Introduction

ANCA-associated vasculitis (AAV) constitutes a group of vasculitides associated with ANCA and comprises WG, microscopic polyangiitis (MPA), renal limited vasculitis (RLV) and the Churg–Strauss syndrome (CSS). It is tempting to assume that the HLA system is involved in the aetiology of AAV as this system distinguishes between self and non-self and is involved in antigen presentation. It is also possible that other immune-modulating genes, like TNF-α and C4A, located near the HLA genes on chromosome 6, are involved in the aetiology of autoimmune diseases. The finding of associations between HLA antigens and severe autoimmune diseases suggests that HLA is indeed involved in the development of these diseases.

No definite answers concerning the possible relationship between HLA antigens and (the course of) AAV exist. In several small studies, conflicting data have been presented (review in [1]; supplementary Table 1, available as supplementary data at Rheumatology Online). Identifying patients at risk for relapses or more serious disease before start of treatment will provide tools to tailor the treatment of these AAV patients.

In this study, we investigated the possible association between HLA antigens and AAV and patients with WG in particular.

HLA-DR4, DR13(6) and the ancestral haplotype A1B8DR3 are associated with ANCA-associated vasculitis and Wegener’s granulomatosis

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Concise Report

Objectives. As the HLA system is involved in recognition of self and non-self, an association with the development of ANCA-associated vasculitis (AAV) seems probable. In this study, the relation between HLA antigens and AAV and its severity were investigated.

Methods. Consecutive patients diagnosed with AAV at our centre, who were followed for at least 2 years, were included. The frequency of HLA antigens of AAV and WG patients was compared with 5872 healthy blood donors from the same region and with 4000 healthy Dutch controls originating from a Eurotransplant database.

Results. From 304 AAV patients, sufficient data were available. We found DR13(6) to be less prevalent and both DR4 and the ancestral haplotype A1B8DR3 more prevalent in patients with AAV compared with controls, particularly in patients with WG. In addition, DR1 was less prevalent in patients with WG in comparison with controls. Further, DR8 was more prevalent in patients with CSS compared with other forms of vasculitis and controls. There were no associations between HLA antigens and disease characteristics or course of AAV or WG.

Conclusions. AAV is associated with increased prevalence of DR4 and the ancestral haplotype A1B8DR3 and with decreased prevalence of DR13(6), particularly in patients with WG. In patients with WG, prevalence of DR1 was decreased, whereas in patients with CSS DR8 was increased. No associations between HLA antigens and disease characteristics or course of AAV were found.

Key words: Autoinflammatory conditions, Vasculitis, Immunogenetics and HLA, Major histocompatibility complex, Physiology, ANCA-associated vasculitis, Wegener's granulomatosis.

Methods

Our cohort included all patients who were diagnosed with AAV in our hospital (tertiary referral centre for vasculitis) in the period 1990–2005 and they were classified using the Chapel Hill Consensus Conference definitions for WG, MPA, RLV or CSS [2]. All patients had to be followed for at least 2 years and provided informed consent for HLA typing.

From their records, disease manifestations and disease severity at diagnosis were collected. Disease severity was scored using the BVAS (Birmingham Vasculitis Activity Score) [3]. During follow-up, the patients were checked (both by clinical and laboratory investigations, including ANCA titres) for disease activity at least every 3 months. Relapse was defined as new or increasing disease activity, requiring use of renewed or intensified immune suppressive therapy [4].

HLA-A, -B and -DR antigens were typed serologically, using a microcytotoxicity assay. In addition, DNA-based HLA typing by sequence specific priming (SSP) or sequence specific oligonucleotides (SSO) was performed for all apparent HLA Class II homozygotes and to resolve any problematic serological typing. The HLA laboratory is accredited by the European Federation for Immunogenetics for all techniques employed. To investigate the possible relationship between HLA antigens and AAV and WG, the prevalence of HLA antigens in patients with AAV/WG was compared with that of 5872 healthy blood donors, coming from the same region as the majority of our patients. Additionally, we investigated the prevalence of the ancestral haplotype A1B8DR3 in our patients, compared with 4000 healthy Dutch controls originating from a Eurotransplant database. We used another
control group for this analysis, for we had no data on haplotypes in the first control group.

In addition, we investigated the relationship between HLA antigens and disease manifestations at diagnosis, ANCA specificity and the occurrence of relapses within 5 years of diagnosis. Furthermore, the relapse-free survival was calculated per HLA antigen for all AAV patients and for WG patients only, both per ANCA specificity. Finally, the relation between HLA antigens and persistence of ANCA was evaluated by comparing patients who became negative for ANCA during the first 12 months after diagnosis with those who did not, both for all patients and for the subgroups of patients with PR3- and MPO-ANCA at diagnosis [4]. ANCA titres were measured by IIF assay, whereas ANCA specificity was confirmed by an antigen-specific capture ELISA assay.

Statistics
Numerical data between groups were compared using the Mann–Whitney U-test. To compare proportions of groups, the chi-square test and Fisher’s exact test were used. Corrections for multiple comparisons were made using the Bonferroni method. Actuarial relapse-free survival was calculated using the Kaplan–Meier method and the log rank test. A corrected P-value of <0.05 was considered significant and P-values reported are corrected for the number of comparisons, unless indicated otherwise. All data were analysed retrospectively.

Results
Patients
Between 1990 and 2005, 418 patients were diagnosed with AAV. In 88 of these patients, HLA typing was not performed, mainly because these patients were seen only once for second opinion (n = 76). Of 26 patients, insufficient data were available (10 died shortly after diagnosis, 16 were treated in other hospitals). In total, 304 patients with AAV with sufficient data and at least 2 years of follow-up could be included. Most (n = 241, 79%) were classified as WG and were ANCA positive (94%). Median follow-up was 8 years (range 2.0–16.8) and ended in July 2007 (Table 1).

HLA antigens in patients with AAV
Table 2 shows the significant differences in the distribution of HLA antigens in patients with AAV, the four different forms of AAV and in healthy controls (details in Supplementary Table 2, available as supplementary data at Rheumatology Online). We found a decrease of DR6 in patients with AAV compared with controls, which was attributable to the split DR13(6) [P < 0.0001; OR 0.3 (0.2, 0.4)], as the split DR14(6) was distributed equally between patients and controls. We further found an increase of DR4 [P < 0.0001; OR 1.7 (1.4, 2.2)]. In addition, the so-called ancestral haplotype, consisting of the antigens A1, B8 and DR3, was overrepresented (19%) compared with Dutch controls originating from a Eurotransplant database [12%, P = 0.001; OR 1.8 (1.3, 2.4)].

In contrast to patients with MPA, RLV and CSS, in patients with WG, DR1 was significantly less prevalent than in controls [P = 0.04; OR 0.6 (0.4, 0.8)] (Table 2). Likewise, in these WG patients, DR4 [P < 0.0001; OR 1.7 (1.3, 2.2)] and the ancestral haplotype A1B8DR3 were more prevalent [P < 0.0001; OR 2.0 (1.4, 2.7)] and DR6 and DR13(6) were less prevalent [P < 0.0001; OR 0.4 (0.3, 0.6) and OR 0.3 (0.2, 0.5), respectively]. Compared with controls, there were no significant differences in HLA antigen distribution in patients with MPA, CSS or RLV. However, between the four groups, HLA antigen distribution did not differ, except for DR8, which was more prevalent in patients with CSS than in the other three groups of AAV (P < 0.0001).

HLA antigens and disease characteristics
We found no associations of HLA antigens with disease activity, measured using the BVAS score, at diagnosis, both for all patients with AAV and for patients with WG. We found no associations of HLA antigens and clinical characteristics like ENT, lung and peripheral nervous system involvement or with dialysis- and/or ventilator dependency at diagnosis (data not shown). Likewise, no differences in organ involvement and dialysis and/or ventilator dependency were found between patients with the ancestral A1B8DR3 haplotype and those without this haplotype (data not shown). We also found no association between clinical characteristics in those who were DR4 positive and DR13(6) negative and those who were DR4 negative and DR13(6) positive (see supplementary Table 3, available as supplementary data at Rheumatology Online).

HLA antigens and relapses
Within 5 years after diagnosis, 149 patients (49%) experienced a relapse. We found no significant associations between HLA antigens and the occurrence of relapses or disease-free survival. There were also no differences in the occurrence of relapses or disease-free survival between patients with or without the
ancestral haplotype (data not shown). Finally, we found no association between HLA antigens or the ancestral haplotype and the presence/absence of renal involvement at the moment of first relapse.

**HLA antigens and ANCA**

We found no differences in distribution of HLA antigens between patients with PR3-ANCA and those with MPO-ANCA. Likewise, there were no differences in HLA antigens between patients who were ANCA negative and those with PR3- or MPO-ANCA at diagnosis. There were also no differences between those who became ANCA negative at least once during the first year of follow-up and those who were persistently ANCA positive. This was true for both AAV and WG patients who were ANCA-positive at diagnosis and for patients with PR3- and MPO-ANCA separately.

**Discussion**

As the HLA system plays a central role in the distinction between self and non-self, an association of this system with the autoimmune disease AAV could be present. In our study involving 304 patients with AAV, we found DR13(6) less and DR4 more prevalent compared with healthy controls. In addition, the ancestral haplotype A1B8DR3 was more prevalent in AAV patients than in controls. These results were also true for patients with WG. In addition, in patients with WG, DR1 was less prevalent than in controls. These associations were not found in patients with MPA, RLV or CSS, which may be due to the small number of patients in these groups, as the HLA antigens were distributed similarly between the diagnoses, with the exception of DR8. DR8 was more prevalent in patients with CSS than in patients with other forms of vasculitis or controls. Furthermore, we found no associations between HLA antigens or combinations of antigens [DR4/DR13(6)] and disease characteristics at presentation or course of AAV. As treatment protocols have changed over time, differences in treatment may have influenced our findings. However, with the exception of patients with CSS, who were mainly treated with corticosteroids only, the majority of our cohort received induction therapy with oral cyclophosphamide and corticosteroids and, from 1996 onwards, 2 years of AZA once stable remission was achieved. Lastly, there were no differences in HLA antigen distribution between patients with PR3- and MPO-ANCA.

So far, divergent results on the association of HLA antigens with AAV have been published [review in [1]: overview in supplementary Table 1, available as supplementary data at *Rheumatology* Online]. The divergency of these results may be explained by the small and heterogeneous patient populations with racial and geographical differences that were studied. The findings in our patients, a large homogeneous cohort of Caucasian AAV patients that was compared with controls from the same region, were similar to other studies including Caucasian (mostly WG) patients. A decrease of DR13(6) has been reported before [5–7], although it should be mentioned that 106 of our 304 patients were included in one of these studies [5]. In agreement with our findings, an increase of DR4 was reported before [5, 6, 8–11]. An increase of DR8 has also been described in WG and MPA patients [12], but not in patients with CSS, like in our study. Our cohort of CSS patients was small, so this finding has to be interpreted with caution. The decrease of DR1 and increase of the ancestral haplotype have not been reported before in WG patients. The increase of the ancestral haplotype, which includes the allele DR3, contrasts reports of a decrease of DR3 in other studies [8–10].

We, like others [5, 13], found no clear associations between HLA antigens (or combinations) and disease characteristics at presentation or course of AAV. In one study, however, DR4 was highly prevalent in a subgroup of PR3-ANCA patients with end-stage renal disease [6]. This contrasts our study, in which we found no association between DR4 and dialysis dependency at diagnosis, although we did not evaluate the long-term outcome on renal function. Interestingly, linkage between a TNF-α2a microsatellite and DR4 was found in that study [6]. This may indicate that functional polymorphisms, located in the vicinity of TNF and linked to HLA genes, produce higher levels of TNF. In agreement with this hypothesis, more constitutional and (a trend to more) vasculitis symptoms were seen in CSS patients carrying DR4 [9]. In contrast, DQ7DR4 was associated with a more favourable course (non-persistence) of ANCA in another study [8].

The role of HLA antigens in the development of autoimmune diseases, like AAV, is unclear. Investigating HLA antigens and genes linked to these antigens using sophisticated methods and relating these findings to clinical parameters will presumably lead to more insight in the aetiology of AAV. Studies using this strategy have already revealed interesting results, which can guide further investigations. For instance, the ancestral haplotype A1B8DR3 leads to prolonged persistence of (auto-)antigens by increasing apoptosis of lymphocytes and TNF-α production, while complement function is decreased [14, 15]. It is therefore interesting that we found significantly more carriers of the ancestral haplotype in patients with WG, although we found no relation with clinical parameters. Furthermore, the percentage of neutrophils expressing PR3 is influenced by a group of 34 HLA antigens [16]. A high percentage of neutrophils expressing PR3 is a risk factor for AAV [17]. Moreover, absence of DR13(6) may reduce the ability to prevent infections and thus predisposes to colonization with *Staphylococcus aureus* [18], which is increased in WG and an established risk factor for relapses of AAV [19].

This hypothesis must still be confirmed at the moment. In addition, using an extended association screen, linkage was found between DPB1*0401 and the retinoid X receptor b allele 3, which is involved in several immune functions, such as induction of apoptosis [20]. In that study, microsatellites, representing apoptosis-related genes and genes that are down-regulated in apoptotic neutrophils, were used to perform this association screen. Further typing of these alleles identified the retinoid X receptor b allele 3 that was linked to DPB1*0401.

In conclusion, we found an association between AAV and the HLA antigens DR4 and DR13(6) and the ancestral haplotype A1B8DR3, particularly in patients with WG. In addition, we found an association between DR1 and WG, and between DR8 and CSS. How these associations lead to the development of AAV is still unclear. We found no association with HLA antigens and disease characteristics or course of AAV.

**Rheumatology key messages**

- AAV, and WG in particular, are associated with several Class II HLA antigens and the ancestral haplotype A1B8DR3.
- HLA antigens are not associated with disease characteristics or course of AAV/WG.

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**Supplementary data**

Supplementary data are available at *Rheumatology* Online.
References