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Immunopathological abnormalities in the normal skin of patients with rheumatoid arthritis in relation to clinical and serological findings: a one year follow up study

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SUMMARY Fifty two patients with seropositive rheumatoid arthritis (RA) were studied over a period of one year to investigate possible relationships among changes of circulating immune complexes (CIC), deposits of immunoglobulins and complement around the cutaneous blood vessels, clinical activity of the disease, and the presence of extra-articular manifestations (EAM). The presence or absence of IgM and C3 in and around the cutaneous blood vessels correlated significantly with the presence or absence of extra-articular features in cross sectional and longitudinal studies. Patients with evidence of these cutaneous immune deposits also had a greater prevalence of CIC as determined by the C1q binding assay (C1qBA) or polyethylene glycol (PEG) assay for IC containing IgM (IgM IC). Although the degree of perivascular mononuclear cell infiltration around the blood vessels in the papillary dermis was related to the patients' clinical state at the initial assessment, it did not correlate with the later changes in the activity of the joint disease or the occurrence of EAM. Thus the deposition of immunoglobulin or complement, or both, seems to be independent of cellular infiltration. The meaning of these cellular infiltrates is not yet fully understood. Our study has shown that many patients with RA who appeared to have only joint disease in fact had subclinical systemic disease as reflected by a positive skin biopsy or CIC. Moreover, the disappearance of IgM deposits from the skin correlated with the disappearance of EAM and improvement of joint disease.

Key words: perivascular immune deposits, immune complexes, perivascular mononuclear cell infiltrates.

The presence of immunoglobulin (IgM) and complement components in and around the cutaneous blood vessels of the clinically uninvolved skin of patients with RA has frequently been reported.[1–5] These deposits have been found to correlate with a higher prevalence of extra-articular manifestations (EAM), especially rheumatoid vasculitis (RV),[2 3 5] and with increased disease activity.[6] Raised levels of circulating immune complexes (CIC) are also associated with an active disease process and with the presence of EAM in patients with seropositive RA.[7 8]

Patients with RV have significantly higher levels of CIC than do patients without clinical signs of vasculitis.[9] It is possible that the IC present in patients without clinically manifest vasculitis cause subclinical damage to dermal blood vessels that is insufficient to produce vasculitis. This hypothesis is supported by the finding of perivascular deposits of immunoglobulins in clinically uninvolved skin of seropositive RA patients with CIC, many of whom also show perivascular lymphocytic infiltrates.[6] Furthermore, the injection of histamine into the skin of seropositive RA patients with detectable IC can produce overt vasculitis.[10] In an attempt to find

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out whether the presence of these vascular abnormalities (IgM deposition and perivascular lymphocytic infiltrate) has clinical relevance we followed up 52 patients with seropositive RA for one year. Uninvolved skin of the upper arm was biopsied at entry to the study and at the end of the study year. The patients were also given a physical examination for scoring of joint activity and the presence of EAM and, at the time of the biopsies, blood was drawn for determination of IC and for other laboratory tests.

Patients and methods

Fifty two patients with definite or classical seropositive RA[11] (mean age 51 years, range 18–73 years) were followed up for one year. Informed consent was obtained from all patients.

In 30 of these patients the duration of the disease was less than one year. They entered the study before of just after therapy with a second line drug (either an antimalarial agent, gold, or D-penicillamine) was started. Fifteen patients, designated as established RA, had a disease duration of more than two years, 12 of them were already receiving disease modifying drugs (antimalarial drugs, gold, or D-penicillamine). One patient was given an antimalarial drug after the first assessment and two patients were given only non-steroidal anti-inflammatory drugs (NSAIDs). Seven patients had leucocytoclastic vasculitis in venules or small arteries, or both, as established in tissue sections of biopsy material (skin or muscle). Four of these latter patients were treated with corticosteroids (prednisone 10 mg/day) combined with cytostatics (two patients azathioprine 2 mg/kg/day and one patient cyclophosphamide) and one patient received corticosteroids (prednisone 5 mg/day) combined with D-penicillamine. The other two patients with vasculitis received only NSAIDs.

All patients were examined at entry to the study and a year later, with special attention to the presence of EAM. Joint activity was scored according to the Ritchie articular index.[12] Active joint disease was considered to be present when the Ritchie articular index score was higher than 15. Serum samples were obtained and a biopsy specimen of clinically uninvolved skin of the upper arm was taken on the day of the physical examination. IC were detected by the ^{125}I-C1q binding assay (C1qBA)[13] and a polyethylene glycol test detecting IC containing IgM (IgM IC).[14]

The lower normal limit for C1qBA was considered to be 20 μg eq AIgG/ml. One part of the biopsy specimen of the skin was snap frozen in liquid nitrogen, cut into 4–6 μm thick sections at −20°C, and stained with monospecific fluorescein isothiocyanate conjugated rabbit antisera to human IgG (Nordic Immunological Laboratories, Tilburg, The Netherlands), IgM, IgA, or C3 (Dakopatts, Copenhagen, Denmark) according to established methods.[15] The other part of the biopsy specimen was fixed in 10% formaldehyde, and the sections were stained with haematoxylin and eosin for scoring the infiltration of mononuclear cells into the dermal vessels as described eslewhere.[6 15] The observer evaluated the biopsy results without knowledge of the clinical state of the patient.

Results

Of the total group of 52 patients, 19 showed deposition of IgM or C3, or both, in and around the blood vessels in the papillary dermis both at the start and after one year of observation (Table 1). Of these 19 patients, eight had an 'early' disease process, seven had established RA, and four patients had an active and recurrent vasculitis despite treatment.

Fifteen other patients showed IgM or C3 deposits,

Table 1 *Signs of deposition of IgM or C3, or both, in skin biopsy specimens in relation to extra-articular manifestations (EAM) in 52 patients with RA during a follow up period of one year*

Immuno-fluores-cence	*No of patients*	*Patients with:*			*EAM*			
		Early RA	*Established RA*	*RV*	+/+*	+/−	−/+	−/−
+/+*	19	8	7	4	**15**	1	1	2
+/−	15	11	1	3	1	**14**	—	—
−/+	2	2	—	—	—	—	**1**	1
−/−	16	9	7	—	3	—	1	**12**
Total	52	30	15	7	19	15	3	15

*Start/end of the study; +=present; −=absent.
Association between the presence of IgM or complement, or both, and AEM: $p<0.001$ (χ^2 test).

Table 2 *Signs of deposition of IgM or C3, or both, in skin biopsy specimens in relation to the articular disease activity in 52 patients with RA during a follow up period of one year*

Immuno-fluorescence	*No of patients*	*Activity**			
		+/+†	+/−	−/+	−/−
+/+†	19	**7**	5	4	3
+/−	15	2	**9**	—	4
−/+	2	—	—	**1**	1
−/−	16	2	5	1	**8**
Total	52	11	19	6	16

*+=Ritchie index >15, −=Ritchie index ≤15.
†Start/end of study.
Association between the prescence of IgM or C3, or both, and disease activity: $p<0.05$ (χ^2 test).

or both, in the skin at the initial assessment but none a year later. Of these 15 patients, 11 had 'early' disease, one had well established RA, and three had RV. In these last three patients, who had received corticosteroids and cytostatics, the signs of vasculitis had disappeared. Two of the 52 patients showed no signs of deposition of IgM or C3 at the start of the study, but such deposits were present a year later. These patients belonged to the 'early' RA group. Sixteen patients were negative initially and still negative after a year; nine of these patients belonged to the 'early' disease group and seven had established RA.

A strong positive association was found for all patients between the presence of EAM and the presence of deposits of IgM and C3 in and around the blood vessels at both assessments (Table 1) and changes in these two parameters were usually in parallel ($p<0.001$). For example, of the 19 patients showing positive immunoglobulin deposition in and around the blood vessels both at the beginning and at the end of the study, 15 also showed persistent EAM. Of the 15 patients showing positive immunoglobulin deposition only at the start of the study, there were 14 patients in whom the EAM disappeared after one year, etc. A less significant association was seen between changes in the activity of the joint disease and changes in the deposits ($p<0.05$) (Table 2).

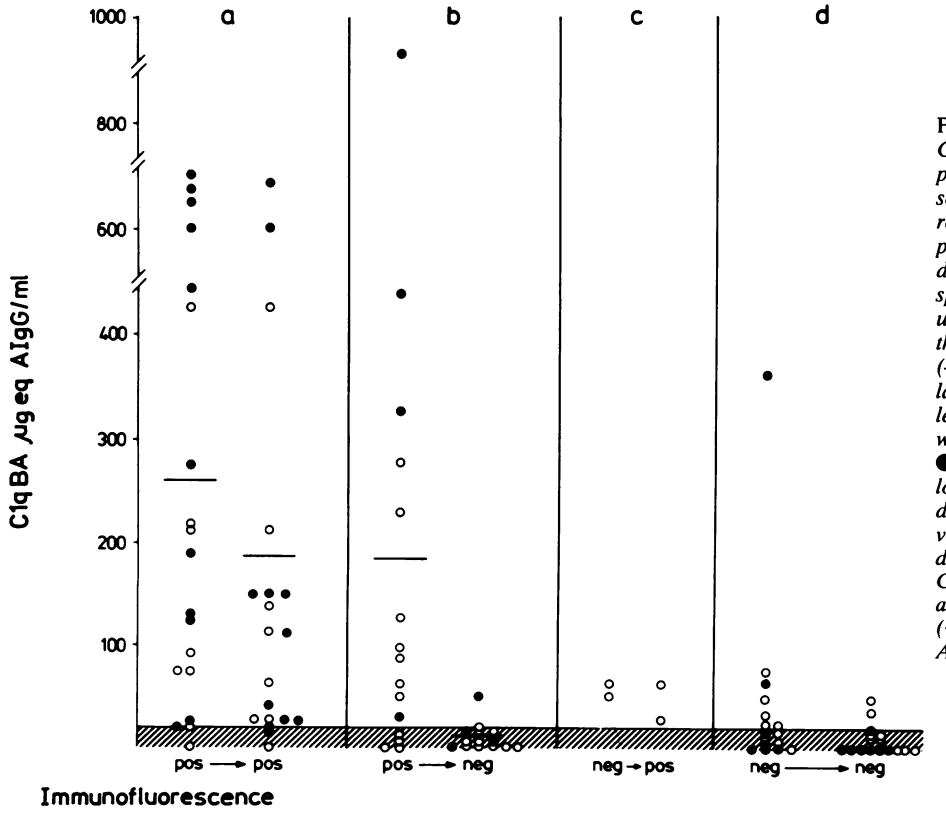

Fig. 1 *Levels of CIC (C1qBA) in patients with seropositive RA in relation to the presence of IgM deposits in biopsy specimens of uninvolved skin at the initial assessment (→) and one year later. Bars=mean levels; ○=patients with early disease; ●=patients with longstanding disease. The normal value for CIC determined by C1qBA is indicated as a hatched area (<20 µg eq AIgG/ml).*

The presence of IC assayed with the C1qBA showed correlation with the presence of deposits in the dermal blood vessels (Fig. 1). Of the 19 patients with IgM or C3 deposits, or both, in both skin biopsy specimens, 18 had circulating IC detected by the C1qBA at the initial evaluation (Fig. 1a) and 17 were still positive in this respect at the second evaluation. Of the 15 patients whose biopsy specimens converted from positive immunofluorescence (IF) to negative IF, 11 had circulating IC at the initial assessment and two also at the second, whereas 13 patients showed neither IF nor IC at the second evaluation (Fig. 1b). Two patients who initially lacked but later showed immunoglobulin deposits in the skin had low levels of IC on both occasions (Fig. 1c), but the 16 patients with no detectable IgM or C3 at both assessments had significantly lower levels of circulating IC compared with patients with immunoglobulin deposits around the vessels. Similar associations were found for the IC containing IgM. Although fewer patients had circulating IgM IC when IgM and C3 deposits were found in the skin biopsy specimen, those with negative C1qBA results and positive skin biopsies nevertheless had IC containing IgM in the circulation. Thus when IgM and C3 were demonstrated in and around the dermal blood vessels, IC were always detectable by the C1qBA or IgM PEG assay, or both. The reverse did not always hold true: positive IC tests were not always accompanied by demonstrable amounts of IgM and C3 in the skin.

Deposits of IgG or IgA were rarely found in the non-involved skin biopsy specimen from these RA patients. When present, the intensity of fluorescence was very weak and limited to only a few vessels.

Perivascular mononuclear cell infiltrates were found in 36 of the 52 patients (69%) at the initial observation and at the second biopsy in 36 out of 50 patients (72%) (for the other two patients the second formalin fixed biopsy specimen was not available). As reported earlier,[6] the degree of mononuclear cell infiltration was related to the patient's clinical status at the initial assessment. No correlation was found between changes in the score for infiltrate at the second biopsy and either joint activity or the presence of IgM or C3 deposits (Table 3). In 14 patients the infiltrate score changed in the same direction as the disease activity, and nine patients showed no change in either activity or score. Seventeen patients with RA showed no change in clinical status but the infiltrate score changed (an increase in 10 and a decrease in seven), whereas in 10 the score was unaltered but the disease activity changed from active to inactive (n=7) or from inactive to active (n=3) (Table 3).

Table 3 *Changes in joint activity in relation to changes in infiltrate score*

Activity	*Infiltrate score*		
	Increase	*Decrease*	*No change*
Increase (RI ↑ ≥15)*	3	0	3
Decrease (RI ↓ <15)†	0	11	7
No change	10	7	9

*Joint activity increased from inactive to active disease.
†Joint activity decreased from active to inactive disease.
RI=Ritchie index.

In addition, no significant correlation was found when the individual values for joint activity were analysed in relation to the corresponding infiltrate score.

Discussion

The results of the present longitudinal study confirm our earlier findings and those of others showing immune components (mainly IgM or C3, or both) in and around blood vessels of the clinically uninvolved skin of patients with RA.[1–6] In our patients the deposition of immunoglobulin or complement, or both, in the dermal blood vessels was not only associated with EAM at the first observation but also ran parallel with the occurrence of EAM and circulating IC with time. These changes were most prominent in the patients with 'early' RA, probably because they had just started treatment with antiphlogistic and remission inducing drugs. The same associations were, however, seen in the group of patients with vasculitis, who were given a more aggressive form of therapy to control the vasculitis at the beginning of the study. Correlations between the presence of perivascular immunoglobulin deposits and clinical features have been found by several authors, though the frequency and the association with specific kinds of EAM have varied widely (25–70%) between studies.[1–6] In our patients this variation may have been related to the severity of the disease or to the large number of patients with an early disease process.

The finding that circulating IC (C1qBA or IC containing IgM, or both) showed correlation with the presence of complement or IgM deposits, or both, provides indirect evidence that rheumatoid factor (RF) containing IC may be involved in the pathogenesis of damage to small blood vessels since human RF can react with heterologous immune complexes in vivo and contribute to inflammation in experimental immune vasculitis.[16] This hypothesis too is supported by the finding that vasculitis and

deposition of IgM in the vessel walls can be elicited by intradermal injection of histamine into seropositive RA patients with circulating IC demonstrated by the C1qBA[10] but not in RA patients without detectable circulating IC and RF.

The perivascular infiltration of mononuclear cells found in the upper dermal area in uninvolved skin, which has also been observed by others,[4 17] showed only a statistically non-significant tendency to correspond with disease articular activity. The mechanism responsible for the appearance of these infiltrates is not known, but could be a response to chemotactic factors released by cell degeneration or a reaction to deposition of immunoreactants in the vessel walls. Immunohistological stains showed that most of the mononuclear cells in the initial biopsy specimens bore the pan-T-lymphocyte marker (Leu 1) and only a few were monocytes.[6]

T cells are able to enter inflammatory sites in the skin much more easily than B cells.[18] A similar subclinical vasculopathy has been found in renal transplant recipients with an active cytomegalovirus infection. In these patients the vasculopathy was correlated with RF production and circulating IC containing IgM (Baldwin, manuscript submitted). Furthermore, it is possible that in the last mentioned study the lymphocytic infiltrate appeared after the deposition of globulins and reflected a secondary event.

The present findings raise the question as to whether rheumatoid disease is a continuous spectrum with systemic manifestation occurring in a few patients or represents a composite of separate diseases. Our study has shown that many patients who appear to have only joint disease can in fact have a concurrent subclinical systemic disease. In particular in the early cases it was confirmed by 'laboratory signs' of systemic disease, i.e., a positive skin biopsy and the presence of CIC, but in most of these patients clinical manifestations remained absent during the year of the study. Furthermore, CIC which correlated with the activity of the joint disease[19] were present in some patients who had not developed clinically manifest EAM during a longitudinal study lasting two years. If RA is a continuous spectrum, why do only some of the patients develop clinical vasculitis or systemic disease? The localisation of immune complexes can be influenced by many factors; for example, the composition of the CIC may differ between patients with prominent vasculitis and those without clinical vasculitis.[19]

In studies on mononuclear cells from patients with active RV the C3b and Fc receptors showed a defective ability to ingest and degrade soluble complexes of IgG in vitro.[23] Some patients with active joint disease and raised levels of CIC had similarly defective mononuclear cells.[23] There is also some evidence indicating that immunogenetic factors can regulate the occurrence of certain aspects of systemic disease in patients with RA.[20–22]

More studies must be performed before it can be determined whether a group of RA patients with a high risk of developing clinical vasculitis can be defined prospectively by characteristics such as repeated demonstration of immune deposits in the papillary vessels of uninvolved skin, defective degradation of IC by mononuclear cells in vitro, and a HLA-DR4 phenotype. If such definition proves possible, the institution of a more aggressive therapy at the onset of the disease might be warranted in these patients.

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