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Association between rapidly progressive glomerulonephritis and the properdin factor BfF and different HLA-D region products

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Association between rapidly progressive glomerulonephritis and the properdin factor BfF and different HLA-D region products. Frequencies of the HLA antigens ABC, DR and MT, as well as of the properdin factor alleles were determined in 24 unrelated patients presenting with immune complex mediated idiopathic rapidly progressive glomerulonephritis (RPGN) type II. As in Goodpasture syndrome (RPGN type I with pulmonary hemorrhage), a significant association with the B-cell alloantigen HLA-DR 2 was demonstrated (relative risk for HLA-DR 2 positive individuals was 3.54; $P < 0.01$). In addition a marked increase of the HLA specificity MT 3 was shown, which is supposed to belong to an antigen system of a second HLA-D region locus. The highest relative risk of 14.67 ($P < 0.00001$), however, was calculated for all patients carrying the BfF phenotype. Increased numbers of patients positive for HLA-DR 2 and -MT 3, as well as BfF suggested immune response genes or disease-related mutations on different haplotypes responsible for a MHC (major histocompatibility complex) associated predisposition of RPGN type II.

Association entre la glomérulonéphrite rapidement progressive et le BfF du facteur de la properdine et les produits des différentes régions HLA-D. Les fréquences des antigènes HLA, ABC, DR et MT, ainsi que les allèles du facteur de la properdine ont été déterminées chez 24 malades non consanguins ayant une glomérulonéphrite rapidement progressive idiopathique médiée par des immunescomplexes (RPGN) de type II. Comme dans le syndrome de Goodpasture (RPGN de type I avec hémorragie pulmonaire), une association significative avec l'alloantigène cellulaire B HLA-DR 2 a été démontrée (le risque relatif pour des individus positifs à HLA-DR 2 était 3,54; $P < 0,01$). En plus, une augmentation marquée de la spécificité HLA-MT 3 a été montrée, laquelle est supposée appartenir à un système antigénique d'un second locus HLA-D. Le risque relatif le plus élevé, 14,67 ($P < 0,00001$), cependant, était calculé pour tous les malades porteurs du phénotype BfF. Les nombres augmentés de malades positifs pour HLA-DR 2 et-MT3, comme pour BfF suggéraient une réponse immune de gènes ou de mutations induites par la maladie sur différents haplotypes responsables d'une MHC (complexe principal d'histocompatibilité) associés à la prédisposition à une RPGN de type II.

Rapid progression to renal failure in some patients with nephritis of acute onset was first observed by Volhard and Fahr [1] in 1914 and later on described as a characteristic symptom of rapidly progressive glomerulonephritis (RPGN) [2]. This disease mainly affects adults of a mean age of 50 years [3]. It causes glomerular damage either as a primary (idiopathic) kidney disease or occurs in association with systemic, infectious, or drug allergic diseases [4].

Idiopathic RPGN is histologically characterized by extensive, obliterating crescent formations within the Bowman's space of the glomeruli. Three subsets of this disorder have been identified with immunostaining: Linear deposits of IgG and less often of C3 along the glomerular basement membrane (GBM) are demonstrated in idiopathic RPGN of type I (anti-GBM disease), whereas a granular staining pattern of IgG and/or IgM, as well as C3 is found mainly within the mesangial capillary wall of type II [3, 5]. The third form of idiopathic RPGN shows no apparent deposition of immunoglobulins [6].

Different immunological mechanisms are thought to underly this primary glomerular disorder, since patients with idiopathic RPGN of type I produce anti-GBM antibodies, whereas circulating immune complexes are detected in the second form of this disease. Recently, Goodpasture syndrome, which presents as an anti-GBM disease accompanied by pulmonary hemorrhages, has been reported to show a strong association with the antigen HLA-DR 2 of the human major histocompatibility complex (MHC) [7]. This observation suggests that genetic factors contribute to abnormal immune responsiveness and may be involved in the pathogenesis of this kidney disease.

In this study HLA phenotypes of patients with idiopathic RPGN of type II were determined to evaluate the influence of the MHC on a genetic predisposition of this disorder. Particularly the relationship between disease susceptibility and different HLA-D region products, as well as the HLA-linked properdin factor (Bf) alleles was investigated in view of presumed immune response genes mapping within the MHC system.

Methods

Patients. Twenty-four unrelated Caucasian patients with the clinical symptoms of idiopathic RPGN (type II) were investigated. The diagnosis of the immune complex-mediated idiopathic RPGN (type II) was verified by histological evaluation of renal biopsies in all patients. As the typical pathomorphological manifestation, crescent formations in all glomeruli were demon-

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strated in all kidney sections. In addition granular deposits of IgG, and/or IgM were observed in all kidney biopsy specimens analyzed either by immunofluorescence studies or immunoperoxidase stainings. In four patients IgA was also found in the glomeruli. Deposits of C3 were detected in 11 kidney biopsy specimens.

Ten patients were females with a mean age of 44.4 years (14 to 72 years) and 14 were males with a mean age of 47.6 years (22 to 76 years). None of these patients presented with pulmonary symptoms as a sign of an underlying Goodpasture syndrome. Anti-GBM antibodies were not detected in the sera of all patients. Secondary RPGN due to systemic lupus erythematosus was excluded by the lack of anti-DNA antibodies in the sera of all patients, as well as by the absence of further organ involvements. No clinical signs of Wegener granulomatosis or malignoma as another cause of secondary RPGN were detected by extensive physical examination of the patients. Furthermore, the clinical investigation of all patients did not reveal a history of drugs related to the onset of this disease. HLA antigens of all patients were investigated and compared with HLA phenotypes of a local control group of 125 normal European Caucasians.

HLA-ABC, -DR, and -MT tissue typing. HLA-ABC antigens were determined on peripheral blood lymphocytes according to the standard microlymphocytotoxicity assay of the National Institutes of Health [8] with locally well characterized anti-HLA antisera defining all known HLA-ABC specificities. HLA-DR and -MT typing was performed on B-lymphocytes in a modified microlymphocytotoxicity assay with prolonged incubation times for the antibody (1 hr) and the complement phase (2 hr) [9]. A local anti-HLA-DR, -MT antiserum set was used which included the best antisera of the 8th International Histocompatibility Workshop for the definition of the HLA-DR specificities 1 - 10 and of the HLA-MT antigens 1 - 3. B-lymphocytes were enriched from peripheral blood lymphocytes by filtration through nylon wool columns as previously described [9].

Properdin factor B (Bf) determination. The Bf alleles were determined by starch gel electrophoresis as reported previously [10].

Statistical analysis. The frequencies of the HLA-ABC, -DR, and -MT antigens as well as the Bf alleles were calculated in the patient group. They were compared with the HLA antigen frequencies of our local control group of 125 normal European Caucasians. Differences between patient and normal group were statistically evaluated by χ^2 analysis with Yates correction. The relative risk to develop idiopathic RPGN (type II) was calculated according to the method of Svejgaard and Ryder [11].

Results

HLA-ABC, -DR, -MT typing results given in Table 1 showed that the frequency of distinct HLA alleles in 24 investigated patients with idiopathic RPGN (type II) was increased compared with their incidence in a local population of 125 normal European Caucasians.

In particular, a statistically significant higher frequency of the antigen HLA-DR 2 was observed in the patient than in the control group (Table 2). Of the patients 62.5% were found to be positive for this antigen, whereas only 32.0% of the normal

Table 1. HLA-ABC, -DR, -MT antigens and Bf-phenotypes in 24 patients with idiopathic RPGN type II

Patient	HLA-phenotype A	B	Cw	DR	MT	Bf-phenotypes
1	2, 3	w44, w35	4, –	w6, 7	2, 3	FS
2	3, w24	18, w60	3, –	2, 6	1, 2	FS
3	1, 2	7, w62	1, 4	2, 4	1, 2	F
4	2, w32	w37, w50	6, –	5, 7	2, 3	F
5	11, w32	w35, w60	3, 4	2, 4	1, 3	FS
6	28, –	27, w60	1, 3	4, 5	2, 3	FS
7	2, –	w60, –	3, –	1, 2	1, –	FS
8	1, w32	7, w44	2, 3	2, 7	1, 3	FS
9	2, 3	7, w51	–, –	2, 7	1, 3	F
10	1, 2	8, 18	7, –	3, 7	2, 3	F
11	2, 26	7, w38	7, 8	2, –	1, –	S
12	2, w32	27, –	2, –	2, w8	1, 2	F
13	2, w24	27, w35	1, 4	5, –	2, –	S
14	11, w23	7, w57	2, 7	2, 7	1, 3	F
15	3, –	7, –	1, –	2, –	1, –	FS
16	2, –	7, w62	3, 7	2, 4	1, 3	S
17	11, w24	5, 18	–, –	2, 4	1, 3	FS
18	2, –	7, w62	–, –	2, 4	1, 3	S
19	2, –	14, w51	–, –	5, w6	2, –	ND
20	2, 11	w35, w57	4, 7	1, 5	1, 2	S
21	1, 3	8, w35	4, 7	3, 4	2, 3	FS
22	1, 2	w53, w57	–, –	2, 7	1, 3	S
23	2, 3	13, w35	6, –	2, 4	1, 3	FS
24	2, w32	w35, w63	4, –	1, w6	1, 2	F

Abbreviation: ND, not done.

European Caucasians carried the HLA-DR 2 allele ($\chi^2 = 6.787$; $P < 0.01$). Thus an increased relative risk of 3.54 for HLA-DR 2 positive individuals was calculated. The influence of all other defined HLA-DR alleles did not differ significantly from the percentage of positive individuals found in the normal control group.

Frequency analysis of the HLA-MT antigens, the most likely products of a second serologically defined HLA-D region locus [12, 13] showed an increase of the specificity HLA-MT 3 in the patient group (Table 2). The specificity HLA-MT 3 was present in 58.3% of the patients and in 29.6% of the normal European controls. This difference in the HLA-MT 3 frequency was statistically significant and reached a relative risk of 3.33 ($P < 0.025$) for all HLA-MT 3 positive individuals to develop idiopathic RPGN of type II. Furthermore, nine of 24 (37.5%) patients carried an HLA phenotype positive for HLA-MT 3 as well as HLA-DR 2, whereas only 13 of 125 (10.4%) normal individuals were found to express both antigens (Table 2, $\chi^2 = 9.695$; $P < 0.005$).

Among products of the HLA-ABC loci HLA-Cw 7 was observed more frequently in the patients than in the normal controls (Table 2). During the 8th International Histocompatibility Workshop linkage disequilibria of HLA-Cw 7 with HLA-B 7 and -B 8 were described [14]. In the patient group both individuals positive for HLA-B 8, as well as three of eight HLA-B 7 positive persons carried the HLA-Cw 7 antigen. However, four of six patients typed as HLA-Cw 7 positive showed also an HLA-MT 3 positive phenotype.

The Bf allotypes of 23 patients analyzed are given in Table 1. As blank alleles of the Bf locus have not been described seven of 23 patients only typed as BfF were most likely to be homozygous for this properdin factor allotype. The rare Bf

Table 2. Frequencies of HLA antigens in 24 patients with idiopathic RPGN (type II) and in 125 normal European Caucasians as controls

HLA antigen	Patients (*N* = 24)	European Caucasians (*N* = 125)	Relative risk	χ^{2a}	*P*[b]
B7	33.3%	28.0%	1.29	0.080	NS
Cw7	25.0%	10.4%	3.04	2.657	<0.15
DR 1	12.5%	20.8%	0.54	0.435	NS
DR 2	62.5%	32.0%	3.54	6.787	<0.01
DR 3	8.3%	17.6%	0.43	0.686	NS
DR 4	33.3%	21.6%	1.81	0.959	NS
DR 5	20.8%	28.8%	0.65	0.304	NS
DRw6	16.7%	22.4%	0.69	0.126	NS
DR 7	29.2%	20.0%	1.65	0.533	NS
DRw8	4.2%	7.2%	0.56	0.010	NS
DRw9	—	4.8%	—	—	—
DRw10	—	1.6%	—	—	—
MT 1	70.8%	72.0%	0.94	0.017	NS
MT 2	50.0%	64.0%	0.56	1.131	NS
MT 3	58.3%	29.6%	3.33	6.163	<0.025
DR 2 - MT 3	37.5%	10.4%	5.17	9.695	<0.005

[a] χ^2 is shown with Yates correction.
[b] The *P* value represents significance; NS, not significant.

allotypes BfF_1 and BfS_1 were not detected in the investigated individuals with idiopathic RPGN type II. The BfF allele was detectable in 17 of 23 patients (73.9%) compared to 36.9% in normal controls (relative risk 4.85; $\chi^2 = 11.387$; $P < 0.001$). The calculated relative risk for the presumed BfFF homozygous individuals was thus 14.67 ($\chi^2 = 38.588$; $P < 0.00001$; Table 3 [15]). Analysis of the relationship between the Bf alleles and HLA antigens showed that 11 of 14 HLA-MT 3 positive and 11 of 15 HLA-DR 2 positive individuals with idiopathic RPGN type II carried the BfF allele.

Table 3. Bf phenotypes in 23 patients with idiopathic RPGN (type II) and in 656 normal controls

Bf phenotypes	Patients (*N* = 23)	Controls (*N* = 656)[a]	Relative risk	χ^{2b}	*P*[c]
S	6 (26.1%)	391 (59.6%)	0.24	8.946	<0.005
FS	10 (43.5%)	216 (32.9%)	1.57	0.690	NS
F	7 (30.4%)	19 (3.0%)	14.67	38.588	<0.00001

[a] Normal controls are shown [15] including the rare Bf phenotypes SS_1 (*N* = 15), FS_1 (*N* = 2), SF_1 (*N* = 8), and FF_1 (*N* = 5).
[b] χ^2 is shown with Yates correction.
[c] The *P* value represents significance, NS, not significant.

Discussion

The results of this study demonstrate a genetic predisposition to develop idiopathic RPGN (type II) for individuals expressing the properdin factor allotype BfF and the HLA-D region alleles HLA-DR 2 and HLA-MT 3. An increased frequency of the antigen HLA-Cw 7 also observed in the patient group can be explained by the known linkage disequilibrium of this MHC product with the HLA-B locus antigens HLA-B 7 and -B 8. The shared association of Goodpasture syndrome (idiopathic RPGN, type I) [7] and idiopathic RPGN type II with HLA-DR 2 points to common genetic factors influencing abnormal immune responses.

A similar relative risk for individuals carrying HLA-MT 3 or -DR 2 suggests that the MT specificity may be as important as the HLA-DR antigen for an inherited susceptibility of idiopathic RPGN (type II). Recently, MT antigens have been claimed to represent surface molecules controlled by MHC genes distinct from, but closely linked to the HLA-DR locus [12, 13]. For HLA-DR 2 a strong linkage disequilibrium with HLA-MT 1, but not with -MT 3 has been demonstrated during the 8th International Histocompatibility Workshop [16]. HLA-DR 2 and -MT 3 are therefore most likely to occur on different haplotypes in the analyzed patient group, although genotypes could not be determined in this study. This assumption is supported by the observation that all HLA-DR 2 positive patients expressed HLA-MT 1 in addition. The significantly increased number of patients positive for HLA-DR 2, as well as HLA-MT 3, suggests that gene complementation plays an important role in the manifestation of idiopathic RPGN, type II. A similarly elevated frequency of the antigen HLA-MT 3 might have been present in the reported 15 HLA-DR 2 positive patients with Goodpasture syndrome due to a linkage of this MT product with the determined HLA-DR 4 or -DR 7 positive phenotypes [7].

The highest relative risk to develop idiopathic RPGN of type II was calculated for individuals expressing the BfF allotype. Several disease associations of Bf alleles already have been described [17]. In particular, a high incidence of the rare BfF_1 allele was found in another kidney disorder, the idiopathic membranous nephropathy [18]. Nothing is known whether the properdin factor genes are involved directly in the pathogenesis of these diseases as part of the complement system, or whether they serve as markers for closely linked disease susceptibility genes. Thus, in patients with idiopathic RPGN of type II the BfF allele could belong to a set of disease markers including the associated HLA-D region products on an extended haplotype which was maintained in the population due to cross-over suppression of a human t-like locus on chromosome 6 and is characterized by disease-related gene mutations [17]. Family studies will be needed to delineate the haplotypes found among the patient group and to evaluate which part of the MHC influences abnormal immune responses and is involved in the pathogenesis of this disease.

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