CLINICAL RESEARCH

HLA-DRw6 and renal allograft rejection

G F J HENDRIKS, G M TH SCHREUDER, F H J CLAAS, J D'AMARO, G G PERSIJN, B COHEN, J J VAN ROOD

Abstract

HLA-DRw6-positive patients are "high responders" to certain renal allograft antigens. A study was therefore conducted of the outcome of 247 first renal allografts in 74 DRw6-positive and 173 DRw6-negative recipients. The effectiveness of matching for HLA-DR determinants in both groups was also analysed.

The one-year graft survival in DRw6-positive patients was 59% as compared with 75% in DRw6-negative recipients ($p=0.012$). A striking difference between the two groups was that HLA-DR matching significantly improved renal allograft survival only in the DRw6-positive patients. In those patients the one-year survival of HLA-DR-identical grafts was 95% as compared with only 38% for 2-DR mismatched grafts ($p=0.009$). In DRw6-negative patients only a slight beneficial effect of HLA-DR matching was observed (83% versus 72% at one year for the 0-DR and 2-DR mismatched grafts, respectively) ($p>0.05$).

These findings are clear evidence that DRw6-positive patients (about a quarter of the patients on the waiting list of Eurotransplant) should be given HLA-DR-identical kidney transplants only.

Introduction

A patient receiving dialysis is described as "immunised" or as a "responder" when HLA antibodies are detectable. Recently Baldwin *et al* observed that the formation of antibodies to the endothelial monocyte antigens in patients who rejected their first renal allograft occurred almost exclusively in recipients who were HLA-DRw6-positive.¹ In other words, DRw6-positive patients were "high responders" to these transplant antigens. Before transplantation no difference in the degree of immunisation caused by blood transfusions or pregnancies was observed between the DRw6-positive and DRw6-negative patients. After transplantation, however, a significant increase in responsiveness was observed only in the group of DRw6-positive patients who rejected their grafts. This response was mainly directed against antigens present on B cells and monocytes (Hendriks, paper in preparation). We have, therefore analysed the one-year graft survival and effectiveness of matching for HLA-DR determinants in HLA-DRw6-positive and DRw6-negative renal allograft recipients.

Subjects and methods

A total of 173 DRw6-negative and 74 DRw6-positive Dutch recipients of first renal allografts were studied. All received blood transfusions before transplantation. In order to analyse the influence of matching for HLA-DR, we selected only those donor-recipient pairs who were reported to have two HLA-DR specificities. This was to avoid the possibility of underestimating the number of mismatches, especially in donors who were typed in centres which could not define all the DR antigens. The average number of HLA-AB mismatches was the same for both groups. Most of these donors and recipients were retyped in the National Reference Laboratory in Leiden. Typing for HLA-A, B, and DR antigens was performed with the Eurotransplant standardised serum set. HLA-A and B typings were performed by the standard NIH lymphocytotoxicity test.² HLA-DR typings were performed by the two-colour fluorescence method.³ Operationally monospecific sera were available for recognition of specificities DR1, 2, 3, 4, 5, 7, and w8. HLA-DRw9 could be typed only in the absence of DR4 and DR7. The definition of DRw6 was based on positive reactions of LB-E12, MT2, and DR2+DRw6 antisera (Schreuder *et al*, paper submitted for publication). All patients were followed up for at least one year. Graft survival times were calculated by the

Eurotransplant Foundation, University Hospital, Rijnsburgerweg 10, 2333 AA Leiden, the Netherlands

G F J HENDRIKS, MD, consultant in internal medicine
G G PERSIJN, MD, medical advisor
B COHEN, MEC, administrative director

Department of Immunohaematology, University Hospital, Rijnsburgerweg 10, 2333 AA Leiden, the Netherlands

G M TH SCHREUDER, AGR ENG, head of tissue typing laboratory
F H J CLAAS, BSC, head of screening laboratory
J D'AMARO, MD, PHD, research associate
J J VAN ROOD, MD, PHD, professor and head of department of immunohaematology

actuarial life-table method. The significance of differences between the different groups of patients was tested by a χ^2 test derived from a log rank analysis.⁴

Results

The one-year graft survival in the DRw6-negative patients was 75% (130 cases) and in the DRw6-positive patients 59% (44 cases) (fig 1; $p=0.012$). Analysis of the results at three, six, and 12 months showed that the highly significant difference between the two groups was caused by a large excess of failures in the DRw6-positive group within the first three months after transplantation. After that period there were no significant differences (table).

Log rank analysis of renal allograft survival in DRw6-negative and DRw6-positive recipients. Results expressed as numbers of observed and expected failures

Months	DRw6-negative		DRw6-positive		χ^2	p
	Observed	Expected	Observed	Expected		
3	21	33.62	27	14.38	15.83	0.00023
6	15	12.98	2	4.02	1.33	0.247
12	7	6.02	1	9.98	0.64	0.430
Overall	43	52.62	30	20.38	6.30	0.012

In the group of DRw6-negative patients only a slight beneficial effect of HLA-DR matching was observed (fig 2). The difference in graft survival between the 0-DR and 2-DR mismatched groups was 11% at one year ($p>0.05$). In the DRw6-positive patients, however, a highly significant effect of HLA-DR matching was observed (overall $p=0.009$) (fig 3). In the HLA-DR-identical group of 19 recipients only one graft failure was observed, while of the 26 patients who received a 2-DR mismatched graft 15 had lost their graft by irreversible rejection within three months after transplantation.

Discussion

There was a clear-cut significant difference in the one-year graft survival between the DRw6-negative and DRw6-positive recipients in this series. The inferior outcome in the DRw6-positive group was mainly caused by an excess of irreversible

FIG 1—Actuarial survival curve of 247 first renal allografts in relation to presence or absence of DRw6 (overall $p=0.012$). Numbers on curves are numbers of patients at risk.

FIG 2—Actuarial survival curve of 173 first renal allografts in DRw6-negative recipients in relation to number of HLA-DR mismatches.

FIG 3—Actuarial survival curve of 74 first renal allografts in DRw6-positive recipients in relation to number of HLA-DR mismatches (overall $p=0.009$).

rejections during the first three months after transplantation (table). This finding is consistent with the observation that HLA-DRw6-positive kidney transplant recipients are apparently "high responders" to endothelial monocyte antigens.¹ The increase in reactivity against HLA-DR and monocyte-specific antigens may be triggered by the renal allograft. That recipients lacking the DRw6 gene did not show this kind of response after transplantectomy may explain why matching for the HLA-DR antigens did not significantly improve kidney graft survival in this group. In recipients who have the DRw6 gene, however, a strong humoral response may be observed as soon as an HLA-DR mismatched kidney is transplanted. In this series almost 60% of the 2-DR mismatched grafts were lost by irreversible rejection within three months after transplantation. We have reported elsewhere that mismatching for HLA-A and B antigens may potentiate the detrimental effect of HLA-DR mismatching in kidney graft survival.⁵ Graft survival in DRw6-positive patients may be enhanced by optimal DR matching. In

such cases a strong immune response does not appear to occur. From our data we conclude that DRw6 may represent an important marker for an immune response gene.

HLA-DRw6 specificity is difficult to define.⁶ In the white population of Holland there are two subgroups of HLA-DRw6 related to the cellularly defined specificities HLA-Dw6 and HLA-Dw9, respectively (Schreuder *et al*, paper submitted for publication). In this study we could not distinguish these two DRw6 subgroups serologically. Whether one or both of these subgroups influence the immune response in patients with transplants remains to be studied. Our data suggest that the serological definition of DRw6 as used for this study may be considered as a homogeneous specificity. Possibly DRw6 specificity represents a "blank" on the DR locus, containing only those determinants which are recognised by LB-E12 (MB1, MT1), DR2+DRw6, and MT2 antisera. If so, this would explain why no good monospecific anti-DRw6 reagents have been found. Nevertheless, Dw6-positive and Dw9-positive homozygous typing cells give clear-cut typing responses with cells from DRw6-positive people.

The implications of our findings for clinical renal transplantation are clear. HLA-DRw6-positive patients (about a quarter of the patients on the waiting list of Eurotransplant) should be given only HLA-DR-identical kidney transplants. This policy is less urgent for HLA-DRw6-negative patients. This guideline must remain provisional, however, until we have studied the effect of HLA-A and B matching in these two groups. A larger series of patients will be needed for such an analysis.

This work was supported in part by the Dutch Organisation for

Health Research (TNO); the Dutch Foundation for Medical Research (FUNGO), which is subsidised by the Dutch Foundation for Advancement of Pure Research (ZWO); the J A Cohen Institute for Radiopathology and Radiation Protection (IRS); and the Dutch Kidney Foundation.

We could not have done this analysis without the generous support of the physicians collaborating in Eurotransplant. We thank the staff of the department of immunohaematology for technical help, and Margret Groenewegen for preparing the manuscript.

References

- ¹ Baldwin WM, Claas FHJ, Es van LA, *et al*. Renal graft rejection and the antigenic anatomy of human kidneys. *Thirteenth congress on transplantation and clinical immunology, Lyon, 1981*. Amsterdam: Excerpta Medica, 1981:140-6.
- ² Ray JG. NIH lymphocyte microcytotoxicity technique. *NIAID manual of tissue typing techniques*. (DHEW publication No (NIH) 77-545.) Washington, DC: DHEW, 1977:32.
- ³ Rood van JJ, Leeuwen van A, Ploem JS. Simultaneous detection of two cell populations by two-colour fluorescence and application to the recognition of B-cell determinants. *Nature* 1976;**262**:795-7.
- ⁴ Peto R, Pike MC, Armitage P, *et al*. Design and analysis of randomized clinical trials requiring prolonged observation of each patient. II. Analysis and examples. *Br J Cancer* 1977;**35**:1.
- ⁵ Rood van JJ, Persijn GG, Cohen B, Lansbergen Q, Schuurman RKB. Hierarchy of factors which influence kidney graft survival. *Dialysis and Transplantation* 1982;**11**:111-8.
- ⁶ Terasaki PI, Park MS, Bernoco D, Opelz G, Mickey MR. Overview of the 1980 international histocompatibility workshop. In: Terasaki PI, ed. *Histocompatibility testing 1980*. Los Angeles: UCLA Tissue Typing Laboratory, 1980:1-17.

(Accepted 15 October 1982)

Clinical aspects of delta infection

T MOESTRUP, B G HANSSON, A WIDELL, E NORDENFELT

Abstract

The clinical features of delta infection were analysed retrospectively in 191 hepatitis B surface antigen (HBsAg) carriers and 592 cases of acute hepatitis B seen over 11 years in the Swedish town of Malmö (population 250 000). With a few exceptions delta infections occurred exclusively in drug addicts.

In the chronic HBsAg-carriers the most common clinical manifestation was an episode of acute hepatitis, which in some individuals became severe with a pronounced rise in serum alanine aminotransferase activity for many months. During the period of delta infection the HBsAg titre was lowered and in three out of 26 cases the patient lost HBsAg altogether and developed hepatitis B surface antibodies (anti-HBs). In one patient the acute hepatitis due to delta infection was fulminant and fatal.

In patients with acute hepatitis B the clinical picture did not distinguish between those with and without simultaneous delta infection. The frequency with which acute hepatitis B was succeeded by a chronic carrier state was the same whether or not the patient was infected simultaneously with the delta agent.

The discovery of the delta agent has improved understanding of the natural history of chronic hepatitis B infection in drug addicts. Thus, instances of acute hepatitis in a chronic carrier, previously termed hepatitis non-A, non-B, may actually be episodes of delta infection.

Introduction

The delta antigen/antibody system was first described by Rizzetto and coworkers¹ in Italy in 1977 and has later been associated with a transmissible hepatitis agent, the delta agent, which can cause infection only when the hepatitis B virus genome is present. The delta agent is found all over the world.² It was first discovered in southern Italy, where it is endemic and associated with serious liver damage,¹ while in Northern Italy, as well as elsewhere in the world, it is prevalent among drug addicts (and haemophiliacs).²

Infectivity studies have shown that chimpanzees are susceptible to delta infection.³ The inocula contained both hepatitis B virus and delta agent. In animals without previous markers of hepatitis B a self-limiting acute hepatitis developed with markers of both hepatitis B virus and delta infection, the

Department of Infectious Diseases, University of Lund, Malmö General Hospital, S-214 01 Malmö, Sweden

T MOESTRUP, MD, physician

Section of Clinical Virology, Department of Medical Microbiology, University of Lund, Malmö General Hospital, S-214 01 Malmö, Sweden

B G HANSSON, PHD, microbiologist

A WIDELL, MD, research fellow

E NORDENFELT, MD, associate professor