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Immunogenetic heterogeneity of rheumatoid arthritis

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SUMMARY Association of HLA-DR4/Dw4 with rheumatoid arthritis (RA) is well established, but conflicting data exist on a possible association with the severity of the disease, including its extra-articular manifestations. In order to investigate whether a subgroup of RA is preferentially associated with DR4, HLA typing was performed in two groups of patients with severe extra-articular manifestations (Felty's syndrome and histologically proved leucocytoclastic vasculitis), patients with severe joint destruction (seropositive and seronegative), a group with only mild joint destruction, and in healthy controls. The frequency of HLA-DR4 was significantly raised in all patient groups compared with that in healthy controls. The two groups with severe extra-articular manifestations, however, both had a DR4 frequency of 92%, which was significantly ($p=0{\cdot}002$) higher than the 62·7% found in the remaining patients. No significant differences were observed between severe or mild joint destruction and seropositivity or seronegativity in the groups without the above-mentioned extra-articular manifestations. From these data we concluded that DR4 is preferentially associated with severe extra-articular disease manifestations of RA. This observation provides an immunogenetic basis for the disease heterogeneity and for the immunological analogy between RA and leprosy.

Key words: HLA-DR4, extra-articular manifestations, Felty's syndrome, rheumatoid vasculitis, immune suppression gene.

The association of HLA-Dw4/DR4 with rheumatoid arthritis (RA) is well established.[1–4] RA, however, includes a heterogeneous group of conditions with highly variable outcomes. Its spectrum of different subgroups in clinical and immunopathological manifestations has been compared with leprosy.[5] Thus one would expect to observe differences in HLA antigen frequencies between its subgroups.[6] If HLA type is associated with disease heterogeneity or expression this might have important implications for studying the pathogenesis of RA and possibly for the prevention and/or therapy of patients in its subgroups. A preferential association of DR4 with severity of joint disease,[7] the occurrence of extra-articular manifestations[8] like Felty's syndrome (FS)[9–11] or rheumatoid vasculitis (RV),[10 12] or both, and the presence of rheumatoid factor (RF)[13 14] have been suggested by several authors. The results of most of these studies, however, failed to reach statistical significance or have been disputed by others, or both.[15–17] To our knowledge a single centre study reporting a significant difference between clinical subgroups of RA has not been published.

In order to determine whether or not a particular subgroup of RA is preferentially associated with HLA-DR4 we performed HLA-DR typing on patients from five clinically well defined subgroups representing the clinical spectrum of RA from our rheumatology clinic.

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Patients and methods

One hundred and twelve Dutch Caucasoid patients with definite or classical RA[18] who were attending the rheumatology outpatient clinic and fulfilled the criteria of one of the five following subgroups were chosen for the study:

A. Felty's syndrome (FS): classical RA with less than $2{\cdot}0\times10^9$/l granulocytes and splenomegaly (24 patients). All were RF positive.

B. Rheumatoid vasculitis (RV): classical RA with leucocytoclastic vasculitis histologically proved by biopsy from skin or muscle, or both (25 patients). Except for one patient who remained seronegative for more than three years during control in outpatient clinic all patients were strongly positive for RF. Four patients of this RV subgroup were also classified as FS.

C. Rheumatoid factor positive RA with severe joint destruction: classical RA, repeatedly positive tests for rheumatoid factor, functional capacity classification class 3–4[19] with radiographic score of joint destruction[20] of at least grade 3 in several joints, without signs of FS or RV (26 patients).

D. Rheumatoid factor negative RA with severe joint destruction: definite or classical RA, repeatedly seronegative for RF measured by two methods,[21 22] functional capacity classification class 3–4 and radiographic scores of joint destruction at least grade 3 in several joints, without signs of FS or RV (17 patients).

E. Patients with mild joint destruction: classical RA for at least five years, at least once positive for RF, functional capacity classification class 1 or 2 with radiographic scores of joint destruction grade 1 or 2 in most of the joints (24 patients). Patients with psoriasis, sacroiliitis, inflammatory bowel disease, and juvenile chronic arthritis were excluded.

A group of 502 randomly selected healthy unrelated Dutch Caucasoid blood donors served as controls for the HLA-DR antigen frequencies.

Rheumatoid factor was determined by human erythrocyte agglutination and latex fixation tests.[21 22]

HLA TYPING AND STATISTICAL ANALYSIS

HLA-DR typing was performed by the two colour fluorescence technique[23] and with a battery of well characterised antisera defining all recognised DR specificities. Comparison of HLA-DR4 frequencies between the patient subgroups and controls was performed by the Woolf-Haldane method.[24]

Results

The clinical data of the patients are summarised in Table 1. Age of onset of the disease was similar in all subgroups. The predominance of female over male patients was evident in all subgroups except for the RV subgroup. The mean patient age and the duration of the disease were higher in patients with FS and those with seropositive destructive RA than in other patients. The type of therapy also differed between the groups: those with mildly destructive RA received only non-steroidal anti-inflammatory drugs (NSAIDs) and chloroquine derivatives, while the patients in the other groups often received gold, D-penicillamine, cytostatics, or corticosteroids. Patients with vasculitis required corticosteriods and cytostatic drugs more frequently than patients of the other groups.

The frequency of HLA-DR4 was significantly higher in all RA groups than in the healthy controls (Table 2). The patients with FS and vasculitis both had a markedly raised DR4 frequency (92%). Thus

Table 1 *Clinical data of patients studied*

Patients	*Group A: Felty's syndrome*	*Group B: Rheumatoid vasculitis*	*Group C: Severe joint destruction RF positive*	*Group D: Severe joint destruction RF negative*	*Group E: Mildly destructive*
Number	24	25	26	17	24
(Female:male)	(18:6)	(11:14)	(15:11)	(12:5)	(18:6)
Mean age (years)	67·6	58·4	63·1	56·6	52·5
(range)	(37–82)	(34–80)	(34–80)	(43–75)	(30–77)
Mean age of onset (years)	45·2	48·1	47·4	46·4	39·9
(range)	(16–68)	(22–64)	(28–71)	(28–71)	(23–65)
Duration of disease (years)	22·4	10·3	16·0	10·1	12·6
(range)	(4–42)	(1–20)	(2–40)	(2–25)	(5–20)
Therapy required:					
NSAIDs* only (n)	0	2	0	1	4
Antimalarials (n)	19	24	17	6	20
Gold (n)	16	15	23	10	1
D-Penicillamine (n)	4	12	13	5	—
Cytostatics (n)	6	15	9	4	—
Corticosteroids (n)	3	16	10	2	—

*NSAIDs=non-steroidal anti-inflammatory drugs.

Table 2 *Preferential association of HLA-DR4 with extra-articular disease in RA patients*

Group A: Felty's syndrome (n=24)	*Group B: Rheumatoid vasculitis (n=25)*	*Group C: Severe joint destruction RF positive (n=26)*	*Group D: Severe joint destruction RF negative (n=17)*	*Group E: Mildly destructive (n=24)*	*Group F: Healthy controls (n=502)*
0·92*	0·92	0·69	0·59	0·58	0·28

A+B ← RR=5·53†; χ^2=10·25, p=0·002 → C+D+E

C+D+E ← RR=4·3; χ^2=29·73 → F

A+B ← RR=23·8; χ^2=42·992 → F

*HLA-DR4 phenotype frequency.
†RR=relative risk.

the occurrence of RA with severe extra-articular manifestations is strongly associated with HLA-DR4, giving a relative risk (RR) of 23·8. All but one of these patients were strongly seropositive, and exclusion of this atypical patient, who also happened to be DR4 negative, increased the RR to 30·6. The differences in HLA-DR4 frequency between groups C (69%), D (59%), and E (58%) were not significant, and taken together the last three (C, D, and E) had a significantly lower frequency of DR4 than groups A and B (p=0·002). Thus the relative risk of HLA-DR4 for the development of FS and RV among our RA patients was 5·53. HLA-DR4 was not associated with severe joint destruction per se (groups C, D *v* E) or with the mere presence of rheumatoid factor (group C *v* D). The frequencies of the other HLA-DR antigens in the five RA subgroups were not significantly different from those in the controls.

Discussion

The most striking observation of the present study is that in the patients with RA complicated by FS or vasculitis, or both, a significantly higher frequency of HLA-DR4 was found compared with that found for RA patients without these extra-articular manifestations. This observation indicates that there is an immunogenetic basis for the clinical heterogeneity of the disease, i.e., between groups of RA patients complicated with severe extra-articular manifestations or systemic RA and those who have mainly articular joint involvement. Although incidental reports have claimed an increased frequency of HLA-DR4 among FS patients,[9 11] and one study has reported the same for FS and RV,[10] the number of patients studied was small. Scott *et al* also observed a high frequency of HLA-DR4 among 32 RV patients, but he did not compare his results with those of RA patients without vasculitis from the same clinic.[12]

It should be noted that joint destruction per se was not found to be associated with HLA-DR4 in the present study (C, D *v* E), which is in agreement with one study[17] and in contrast with another.[7] Thus HLA-DR4 appears to be associated with severe *extra-articular* rather than with articular disease. In this context it may be of importance that in patients with severe articular involvement, but without FS or documented vasculitis, or both, the presence of RF was not associated with HLA-DR4. In a previous study we showed that the titres of circulating RF were not associated with the severity of joint involvement, but a strong association was found with the presence of extra-articular disease (e.g., RV).[25] From those results and the present data we therefore conclude that the presence of high titres of RF and HLA-DR4 may both contribute to the development of extra-articular involvement in RA.

There are two explanations for the preferential association of DR4 with extra-articular or 'systemic' RA. Both explanations assume that its less striking but still increased frequency in the groups without FS and/or histologically proved vasculitis is caused by 'dilution' of this group with less prominent extra-articular manifestations not classified as FS or RV. The first explanation is that DR4 is a marker for a disease modifying gene, RA as such not being associated with DR4. This explanation was suggested earlier by de Jongh *et al*, based on the results of a study of RA patients not selected via hospitals, which did not show an increased frequency of DR4.[26] The second explanation is that DR4 is only associated with a subgroup of RA,

which is also characterised by extra-articular manifestations. In this latter case DR4 is not a disease modifying but really a disease susceptibility gene. A recent family study provided evidence in favour of the second explanation.[27] In that study cosegregation of HLA-DR4 with susceptibility to RA was observed, which would not have been the case if DR4 was only a marker for a disease modifying gene.

The significant difference in DR4 frequency between RA patients selected for two severe extra-articular disease manifestations and those that did not display those manifestations extends the immunological analogy drawn between RA and leprosy.[5] In leprosy it has now been clearly shown that the type of the disease is controlled by HLA linked genes.[28] This HLA linked control of leprosy type is in all probability caused by HLA class II immune response (Ir) and immune supression (Is) genes, which control the cellular immune reactivity of the host against *Mycobacterium leprae*,[29] the main factor defining leprosy type.[30] If RA is now considered it can be seen that the immunological analogy between rheumatoid disease and lepromatous leprosy[5] might also have an immunogenetic basis, namely the preferential association with DR4. Thus the HLA and leprosy studies might also give a lead for studies on the mechanism of the association between (extra-articular) RA and DR4. The most probable mechanism of the association between HLA and lepromatous leprosy is an HLA class II Is gene.[31] Therefore it might be more pertinent to look for an HLA linked Is gene rather than for an Ir gene associated with DR4 in RA patients. Such an approach might lead to the identification of a possible inciting antigen, which might even be a mycobacterial antigen.[32]

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