of clinical events and possibly for the design of therapeutic strategies.

Acknowledgments The authors thank Prof Dr R A P Koene, Dr J M Wilmink, and Dr W Weimar, whose patients were included in the investigations, and Mrs I Cuiel and Mrs E van der Wilk for editing the manuscript.

REFERENCES

- 1 Wonigeit K, Pichlmayr R. Specific defect in the capability to generate cytotoxic effector cells *in vitro* after organ transplantation in man. *Dial Transplant* 1977, 6: 58.
- 2 Thomas J, Thomas F, Mendez-Picon G, Lee H. Immunological monitoring of long surviving renal transplant recipients. *Surgery* 1977, 81: 125.
- 3 Liburd EM, Pazderka V, Kovithavongs T, Dossetor JB. Evidence for suppressor cells and reduced cell induction by the donor in transplant patients. *Transplant Proc* 1978, 10: 557.
- 4 Goulmy E, Persijn G, Blokland E, van Rood JJ. Cell mediated lympholysis studies in renal allograft recipients. *Transplantation* 1981, 31: 210.
- 5 Pfeffer PF, Hirschberg H, Thorsby E. Donor specific decreased primary and secondary cell mediated immune responses in patients with well functioning grafts. *Transplant Proc* 1981, 13: 1604.
- 6 Harmon WE, Parkman R, Lavin PT, et al. Comparison of cell mediated lympholysis and mixed lymphocyte culture in the immunologic evaluation for renal transplantation. *J Immunol* 1982, 129: 1573.
- 7 Ono E, Dohi K, Fukuda Y, et al. Donor specific CML unresponsiveness in successful living related kidney transplant recipients. *Transplant Proc* 1983, 15: 814.
- 8 Goulmy E, Blokland E, Persijn G, Paul LC, Wilmink JM, van Rood JJ. HLA regulates postrenal transplant CML non reactivity. *J Immunol* 1985, 135: 3082.
- 9 Thomas J, Johns C, Hoffmann S, Thomas F, Lee HM. Suppressor cells inhibiting the generation of cytotoxic T lymphocytes in long term human transplant. *Proc Dialysis Transplant Forum* 1978, 7: 87.
- 10 Dossetor JB, Liburd EM, Kovithavongs T, Pazderka V. Suppressor cells and loss of *in vitro* killer cell inductiveness to donor cells in the peripheral blood lymphocytes of allograft recipients. *Transplant Proc* 1979, 11: 1260.
- 11 Charpentier B, Lang Ph, Martin B, Fries D. Specific recipient donor unresponsiveness mediated by a suppressor cell system in human kidney allograft tolerance. *Transplantation* 1982, 33: 470.
- 12 Seki Y, Sakagami K, Takeuchi H, et al. Evidence for donor specific cell-mediated lympholysis unresponsiveness and suppressor cells in well functioning kidney transplants. *Transplant Proc* 1983, 15: 2131.
- 13 Cohen DJ, Lee HM, Mohanakumar T. Mechanisms of CML by porespensiveness in long term renal allograft recipients. *Hum Immunol* 1985, 14: 279.
- 14 Wramner L, Olansson M, Soderstrom T, Lindholm L, Rijdberg L, Brynger H. Evidence of donor specific cellular suppressor activity in donor specific cell mediated lympholysis unresponsiveness in renal transplant patients. *Transplantation* 1987, 44: 390.
- 15 Miyajima T, Higuchi R, Kashiwabara H, Yokoyama T, Fujimoto S. Anti idiotypic antibodies in a patient with a functional renal graft. *Nature* 1980, 283: 306.
- 16 Singal DP, Joseph S. Role of blood transfusions on the induction of antibodies against recognition sites on T lymphocytes in renal transplant patients. *Hum Immunol* 1982, 4: 93.
- 17 Shohat B, Cytron S, Boner C, Danon YL. Blocking antibodies and T cell subsets in longterm survivors of renal allografts. *Transplantation* 1987, 44: 34.
- 18 Lancaster F, Chui YL, Batchelor JR. Anti idiotypic T cells suppress rejection of renal allografts in rats. *Nature* 1985, 315: 336.
- 19 Rammensee HG, Fink PJ, Bevan MJ. Functional clonal deletion of class I-specific cytotoxic T lymphocytes by veto cells that express antigen. *J Immunol* 1984, 133: 2390.
- 20 Pfeffer PF, Thorsby E, Hirschberg H. Cell mediated cytotoxicity toward the donor in patients with well functioning kidney grafts: possible mechanism of specially reduced cytotoxic response. *Transplantation* 1983, 35: 546.
- 21 Herzog WR, Zanker B, Irschick E, et al. Selective reduction of donor specific cytotoxic T lymphocyte precursors in patients with a well functioning kidney allograft. *Transplantation* 1987, 43: 384.
- 22 Goulmy E. HLA A, B restriction of cytotoxic T cells. In Ferrone S, Solheim BG, eds. *HLA typing methodology and clinical relevance*. West Palm Beach: CRC Press, 1982: 2: 105.
- 23 Mantel N, Byar DP. Evaluation of response time data involving transient states: an illustration using heart transplant data. *J Am Stat Assoc* 1974, 69: 81.
- 24 Persijn GG, Cohen B, Lansbergen Q, et al. Effect of HLA A and B matching on survival of grafts and recipients after renal transplantation. *N Eng J Med* 1982, 309: 905.
- 25 Dallman MJ, Wood KJ, Morris PJ. Specific cytotoxic T cells are found in the non rejected kidneys of blood transfused rat. *J Exp Med* 1987, 165: 566.
- 26 Michel MC, Barry TS, Finu OJ. Human allograft derived T cell lines donor class I and class II directed cytotoxicity and repertoire stability in sequential biopsies. *Hum Immunol* 1988, 22: 185.
- 27 Bonneville M, Moreau JF, Blokland E, et al. T lymphocyte cloning from rejected human kidney allograft. II. Recognition repertoire of alloreactive T cell clones. *J Immunol* 1988, 141: 4187.
- 28 Loveland BE, Hogarth PM, Ceredig RH, McKenzie IFC. Cells mediating graft rejection on the mouse. I. Lyt 1 cells mediate skin graft rejection. *J Exp Med* 1981, 153: 1044.
- 29 Mason DW, Morris PJ. Effector mechanism in allograft rejection. *Annu Rev Immunol* 1986, 4: 119.
- 30 Hendriks GFJ, Schreuder GMTh, Claas FHJ, et al. HLA DRw6 and renal allograft rejection. *Br Med J* 1983, 286: 85.
- 31 Paul LC, Van Es LA, Van Rood JJ, van Leeuwen A, Brutel de La Riviere G, de Graeff J. Antibodies directed against antigens on the endothelium of peritubular capillaries in patients with rejecting renal allografts. *Transplantation* 1979, 27: 175.
- 32 Hall BM, Duggin GG, Bishop G, Horvath JS, Fuller DJ. Increased

- expression of HLA-DR antigens on renal tubular cells in renal transplants: relevances to the rejection response. *Lancet* 1984, 2: 247
- 33 Opelz G, Sengar DPS, Mickey MR, Terasaki PI. Effect of blood transfusions on subsequent kidney transplantation. *Transplant Proc* 1973, 5: 253
- 34 Persijn GG, Cohen B, Lansbergen Q, Van Rood JJ. Retrospective and prospective studies on the effect of blood transfusions in renal transplantation in The Netherlands. *Transplantation* 1979, 28: 396.
- 35 Lagaay EL, Termijtelen A, Goulmy E, Van Rood JJ. The presence of "self" MHC class II (HLA-DR) antigens determines whether blood transfusions immune or suppress. *J Cell Biochem [Suppl]* 1989, 13A: 200
- 36 McWhinnie DL, Thompson JF, Taylor HM, et al. Leucocyte infiltration patterns in renal allografts assessed by immunoperoxidase staining of 245 sequential biopsies. *Transplant Proc* 1985, 17: 560
- 37 Hayry P, von Willebrand F. Transplant aspiration cytology. *Transplantation* 1984, 38: 7

Received 15 December 1988

Accepted 2 May 1989

0041-1337/89/4804-0563\$02.00/0

TRANSPLANTATION

Copyright © 1989 by Williams & Wilkins

Vol 48, 563-568, No 4, October 1989

Printed in U.S.A.

MECHANISMS OF INSULIN RESISTANCE AFTER KIDNEY TRANSPLANTATION¹

AGNETA EKSTRAND, JUHANI AHONEN, CAROLA GRONHAGEN-RISKA, AND LEIF GROOP²

Fourth Department of Medicine and Fourth Department of Surgery, Helsinki University Hospital, Helsinki, Finland

In order to study the effect of corticosteroids on energy metabolism in immunosuppressed patients after kidney transplantation, we have examined glucose utilization, energy expenditure, and lean body mass in 10 kidney-transplanted patients receiving steroids (methylprednisolone 8.2 ± 1.5 mg/day) and in 10 healthy age- and weight-matched control subjects. Glucose utilization was measured during euglycemic insulin clamp in combination with indirect calorimetry and infusion of [³H-3]-glucose, while β -cell function was measured during a hyperglycemic clamp. The kidney-transplanted patients were resistant to the glucoregulatory effect of insulin, as demonstrated by a 25% reduction in total glucose disposal compared to control subjects. This defect was almost completely accounted for by a defect in storage of glucose as glycogen (3.3 ± 0.5 vs. 5.0 ± 0.5 mg/kgLBM-min; $P < 0.05$). The reduction in nonoxidative glucose disposal was associated with reduced lean body mass and incapacity to release energy as heat after infusion of insulin, i.e. thermogenic defect. In contrast, oxidation of glucose and lipids was not influenced by steroid therapy. Furthermore, suppression of hepatic glucose production was normal, and insulin secretion was normally enhanced in relation to the degree of insulin resistance in the steroid-treated patients. In conclusion, steroid-induced insulin resistance in kidney-transplanted patients is due to alterations in the nonoxidative pathway of glucose metabolism. These findings raise the question

of whether steroid therapy directly influences glycogen synthase in man.

Despite the introduction of cyclosporine and other new drugs, corticosteroids have been the cornerstone in immunosuppressive therapy after kidney transplantation. Steroids have been known to induce insulin resistance in experimental animals (1, 2) and in man (3-5). Several mechanisms have been suggested to explain the steroid-induced insulin resistance, e.g. decreased insulin receptor number and affinity (6, 7), impaired peripheral glucose uptake (primarily in muscle) (8), impaired suppression of endogenous glucose production (9), and activation of the glucose/fatty acid (FFA) cycle initially proposed by Randle et al. (10). The glucose/FFA cycle is based upon the concept of substrate competition, i.e. increased utilization of FFA leads to decreased utilization of glucose and vice versa. In addition to effects on glucose metabolism, steroids are known to induce protein catabolism (11). The clinical consequence is wasting of muscle tissue. The majority of glucose after a meal is taken up by muscle tissue (12, 13), where it is metabolized by either of two pathways: oxidation to carbon dioxide and water, or storage as glycogen. Total body glucose metabolism has been shown to correlate with the muscle mass (14), but it is not known whether insulin resistance correlates with muscle wasting during chronic steroid therapy.

The clinical endpoint of insulin resistance is diabetes mellitus. The incidence of secondary diabetes after kidney transplantation has markedly increased over the past years (15, 16). There may be several explanations for this phenomenon: inclusion of older patients in transplantation programs and the use

¹ Abbreviations: FFA, glucose/fatty acid, HGP, hepatic glucose production

¹ This work was supported in part by the Finska Lakaresällskapet (Finnish Medical Society), Munuaissaatio (Kidney Foundation), the Sigrd Juselius Foundation, and the Farnos Science Foundation

² Address correspondence to Leif Groop, Fourth Department of Medicine, Helsinki University Hospital, Unioninkatu 38, SF-00170 Helsinki, Finland