Perivascular infiltration in normal skin of patients with rheumatoid arthritis: association with rheumatoid factors and HLA-DR antigens

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SUMMARY The relation between immunohistological findings in biopsy specimens of apparently normal skin, HLA antigens, and rheumatoid factors (RF) was studied in 120 patients with rheumatoid arthritis (RA), selected for treatment with D-penicillamine. Perivascular infiltration (PVI) of more than three mononuclear cells was present in 77 (68%) of 114 patients, accompanied usually by the presence of IgM or C3, or both, in immunofluorescence studies. The number of perivascular cells was associated significantly with the titre of circulating RF. A weak relation of both perivascular cellular infiltration and RF with HLA-DR3 and DR4 did not reach statistical significance. It is concluded that the histological presence of perivascular inflammation is associated mainly with deposition of RF. It is suggested that the first is merely an epiphenomenon of the latter. PVI was not prognostic for the occurrence of the clinical syndrome of rheumatoid vasculitis. For practical purposes skin biopsies do not appear to be useful in the evaluation of individual patients with RA.

Perivascular infiltrates of mononuclear cells, accompanied frequently by deposition of IgM or C3, or both, in immunofluorescence studies, are found in normal appearing skin of 25–65% of patients with rheumatoid arthritis (RA).^{1–6} In healthy controls and patients with osteoarthritis such abnormalities are absent.^{3, 5, 7} The presence of these infiltrates has been associated with extra-articular disease manifestations, seropositivity, and circulating immune complexes, but does not necessarily indicate the clinical syndrome of rheumatoid vasculitis with skin ulcers, nail fold lesions, neuropathy, or gangrene.^{1–9}

The importance of these findings is subject to discussion. Some regard it as an epiphenomenon, whereas others think they play a central part in the pathogenesis of RA.^{3, 9, 10} The genetic basis of RA has been demonstrated by the association with HLA-DR4 and also in twin studies.^{11, 12} HLA-DR4 has been associated with manifestations of RA that indicate a more severe disease course: seropositiv-

ity, erosions, and extra-articular features like Felty's syndrome and rheumatoid vasculitis.^{12–21} Studies of a direct relation between HLA antigens and the presence of PVI have to our knowledge not been reported, though an association of PVI with HLA-DR4 has been mentioned.²²

We took biopsy specimens of apparently normal skin from patients with RA who entered an open study of effectivity and toxicity of D-penicillamine.²³ To evaluate possible associations with side effects HLA typing was performed in most of these patients. These data enabled us to study the relation between histological findings in normal skin, rheumatoid factors (RF), and HLA antigens—subject of the present report.

Patients and methods

One hundred and twenty consecutive patients with classical or definite RA, eligible for treatment with D-penicillamine, were studied. The male/female ratio was 45/75, mean age 58 (range 24–80) years and mean duration of disease 14 (range 1–44) years. Eighty six patients (72%) were seropositive (Rose-

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Waal test titre $\geq 1/16$), the others being seronegative on at least three occasions. Extra-articular features were present in seven patients: a combination of splenomegaly, episcleritis, and lymphadenopathy in one patient, episcleritis alone in two, and pleuritis with lymphadenopathy in one. Leg ulcers were present in two patients and a mild polyneuropathy in one. These patients did not have other manifestations of the clinical syndrome of rheumatoid vasculitis. Skin biopsy was performed in six of these seven patients.

Erythrocyte sedimentation rate (ESR), RF, and antinuclear factors (ANF) were measured with standard methods at the time the skin biopsy specimen was taken, i.e., before treatment with D-penicillamine was started. HLA typing with a standard microlymphocytotoxicity assay was performed in the laboratory of the Blood Transfusion Service, University of Nijmegen (Dr P Reekers) and the central laboratory of the Netherlands Red Cross Blood Transfusion Service (Dr L P de Waal). As the decision to evaluate possible associations with tissue antigens was taken after the beginning of the study HLA-DR typing was not performed on 30 patients.

One hundred and fourteen patients consented to a skin biopsy. Punch biopsy specimens were taken from the medial side of the forearm. One specimen was snap frozen in liquid nitrogen for immunofluorescence studies, another was fixed in 4% formaldehyde and stained with haematoxylin and eosin (H&E). The sections were initially assessed by the routine methods of the pathology department. Direct immunofluorescence was performed with monospecific rabbit antisera against IgG, IgM, IgA, IgE, and C3. Immunoglobulins or C3, or both, were considered present when granular deposits in the subepidermal vessel walls exceeded the intensity of background staining. Previous studies in our laboratory of skin biopsy specimens from non-rheumatoid patients and a few patients who died from traffic accidents did not show deposition of C3, and rarely a slight deposition of IgM.²⁴ To obtain an objective

measure of perivascular infiltration all H&E sections were reassessed using a semiquantitative scale ranging from 0 to 3 as described by others.⁵ Less than three mononuclear cells per vessel were scored as 0, with three to six cells the score was 1, 7–10 cells: 2, more than 10 cells: 3. Skin biopsies, examinations of immunofluorescent sections, and the reassessment of H&E sections were performed by one of us (TMV).

Statistical analysis was performed with the χ^2 test with Yates's correction.

Results

Characteristics of all patients, patients who had skin biopsies, and those who were HLA typed showed no important differences. Thus the probability of selection bias in these respects is small (Table 1).

Perivascular infiltration of more than three mononuclear cells was present in 77 (68%) of 114 patients and of more than 10 cells in only two patients, both without clinical signs of vasculitis at the time of the biopsy. Immunofluorescence studies showed deposition of IgM, almost invariably together with C3 in 58 (51%) patients. Deposition of IgA was found in one patient. IgE, bound to mast cells, was seen in five patients. As expected the prevalence of HLA-DR4 was significantly higher than in healthy Dutch blood donors (71% v 20%). This was not the case for HLA-DR3 (16% v 23%). Results of both HLA typing and a skin biopsy were available from 86 patients.

The number of perivascular cells was significantly increased in patients who also had deposition of IgM or C3, or both, men, seropositive patients, patients with rheumatoid nodules, and patients possessing HLA-DR3 (Table 2). PVI was also more prevalent in patients with ANF or HLA-DR4, but the level of statistical significance was not reached.

The prevalence of serological abnormalities and rheumatoid nodules was increased slightly in patients having either HLA-DR3 or HLA-DR4, but

Table 1 Characteristics of all patients consenting to a skin biopsy or HLA typing, or both

Characteristic	All patients	Skin biopsy	HLA typed
Number	120	114	90
Women	75 (63)*	71 (62)	58 (64)
Mean age in years (SD; range)	58 (12; 24–80)	58 (12; 24–80)	57 (12; 24–77)
Mean duration in years (SD; range)	14 (10; 1–44)	13 (10; 1–44)	14 (9; 1–44)
Rheumatoid nodules	38 (32)	35 (31)	28 (31)
Radiological erosions	110 (92)	104 (91)	83 (92)
Mean ESR in mm/h (SD; range)	52 (28; 6–130)	53 (27; 6–130)	52 (28; 6–130)
Rose-Waaler test titre $\geq 1/16$	86 (72)	82 (72)	65 (72)
Antinuclear factors	32 (27)	30 (26)	24 (27)

*Values are Nos (%).

Table 2 Characteristics of 114 patients divided into three groups according to semiquantitative scoring of perivascular mononuclear cells

Characteristic	0 (0-2 cells)	1 (3-6 cells)	2/3 (>6 cells)
Number	37 (32)†	49 (43)	28 (25)
IgM/C3 deposition	6 (16)	29 (59)	23 (82)**
Men	11 (30)	17 (35)	15 (54)*
Mean age (years)	61	57	58
Mean duration (years)	16	13	11
Rheumatoid nodules	6 (16)	16 (33)	13 (46)*
Other EAF‡	1 (3)	4 (8)	1 (4)
Radiological erosions	34 (92)	44 (90)	26 (93)
Mean ESR (mm/h)	58	47	57
Rose-Waaler test titre $\geq 1/16$	23 (62)	32 (65)	27 (96)**
Antinuclear factors	6 (16)	14 (29)	10 (36)
Number HLA typed	30 (81)	35 (71)	21 (75)
HLA-DR4 positive	20 (67)	23 (66)	17 (81)
HLA-DR3 positive	1 (3)	8 (23)	5 (24)*

* **Significantly increased (* $p < 0.05$, ** $p < 0.01$; χ^2 test) if compared with patients with ≤ 6 cells.

†Values are Nos (%).

‡EAF=extra-articular features: see text.

again differences did not reach statistical significance (Table 3).

To analyse further the relation of RF and perivascular inflammation as well as HLA-DR antigens patients were divided into groups according to the

Table 3 HLA-DR3 and HLA-DR4 and some disease parameters

	No	RF+	ANF+	Nodules	Erosion
DR4+	64	49 (77)*	17 (27)	22 (34)	58 (91)
DR4-	26	16 (62)	7 (27)	6 (23)	25 (96)
DR3+	14	12 (86)	6 (43)	4 (29)	13 (93)
DR3-	76	49 (64)	18 (24)	24 (32)	70 (92)

*Values are Nos (%).

Differences do not reach statistical significance.

Table 4 Relation between titre of the Rose-Waaler test at the time of the biopsy, semiquantitative scoring of perivascular infiltration, and deposition of IgM or C3, or both

Rose-Waaler	No	0-2 Cells	3-6 Cells	>6 Cells	IgM/C3
Negative	37	16 (43)†	17 (46)	4 (11)	7 (19)
1/8-1/16	11	5 (45)	4 (36)	2 (18)	5 (45)
1/32-1/64	17	6 (35)	8 (47)	3 (18)	12 (71)
$\geq 1/128$	49	10 (20)	20 (41)	19 (39)	34 (69)
All patients	114	37 (32)	49 (43)	28 (25)	58 (51)

If all patients with Rose-Waaler test titres $\leq 1/16$ are compared with those with titres $\geq 1/32$ significant differences are found for the number of perivascular cells ($\chi^2=8.2$, $p < 0.02$) and deposition of IgM/C3 ($\chi^2=24.6$, $p < 0.01$).

Table 5 Relation between titre of the Rose-Waaler test at the time of biopsy and HLA-DR3 and HLA-DR4

Rose-Waaler test titre	No	DR3+	DR4+
Negative	21	2 (10)*	15 (71)
1/8-1/16	13	0 (0)	8 (62)
1/32-1/64	14	2 (14)	8 (57)
$\geq 1/128$	42	10 (24)	33 (79)
All patients	90	14 (16)	64 (71)

*Values are Nos (%).

Differences do not reach statistical significance.

titre of RF as estimated at the time of biopsy (Tables 4 and 5). Deposition of IgM or C3, or both, as well as PVI were significantly associated with high titres of RF. HLA-DR3 and DR4 were somewhat more prevalent in patients with high titres of RF, but the difference did not reach statistical significance.

Discussion

Within the broad spectrum of RA, seropositive disease with extra-articular features has been proposed as a separate disease entity. From many studies the clustering of RF, nodules, Felty's syndrome, and—less pronounced—radiological erosion of para-articular bone has become clear. This concept is further supported by the association of these symptoms with HLA-DR4. It is likely that PVI and deposition of IgM or C3, or both, are also associated with this cluster.¹⁻⁷ In this study PVI of mononuclear cells in normal skin was associated with seropositivity and the presence of rheumatoid

nodules, confirming the above mentioned observations. A direct relation between extra-articular disease manifestations could not be shown as the latter were present in only six of 114 patients. Association of PVI with male sex (Table 2) is likely to be secondary to the association with RF and nodules, the latter being more often present in male patients with RA. Our data indicate a weak relation between ANF and HLA-DR3 as has been reported by others, both being weakly associated with PVI.¹⁵⁻¹⁷ Perhaps these should also be regarded as markers for a more severe form of RA. PVI was present in 77/114 (68%) of our patients with RA selected for treatment with D-penicillamine. Compared with other studies this figure is high, which may reflect partly rather serious and long-standing disease in our patients. More important are differences in the criteria that were used: a cut off point of six cells per vessel reduces the figure to 25%.

The number of perivascular cells correlated with deposition of IgM or C3, or both, in the vessel wall, as reported in other studies.¹⁻⁴ Both PVI and IgM/C3 deposition were associated with RF. The increasing number of perivascular cells in patients with high titres of RF (Table 4) can be regarded as an argument for a causal relation. Our data indicate a weak association of RF with HLA-DR3 and DR4 (Tables 3 and 5), not reaching statistical significance. As the connection between HLA-DR4 and RF is in agreement with observations of others¹³⁻¹⁷ we believe it is not likely to have resulted from chance. As higher titres of RF are associated with both PVI and HLA-DR4 an increased prevalence of this genetic marker in patients with dense infiltrates is expected. Although not reaching statistical significance, HLA-DR4 was indeed more frequently present in patients with infiltrates of more than six cells. HLA-DR3 also appears to be associated with PVI, but it is more correct to state that the prevalence of HLA-DR3 was in fact only decreased in patients with 0-3 cells as the overall prevalence in the other groups did not exceed that in healthy controls.

From a pathogenetic point of view as well as from a practical one it is important to address the question of what comes first: RF or vasculitis? Intravenous administration of RF to rats is followed by diapedesis of leucocytes from venules and capillaries and may induce cutaneous inflammation.²⁵⁻²⁶ When this observation is extended to patients with RA one may propose a hypothesis of circulating (IgM) RF being trapped or deposited in the vessel wall, activating the complement system, and attracting leucocytes.³ The demonstration of IgM or C3, or both, in most patients with PVI (Table 2) is in

agreement with this hypothesis but does not prove that deposition or formation of immune complexes in the vessel wall precedes cellular infiltration.

If HLA-DR4 is a marker for the genetic basis of a more severe disease process, including high titres of RF and PVI, one would expect a stronger association with the primary disease manifestation. Our finding that HLA-DR4 is more prevalent in patients with RF than in patients with perivascular inflammation supports the hypothesis that PVI is secondary to deposition of circulating RF.

In the management of patients with RA information on the presence of PVI will only be helpful if clear associations with certain disease manifestations or prognosis can be demonstrated. Westedt *et al* found correlation of PVI with active joint inflammation using the Ritchie index.^{4,7} Disease activity in our patients, as measured by the ESR, was not associated with the presence or degree of perivascular inflammation. A possible prognostic significance of PVI has been evaluated in two studies.^{4,6} Neither PVI nor skin vessel wall immune deposits were predictive of change in activity of joint disease or development of extra-articular manifestations. In our patient group, after an average follow up period of 7 (1-10) years, 2/86 patients with 0-6 perivascular cells, and 3/28 patients with >6 cells had developed minor clinical signs of vasculitis: skin ulcers, combined with nail fold lesions in two. These five patients were all seropositive, two had ANF, while HLA-DR4 was present in 4/4 and HLA-DR3 in 0/4. In our opinion this outcome does not indicate a useful prognostic significance of skin biopsies.

In conclusion, perivascular cellular infiltration in apparently normal skin of patients with RA is associated with some of the symptoms that mark a more severe disease course. HLA-DR4 has also been associated with these symptoms, but its prevalence is only marginally increased in patients with perivascular inflammation. The perivascular infiltrates are probably secondary to the presence and quantity of circulating RF.

It is not likely that skin biopsies will provide a better insight into the prognosis of RA for individual patients. To identify patients with a more severe disease course or a high risk of developing extra-articular symptoms other tests like RF and ANF can be obtained more easily and are probably better discriminators.

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