CLINICAL RESEARCH

HLA-DRw6 as a risk factor for active cytomegalovirus but not for herpes simplex virus infection after renal allograft transplantation

H W ROENHORST, A M TEGZESS, J M BEELEN, J M MIDDELDORP, T H THE

Abstract

To study genetically determined susceptibility to cytomegalovirus and herpes simplex virus infections in patients given renal transplants a prospective study was performed of 68 consecutive patients receiving their first cadaveric kidney allograft. The recipients positive for HLA-DRw6 showed a significantly increased incidence of active cytomegalovirus infection as early as the 10th week after transplantation ($p<0.05$). No relation with other human leucocyte antigens was found, nor did a correlation exist between HLA typing and the incidence of herpes simplex virus infections.

Furthermore, recipients positive for HLA-DRw6 with secondary cytomegalovirus infections excreted infectious virus more often ($p<0.01$) and showed more clinical symptoms ($p<0.01$) than a comparable group of recipients negative for HLA-DRw6. These observations may have practical implications for the treatment of patients who have had renal transplant operations.

Introduction

Herpes virus infections, in particular cytomegalovirus infections, cause considerable morbidity and mortality after renal transplantation.[1-4] Major risk factors for cytomegalovirus infections after operations are: the amount of immunosuppressive treatment, the degree of HLA matching of donors and recipients, and the virus specific serological state of the recipient before transplantation.[1,3,5,6] The HLA system influences the predisposition for several diseases and infections.[7,8]

Little is known of the relation between human leucocyte antigens and cytomegalovirus infections in man, although one report suggested that the humoral immune response induced by active cytomegalovirus infection in recipients of transplanted kidneys is associated with determinants of HLA-DR.[9] This prospective study of patients receiving their first renal allograft examined the existence of an association between the distinct HLA types and the occurrence of cytomegalovirus and herpes simplex virus infections after transplantation.

Patients and methods

PATIENTS, IMMUNOSUPPRESSIVE REGIMEN, AND REJECTION OF GRAFTS

Between January 1981 and July 1982, 68 patients (39 men, 29 women) with chronic renal failure after haemodialysis treatment received their first cadaveric kidney allograft at our transplantation centre. All the patients had received one or more blood transfusions containing leucocytes before grafting. Characteristics of the recipient population were as follows: median age 37 (range 15-64) years; median duration of dialysis treatment 17 (range 0-120) months; 18 patients were negative for cytomegalovirus antibodies (titre <40 with enzyme linked immunosorbent assay) and 50 positive; 18 patients were negative for herpes simplex virus antibodies (titre <8 with a complement fixation method) and 50 positive; and phenotyping was HLA-DR1 (18 cases), HLA-DR2 (20), HLA-DR3 (20), HLA-DR4 (22), HLA-DR5 (18), HLA-DR6 (18), HLA-DR7 (8), HLA-DR8 (3), HLA-DR9 (0), and HLA-DR10 (0). The immunosuppressive regimen consisted of azathioprine 1·5 mg/kg/day and prednisolone at an initial dose of 60 mg/day down to 20 mg/day in two weeks. Maintenance dose of steroids was 10 mg/day after the sixth month. The patients were followed up for one year after transplantation.

Rejection episodes were diagnosed by physical examination and laboratory investigation, with a rise in serum creatinine concentration of at least 50 μmol/l (0·57 mg/100 ml) persisting more than three days and a fall in creatinine clearance and urinary excretion of sodium. Other causes of impaired renal function were excluded by renal scintigraphy, intravenous pyelography, ultrasonography, or biopsy of allografts. Rejection episodes were treated with 200 mg oral prednisolone, which was gradually reduced over three weeks to the initial dose before the rejection episode. No antilymphocyte globulin was given.

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OCCURRENCE OF VIRAL INFECTIONS

This was documented by samples from serum, urine, and saliva taken serially shortly before and at regular intervals (weekly in the first two months and thereafter at least at weeks 10, 12, 16, 26, and 52) after transplantation. Clinical history, especially directed at signs and symptoms of cytomegalovirus infections—that is, fever, arthralgia, coughing, rash, jaundice, and chest and back pain—and laboratory data such as leucocyte and platelet counts, transaminase activity, and concentrations of bilirubin, serum and urine electrolytes, and creatinine, were recorded. In case of a return to hospital because of active cytomegalovirus infection or other causes the initial follow up scheme mentioned above was started again. In case of active cytomegalovirus infection the immunosuppressive treatment was lowered temporarily.

SEROLOGICAL METHODS

Complement fixation with antigens of cytomegalovirus (AD 169 strain) and herpes simplex virus (kos strain) extracted by glycine were performed with a microtitre technique.[10] The antigens were commercially obtained (Flow Laboratories, USA). In addition, antibodies of the IgG class specific to cytomegalovirus were detected with an enzyme linked immunosorbent assay.[11] Titres <8 with the complement fixation method and <40 with the enzyme linked immunosorbent assay were considered seronegative.

Health.[12] The two colour fluorescence method was used for typing 10 HLA-DR specificities.[13]

Actuarial tables were used to compare the course and incidence of active viral infections in different groups of patients. Statistical analyses were done with a log rank test,[14] Wilcoxon's rank sum test, χ^2 tests with continuity correction, and Fisher's exact test. p Values $\leq 0{\cdot}05$ were considered significant.

Results

Fourteen of the 68 patients had an irreversible acute rejection of the allograft within the first two weeks. They were returned to maintenance haemodialysis treatment, and the immunosuppressive treatment was stopped. None of these 14 patients (cytomegalovirus seronegative (2 cases) and seropositive (12)) had evidence of active cytomegalovirus infection before or after rejection. In the course of the first year after transplantation 38 of the remaining 54 patients showed significant rises in antibody titres to cytomegalovirus. Five had primary and 33 secondary infection. Twelve patients had one or more episodes of excreting cytomegalovirus, whereas 21 showed signs of clinical symptomatic cytomegalovirus infections (table I).

In an attempt to establish an association with active cytomegalovirus infection the HLA frequency was analysed in the 54 patients. Log rank analysis with trend correction for the cytomegalovirus state in patients before transplantation showed a significantly higher incidence of active

TABLE I—*Characteristics of recipients with active cytomegalovirus infections*

Case No*	Age (years)	Duration of dialysis treatment (months)	Type of HLA-DR	No of mismatches for HLA-DR	First days of anti-rejection treatment	Cytomegalovirus infections		
						Week No†	Excretion§	Signs and symptoms‖
1	36	6·5	2, 6	1	5	4	111, 372	A
2	23	18	2, 6		1	5		A, B, F, H
3	38	25	4, 6	1	3, 174	5	165, 201, 266	B
4	19	11	1, 4		5, 13	5		
5	36	19	3, 4			5		
6	28	0	4, 7		7, 43	6		A, H
7	25	52	1		21	6		
8	32	16·5	1, 7	1	30	6		
9	31	0	3, 4	1	4, 160	6	48, 62, 69, 83, 154	
10	31	5·5	5, 6		2, 41	6	41, 46, 69	G
11	42	21·5	4, 6	1		6	44, 71, 78	A, B, F, H, R
12	22	5	3, 6	1	5	7	51, 71, 85, 252	A, B, F, H, R
13	54	29	4, 5	1	7, 51, 105 8	8		
14	33	56	4, 5		10, 44, 96	7		
15	18	6	2, 3		8, 35	7		B
16	28	22	2, 6		7	7	55	R
17	42	17·5	6, 7		10	7		B
18	21	6	1, 6	1	9	7		A, R
19	39	17·5	1, 5		3, 35	7	111	
20	33	28	3, 6	1		8		
21	33	48	2, 5	1	9, 46	8		
22	56	34	1, 4	1		8		
23	37	37	5, 6		7, 66	9	78, 143, 199	R
24	40	11·5	2, 6		8, 39	9		A, F
25	53	22	5		4	9		F
26	21	9	1, 3		8, 35	8		B
27	29	16	3	1	16	9		
28	20	13	3, 4		35	11		A, F, R
29	42	33	3		2, 35	12		
30	52	120	1, 4		9	8		
31	54	12	2, 5		265	>26		R
32	51	38	2, 6			>26		
33	49	8	3, 7		256	>26		
34	24	6·5	1, 3		2, 34	6	48, 76, 116, 123	A, F, G, P
35	47	16·5	1, 6		12, 46	6		A, B, F, G, H, R
36	23	30·5	4, 6		10	7	73, 108, 164	A, B, F, H, R
37	48	46·5	1, 2		11	7	64, 90	B, F, G, H, R
38	40	43	1, 3		10	>26		

*Cases 1-33 had secondary cytomegalovirus infection; cases 34-38 had primary cytomegalovirus infection.
†Weeks after transplantation when seroconversion or significant increase of titres of cytomegalovirus antibodies was first shown.
§Days of isolation of infectious virus from urine or saliva, or both.
‖A = arthralgia; B = abnormalities of blood: leucopenia ($<3\times10^9$/l) or thrombocytopenia ($<125\times10^9$/l), or both; F = fever (>38°C); G = gastrointestinal complications such as pneumatosis intestinalis, duodenal ulcer, or perforation of the sigmoid; H = hepatitis (serum alanine aminotransferase activity >50 U/l); P = cytomegalovirus pneumonitis; and R = dysfunction of renal allograft at time of infection.

VIRUS ISOLATION

Specimens of freshly voided urine and mouth swabs were inoculated into tissue culture trays containing human embryonal lung fibroblasts or kos cells. All cultures were monitored regularly for the development of a cytopathological effect. Human embryonal lung fibroblasts were cultured for at least six weeks and kos cells for three weeks. Positive results were confirmed with coverslip cultures stained with haematoxylin and eosin. Herpes simplex virus isolates were typed by determining the size of the pock on egg chorioallantoic membranes.

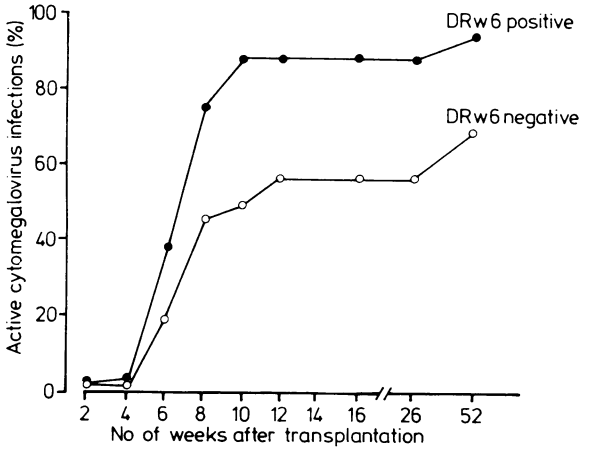
Criteria of active cytomegalovirus or herpes simplex virus infection were, in case of primary infection, a seroconversion and, in case of secondary infection, significant (fourfold or more) rises in antibody titres or isolation of virus from urine and saliva, or both. Tissue typing of HLA-A, B was done by the standard microlymphocytotoxicity assay of the National Institutes of

cytomegalovirus infection in the patients positive for HLA-DRw6 (figure, $\chi^2=4{\cdot}14$, $p<0{\cdot}05$). After one year of follow up 15 out of the 16 recipients positive for DRw6 compared with 23 out of the 38 recipients negative for DRw6 experienced an active infection. Already at the 10th week after renal transplantation the differences were significant (figure, $\chi^2=4{\cdot}62$, $p<0{\cdot}05$). Eight of 15 recipients positive for DRw6 compared with four of 23 recipients negative for DRw6 excreted cytomegalovirus at least once in the year after transplantation ($\chi^2=3{\cdot}69$, $p<0{\cdot}01$). In the group with secondary cytomegalovirus infections the excretion occurred more often in recipients positive for DRw6 (table II, $\chi^2=5{\cdot}24$, $p<0{\cdot}01$ with Fisher's exact test).

Within the total group of recipients with primary and secondary cytomegalovirus infection clinical symptoms occurred more often in those positive for DRw6. Thirteen out of 15 recipients positive for DRw6 compared with eight out of 23 negative for DRw6 were clinically ill

($\chi^2=8{\cdot}10$, $p<0{\cdot}005$). The same difference was noticed in the group with secondary cytomegalovirus infections: the recipients positive for DRw6 were clinically ill significantly more often in association with their active cytomegalovirus infection than were those negative for DRw6 ($\chi^2=7{\cdot}39$, $p<0{\cdot}01$, table II).

No association between active infection and other HLA-A, HLA-B, or HLA-DR antigens was found. Between recipients positive and negative for HLA-DRw6 with active cytomegalovirus infections no significant differences were found with regard to age, duration of haemodialysis treatment before transplantation, number of blood transfusions, and serological state of cytomegalovirus before transplantation. Nor did the amount of immunosuppressive treatment or the incidence and times of early



Incidence of active cytomegalovirus infections in patients positive (n=16) and negative (n=38) for DRw6 in first year after renal allograft transplantation. After 10th week: $p<0{\cdot}05$ (statistical analysis by log rank test with trend correction for cytomegalovirus serological state before transplantation).

TABLE II—*Secondary cytomegalovirus infections: increased incidence of excretion and symptoms in recipients positive for HLA-DRw6*

	Excretion of cytomegalovirus		Symptoms of cytomegalovirus	
	Absent	Present	Absent	Present
Positive for DRw6	6	7*	2	11*
Negative for DRw6	18	2	14	6

*$p<0{\cdot}01$.

and late episodes of rejection differ between these two groups (see table I). Thirty six patients had one or more episodes of excreting herpes simplex virus after transplantation, whereas 30 showed significant serological increases in titres of herpes simplex virus antibodies. No relation was found between HLA-A, HLA-B, or HLA-DR and the incidence of active herpes simplex virus infection after transplantation.

Discussion

This prospective study shows an association between the presence of HLA-DRw6 in the recipient population and an increased incidence of cytomegalovirus infections after transplantation in 68 patients who were followed up for one year after allografting of the kidney. As this association was not detectable for herpes simplex virus, another member of the herpes virus group, the relation with DRw6 seems to be specific to cytomegalovirus. Furthermore, recipients positive for DRw6 actively infected with cytomegalovirus experienced infections earlier after transplantation and showed clinical symptoms in association with these infections more often than did those negative for DRw6. This association holds especially for those patients positive for DRw6 experiencing a secondary cytomegalovirus infection after transplantation, who also excreted cytomegalovirus more often.

Other studies did not find an increased prevalence of HLA-DR determinants in patients with cytomegalovirus infections after transplantation.[9] The incidence of active infections (based on the same criteria) in that study population, however, was low (19%) compared with that in our group (56%) and with other reports.[15,16] The reasons for this low incidence of infection are perhaps related to differences in the techniques of detecting antibodies (immune adherence haemagglutination technique *v* enzyme linked immunosorbent assay) and a shorter follow up time. There are no major differences in immunosuppressive regimens. Like other investigators we could not find a relation with HLA-A or HLA-B.[17]

In the murine model reactivation to cytomegalovirus was triggered by allogeneic reactivity.[18] The reported state of high responders to renal allograft antigens in patients positive for HLA-DRw6 could therefore contribute to a higher incidence of cytomegalovirus infections in these recipients due to immunosuppressive treatment and increased allogeneic reactivity.[19,20] In this study, however, the number and time of occurrence of early reversible rejections were not different in recipient groups positive and negative for DRw6. Furthermore, both groups received the same amount of immunosuppressive treatment.

In murine experiments a genetically determined susceptibility or resistance to cytomegalovirus infection controlled by genes of the H_2 complex was found.[21-23] Studies in man showed an association of HLA-DR antigens with cellular immune responses to infectious agents.[7,24,25] The increased incidence of viral excretion and clinical illness in patients positive for DRw6 with secondary cytomegalovirus infection is therefore probably related to defective or suppressed host immune responses to cytomegalovirus antigens. It would be of interest to investigate whether recipients positive for DRw6 who are seropositive for cytomegalovirus already have disturbances in their general immunity or cytomegalovirus specific immunity, or both, before their transplantation. Sometimes it is difficult to discriminate between clinical symptoms caused by cytomegalovirus infections and those brought by rejection of allografts.[4,26] In conclusion, our study suggests that regular virological and serological monitoring for cytomegalovirus is especially indicated in patients positive for DRw6, as anti-rejection treatment given wrongly could lead to more severe sequels of cytomegalovirus infections. These sequels as well as that of rejection could explain the lower rate of survival after grafting in patients positive for DRw6, as reported elsewhere.[19,20]

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Lack of relation between glycosylated haemoglobin concentrations and number of daily insulin injections: cross sectional study in care of ambulatory diabetes

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Abstract

Diabetic treatment aims at achieving a normal blood glucose concentration as reflected by the glycosylated haemoglobin concentration. Intensive treatment by insulin pump or multiple insulin injections is thought to achieve this. In an unselected group of outpatient diabetics metabolic control was the same after one, two, three, or more injections, which suggests that the mode of treatment was optimal for each group.

Introduction

Evidence of a relation between the degree of glycaemic control and development of late complications in diabetes is increasing. Thus the aim of diabetic treatment is to achieve as normal blood glucose concentrations, reflected by glycosylated haemoglobin concentrations, as possible. Recent studies have shown that intensive treatment, by either insulin pump[1,2] or multiple insulin injections,[3] can achieve this goal. This study aimed at determining any relation between the degree of metabolic control and the number of insulin injections given a day in an unselected group of outpatient diabetics.

Patients, methods, and results

The study included 289 consecutive diabetics treated with insulin who were attending an outpatient clinic. The glycosylated haemoglobin concentration was determined by ion exchange chromatography with microcolumns (BIO-RAD).

The patients were divided into groups according to the number of insulin injections given a day: group A received one injection early in the morning; group B two a day, in the morning and at 4-5 pm; group C two a day, in the morning and at 9-10 pm; and group D three or more a day.

The table shows no difference in glycosylated haemoglobin concentrations between the four groups regardless of the number of insulin injections given a day. No correlation existed between glycosylated haemoglobin concentration and age, age at onset, duration of diabetes, amount of insulin given a day (IU/kg), or body mass index in any of the groups (data not shown). To ensure that the results were not influenced by the type of diabetes, the patients were also divided into groups according to age at onset of diabetes. Patients aged under 30 at onset were considered to have type I diabetes and those over 30 type II diabetes. The table shows no differences between the groups in either type I or type II diabetics. Furthermore, within each group the age at onset did not significantly influence the degree of metabolic control when expressed as glycosylated haemoglobin concentration.

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Mean (SEM) percentages of glycosylated haemoglobin concentrations in four groups of diabetics

Groups	All patients	Patients aged under 30 at onset	Patients aged over 30 at onset
A	7·8 (0·2) (n = 67)	8·1 (0·3) (n = 28)	7·6 (0·2) (n = 39)
B	8·3 (0·1) (n = 133)	8·3 (0·1) (n = 88)	8·1 (0·2) (n = 45)
C	8·3 (0·2) (n = 36)	8·2 (0·3) (n = 28)	8·5 (0·3) (n = 8)
D	8·2 (0·2) (n = 53)	8·3 (0·2) (n = 43)	7·5 (0·5) (n = 10)

Student's *t* test.

Discussion

Recently several studies have shown that intensified insulin treatment, by either multiple insulin injections[3] or subcutaneous insulin infusion systems,[1,2] gives normal or near normal glycosylated haemoglobin concentrations in selected groups of patients. In most studies, however, intensive engagement of the physicians might be as important a factor as the mode of treatment. Thus in everyday practice the question remains of whether metabolic control of diabetes improves with the number of insulin injections given a day in an unselected group of outpatient diabetics.

In this study, regardless of whether the patients received one, two, three, or more injections a day, the degree of metabolic control was the same. In our opinion this reflects the fact that the mode of treatment was optimal for each group. The study does not tell whether further improvement might have been achieved if patients given one or two injections a day had been given more intensive treatment. On the other hand, as the glycosylated haemoglobin concentration was the same in all four groups the number of insulin injections per se does not seem to be the most important factor to improve metabolic control. Other factors, such as intensive attention by the health care team and the patients' self control of blood glucose concentrations, might be just as important.[4,5] The conflicting results between this and other studies showing improved metabolic control with multiple insulin injections may show this.

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