Functional defects of monocyte C3b receptor-mediated phagocytosis in rheumatoid arthritis (RA): evidence for an association with the appearance of a circulating population of non-specific esterase-negative mononuclear phagocytes

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SUMMARY We have previously described a selective defect of monocyte C3b receptor-mediated phagocytosis in patients with rheumatoid cutaneous vasculitis. We have studied a further 15 rheumatoid arthritis patients with other associated diseases and complications and have identified 4 further patients with a similar defect. Serological and cytochemical studies suggest that the defect in phagocytosis is due to the appearance of increased numbers of large nonspecific esterase-negative mononuclear phagocytes with defective C3b receptor phagocytic function rather than to receptor blockade by immune complexes.

Many of the extra-articular manifestations of rheumatoid arthritis including cutaneous vasculitis are thought to be initiated by circulating immune complexes (CIC). The cells responsible for the clearance of CICs are the mononuclear phagocytes of the spleen and liver, which are derived from bone marrow, and whose immediate precursors are the blood monocyte. The episodic nature of clinical manifestations suggests the possibility of intermittent saturation of these clearance mechanisms. Previous studies of in-vitro phagocytic function of blood monocytes in rheumatoid arthritis have shown that during cutaneous vasculitis there is depression of C3b receptor-mediated phagocytosis but no abnormality of Fc receptor phagocytic function and no direct evidence of receptor blockade.

We have therefore examined the alternative possibility that alteration in C3b receptor-mediated phagocytosis in patients with rheumatoid vasculitis is due to the presence of a subpopulation of immature monocytes. In addition we have studied a group of RA patients with miscellaneous complications and associated diseases to determine whether the phagocytic defect is found in clinical conditions other than vasculitis.

Patients and methods

Twenty-three healthy hospital employees (10 male, 13 female), mean age 32-2 years (range 21-64 years), and 24 patients (12 male, 12 female), mean age 59-4 years (range 35-73 years), with classical or definite rheumatoid arthritis were studied. Four groups of patients were studied: group A—active vasculitis (n = 9); group B—inactive vasculitis (n = 5); group C—multiple nodules (n = 9); group D—miscellaneous associated complications and diseases; comprising pyoderma gangrenosum (n = 2), pericarditis (n = 1), primary biliary cirrhosis (PBC) (n = 1), pyarthrosis, sacral ulceration and secondary Sjögren’s syndrome (n = 1), paravertebral abscess (n = 1), Felty’s syndrome (n = 1). The erythrocyte sedimentation rates and rheumatoid factor titres were comparable in each of these 4 groups of patients (Table 1). Details of drug therapy are shown in Table 2.
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Table 1 RA patients—serology

<table>
<thead>
<tr>
<th>RA patient groups</th>
<th>n</th>
<th>ESR, mm in 1st hour</th>
<th>Rheumatoid factor titre</th>
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<tr>
<td></td>
<td></td>
<td>Mean</td>
<td>Range</td>
</tr>
<tr>
<td>A—Active vasculitis</td>
<td>9</td>
<td>82</td>
<td>51–110</td>
</tr>
<tr>
<td>B—Inactive vasculitis</td>
<td>5</td>
<td>79</td>
<td>63–111</td>
</tr>
<tr>
<td>C—Multiple nodules</td>
<td>9</td>
<td>63</td>
<td>21–112</td>
</tr>
<tr>
<td>D—Miscellaneous complications</td>
<td>7</td>
<td>85</td>
<td>27–127</td>
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Table 2 RA patients—drug therapy

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Patient groups†</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
</tr>
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<tbody>
<tr>
<td>NSAID alone</td>
<td></td>
<td>3</td>
<td>1</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>Prednisolone (&lt;10 mg o.d.)</td>
<td></td>
<td>3</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Penicillamine (500 mg o.d.)</td>
<td></td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Prednisolone (&lt;7.5 mg o.d.) + penicillamine (≤500 mg o.d.)</td>
<td></td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Prednisolone (5 mg o.d.) + chloroquine (250 mg o.d.)</td>
<td></td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Myocrisin* (600 mg total dose)</td>
<td></td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

* Sodium aurothiomalate.
† Patient group A—active vasculitis; B—inactive vasculitis; C—multiple nodules; D—miscellaneous complications.

NSAID = nonsteroidal anti-inflammatory drugs

Monocyte separation. Mononuclear cells were separated from 10 ml of venous blood by means of a density gradient, washed, and resuspended in Hanks's balanced salt solution with 0.1% gelatin (HBSS). The concentration of monocytes present was determined by a rapid Coulter sizing technique and, where indicated in the results, by combined nonspecific esterase (NSE) and AS-D chloroacetate esterase staining. The final concentration of monocytes in the phagocytic assay lay between 10⁶ and 2·0 × 10⁶ monocytes/ml.

Yeast opsonisation. Heat-killed yeast particles were preopsonised in bulk, resuspended to 10³/ml, and stored in 1 ml aliquots in liquid nitrogen. The final concentration of yeast in the phagocytic assay was approximately 0·5 × 10⁶ yeast/ml. Preopsonisation was carried out as follows:

(i) Candida albicans opsonised in pooled human IgG (SE Scotland Blood Transfusion Service) in a ratio of 5 × 10⁶ yeast/25 mg IgG/ml of phosphate buffered saline (PBS) provided a particle coated only with IgG;

(ii) Saccharomyces cerevisiae opsonised in serum obtained from a single healthy human donor in a ratio of 10⁶ yeast/0·4 ml serum provided a particle coated only with complement (C3b).

The presence of these opsonins alone was confirmed by direct immunofluorescence. Furthermore, heating the serum at 58°C for 30 minutes resulted in complete loss of opsonising capacity, while similar treatment had no effect on the pooled human IgG. In each case dose response curves were performed to establish the optimum ratio of yeast to opsonin for maximum phagocytic rates. There was no significant phagocytosis of unopsonised yeast, confirming that immune recognition by either the Fc or C3b receptor was essential for rapid uptake.

Phagocytic assay. Phagocytosis was measured by a modification of the method of Leijh et al. Equal volumes (150 μl) of mononuclear cells and yeast particles were dispensed into 6 400-μl wells machined in a Teflon block and incubated at 37°C under rotation. Aliquots of cell suspension were removed immediately after mixing and at timed intervals, diluted in counting fluid (2% acetic acid plus gentian violet), and numbers of yeast remaining extracellular were counted in haemocytometers. The rate of phagocytosis was determined by the fall in extracellular yeast concentration. The method was validated for both C3b receptor and Fc receptor-mediated uptake by means of metabolic controls and electron microscopy. Pilot studies showed the kinetics of phagocytosis to be second order and that a rate constant K, which is a measure of the efficiency of phagocytosis, could be obtained from the expression:

\[ K = \frac{1}{t} \times \frac{1}{[M_0]} \times \ln \frac{N_0}{N(t)} \text{ ml/min/monocyte} \]

by measuring changes in the number of yeast (N) over a 20-minute interval (t) in the presence of a known monocyte concentration (M₀).

The rate constant for C3b-mediated uptake (Kc) was measured in controls and patients with the C3b coated yeast and similarly for Fc-mediated uptake (KFc) with the IgG-coated yeast.

Serological studies. Serum drawn at the time of the phagocytic assay was stored in liquid nitrogen for subsequent measurement of serum C3 and C4 concentrations by radial immunodiffusion, C1q binding activity, and anti-complementary activity. Rheumatoid factor (RF) titres were measured with a sensitised human red cell agglutination assay.


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Statistical methods. Student's t-test and Fisher's exact probability test were used where appropriate.

Results

Fc receptor mediated phagocytosis (KFc)
No significant differences in the rate of Fc receptor-mediated phagocytosis were found between the normal controls and any of the groups of patients studied (Fig. 1).

C3b receptor mediated phagocytosis (Kc)
All patients with cutaneous vasculitis had reduction of Kc compared with normal controls, while 5 patients with inactive vasculitis had normal Kc (Fig. 2, A and B). Two of these patients (I and II) were studied initially when vasculitis was active and again later after cessation of vasculitis; a third (III) was studied before development of vasculitis and later during an episode of vasculitis; and the remaining 2 patients were studied only in the convalescent phase after an episode of vasculitis (Fig. 3).

Two patients (IV and V) with vasculitis; had a further episode some weeks later and were restudied. On each occasion Kc was found to be reduced (Fig. 3).

None of the 9 patients with multiple nodules had reduced Kc (Fig. 2, C). A significant proportion (4/7) with extra-articular manifestations other than vasculitis were found to have rates of C3b-receptor-mediated phagocytosis below the normal range; 2 had pyoderma gangrenosum, one had pericarditis,
and the fourth multiple rheumatoid nodules and primary biliary cirrhosis (Fig. 2, D). Of 3 remaining patients with normal Kc one had Felty's syndrome and 2 had serious pyogenic infections (Fig. 2, D).

Serological Studies

Patients with vasculitis and low Kc had significantly lower mean serum C3 levels and C4 levels and higher ACA titres than patients with normal Kc (Fig. 4), although many of the latter also had subnormal complement levels and elevated ACA titres. Furthermore, serum ACA titres and mean serum C3 and C4 levels in the 4 RA patients with reduced Kc who did not have vasculitis were no different from RA patients with normal Kc (Fig. 4). Thus reduction of Kc was not invariably accompanied by evidence of complement activation and low serum complement levels.

Results of C1qba were available from 29 phagocytic studies (22 patients). Although a negative correlation was found between Kc and C1qba (Fig. 5), it is clear that some patients with gross elevation of C1qba had normal Kc.

Serial studies of 5 patients who had episodes of vasculitis were also carried out to examine the relationship between Kc, serum complement, and tests for immune complexes. The results are shown in Fig. 3. In patient I remission of vasculitis was accompanied by a rise in Kc and serum C3 and concomitant fall in C1qba and serum ACA. Serum C4 levels showed a small but insignificant fall. Patient II was first studied during active vasculitis, and her subsequent clinical recovery was marked by a rise in Kc, serum C3, and C4. However, C1qba and ACA remained high, and 16 weeks later this patient developed pyoderma gangrenosum. The appearance of pyoderma gangrenosum was accompanied by a further fall in Kc and a rise in ACA. In view of the rise in ACA the further rise in serum C3 levels was
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Table 3  Percentage of neutrophil polymorphs (PMN) contaminating mixed mononuclear cell preparations estimated by AS-D chloroacetate esterase staining

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>% PMN (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal subjects</td>
<td>8</td>
<td>0·5 (0·2-0)</td>
</tr>
<tr>
<td>RA—normal Kc</td>
<td>9</td>
<td>0·4 (0·4-0)</td>
</tr>
<tr>
<td>RA—reduced Kc</td>
<td>5</td>
<td>1·0 (0·3-0)</td>
</tr>
</tbody>
</table>

Table 4  Percentage of monocytes (mononuclear cells >250 μm²) in mixed mononuclear cell preparations estimated by Coulter sizing

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>% Monocyte (SD)</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal subjects</td>
<td>13</td>
<td>24·2</td>
<td>3·9</td>
</tr>
<tr>
<td>RA—normal Kc</td>
<td>17</td>
<td>34·8</td>
<td>8·0</td>
</tr>
<tr>
<td>RA—reduced Kc</td>
<td>15</td>
<td>33·9</td>
<td>7·0</td>
</tr>
</tbody>
</table>

SD = standard deviation.

unexpected. In patient III the appearance of vasculitis was accompanied by a marked fall in Kc, serum C3 and C4, and rise in C1qba. However, ACA surprisingly fell to zero. In 2 patients, IV and V, with recurrent vasculitis Kc and serum C3 levels remained depressed and serum ACA and C1qba remained elevated. Serum C4 levels remained unchanged.

Relationship of Kc and KFc to differential monocyte counts determined by Coulter sizing and nonspecific esterase (NSE) staining

AS-D chloroacetate esterase staining of mononuclear cell preparations showed few neutrophil granulocytes (Table 3). Large (>250 μm²) mononuclear cells were more frequent in RA patients than in the normal controls (p<0·001; Student's t test), but there was no significant difference between RA patients with normal Kc and those with reduced Kc (p<0·5; Student's t test) (Table 4). The proportion of monocytes staining with NSE from normal controls and RA patients with normal Kc correlated well with the proportion of large cells (Fig. 6).

In patients with reduced Kc, however, the percentage of monocytes (mean ± SD) determined by NSE staining (19·0 ± 5·3) was significantly less than that obtained by Coulter sizing (33·9 ± 7·0) (p<0·001; Student's t test). Furthermore, the rate of C3b receptor mediated phagocytosis declined in direct proportion to the discrepancy between the Coulter and NSE differential counts (Fig. 7), while KFc remained unchanged.

Effect of drug therapy

Drug therapy did not appear to have any effect on results of phagocytic studies (Table 2). Five patients with reduced Kc and 4 with normal Kc were taking prednisolone in stable dosage either alone or in combination with another drug. Three patients with

Fig. 6  Percentage of monocytes in mixed mononuclear cell populations; percentage of monocytes determined by nonspecific esterase stain (NSE) correlates well with percentage determined by Coulter sizing in both normal controls (n = 19, r = 0·78, p<0·001) and RA patients (n = 24, r = 0·71, p<0·001).

Fig. 7  Correlation between reduction in Kc and percentage of large mononuclear cells (>250 μm²) failing to stain for nonspecific esterase.
reduced Kc and 2 with normal Kc were taking penicillamine. Two further patients with normal Kc were on gold and chloroquine respectively.

Discussion

We have extended and confirmed our original observation of reduction of monocyte C3b receptor-mediated phagocytic function in patients with RA and cutaneous vasculitis.

In addition, we have studied 16 RA patients who did not have vasculitis, and 4 of these had a similar phagocytic defect. Two of the 4 had pyoderma gangrenosum, a known association of both RA and seronegative arthritis, one had nodular RA in association with primary biliary cirrhosis, and the fourth had nodular RA complicated by pericarditis. None of the 12 remaining patients, who included 2 with pyogenic infection, had a phagocytic defect. In contrast to studies which we have carried out in patients with systemic lupus erythematosus we have not found any significant alteration in Fc receptor phagocytic function.

The studies in patients with RA and reduced C3b-mediated phagocytosis do not suggest receptor blockade. Hypocomplementaemia and raised serum ACA and C1qba were features of many of the patients with reduced C3b receptor-mediated phagocytosis. However, reduced Kc did not always correlate with these serological changes, and of 4 patients with reduced Kc without vasculitis 2 had normal serum C3 and C4 levels and 3 had no increase in ACA. Conversely, normal Kc was seen in some patients with very high levels of C1qba, lowered serum C3 and C4 levels, and elevated ACA titres. Thus the association between serological evidence of complement fixing CICs and reduced Kc was not invariant. Furthermore, we have found no evidence for membrane bound or cytoplasmic CICs by direct immunofluorescence on monocytes from patients with vasculitis and reduced Kc.

An alternative explanation is that reduced Kc is due to the presence of a subpopulation of monocytes with reduced C3b receptor function. This is supported by the demonstration that reduced Kc correlated with increasing numbers of NSE negative, large (>250 μm²) mononuclear cells. Since KFc remained normal, the implication is that these NSE-negative cells have a phagocytic Fc receptor but functionally inactive or absent C3b receptors.

Horwitz and Steagall reported increased numbers of nonphagocytic, NSE-negative monocyte precursors in the peripheral blood of patients with RA. They used latex particles to measure phagocytic function, and Fc and C3b receptor function was not tested directly. Thus a direct comparison with our data is not possible. More recent studies of the functional and cytochemical characteristics of monocyte precursors have produced conflicting data. Lohmann-Matthes et al. have identified a murine bone marrow derived monocyte precursor which is nonadherent, nonphagocytic, and NSE-negative and has natural killer cell (NK) activity. They have shown that this NSE-negative cell matures into a typical NSE-positive mononuclear phagocyte on culture. These observations have been extended to human peripheral blood, where a similar nonadherent, NSE-negative population with NK activity has been identified which also matures into an NSE-positive macrophage. The phagocytic function of this cell was not studied. On the other hand studies of human bone marrow cells identified the human promonocyte as a large NSE-positive cell with a phagocytic Fc receptor. These cells carry complement receptors which have little if any phagocytic activity. The cytochemical and functional properties of human monoblasts have not been fully characterised. Studies of murine marrow precursors showed that only 30% of promonocytes are NSE-positive and are less phagocytic than their human counterparts.

Thus, while some human blood monocyte precursors are NSE-positive cells with immature C3b receptor phagocytic function, there is evidence to support the hypothesis that there may also be a population of NSE-negative mononuclear phagocytes.

We suggest that these observations have both practical and theoretical implications. From the practical standpoint it should not be assumed that changes in receptor function are due necessarily to blockade by immune complexes; they may be due to alteration in the composition of the cell population under study. The theoretical implication from these results is that there may be significant alteration in traffic of monocytes in rheumatoid arthritis. Although the subpopulation described may be arriving in the blood stream direct from the marrow, the possibility cannot be excluded that these cells may be derived from the thoracic duct and form part of a recirculating population of mononuclear cells.

Complement assays were performed by Dr K. C. Watson, of the Department of Bacteriology, Western General Hospital, Edinburgh. The work was supported by grants from the Arthritis and Rheumatism Council.

References

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