# CLINICAL RESEARCH

# Evidence for defect of complement-mediated phagocytosis by monocytes from patients with rheumatoid arthritis and cutaneous vasculitis

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## Abstract

**In-vitro measurements of the rate of monocyte phagocytosis of heat-killed yeast preopsonised in human AB serum from 14 patients with rheumatoid arthritis and 14 normal controls showed a significant reduction in five patients with active vasculitis but no change in nine with active arthritis alone. Further studies of complement- and Fc-mediated monocyte phagocytosis in which the rate constants ($K_c$ and $K_{Fc}$ respectively) were determined using complement-coated Saccharomyces cerevisiae and Candida albicans opsonised with IgG in monocytes from nine patients with rheumatoid vasculitis and 12 controls showed a significant reduction in $K_c$ ($p < 0.01$) but normal $K_{Fc}$. $K_c$ was normal in three patients with inactive vasculitis. Low $K_c$ was correlated with low serum C3 concentrations but not with Clq binding or anticomplementary activity, and no evidence of intracytoplasmic or membrane-bound immune complexes was detected in monocytes from patients with active vasculitis.**

**These results show that cutaneous vasculitis in rheumatoid arthritis is associated with selective impairment of complement-mediated monocyte phagocytosis, which does not appear to result from receptor blockade by immune complexes.**


## Introduction

Cutaneous vasculitis in rheumatoid arthritis is associated with hypocomplementaemia,[1] increased complement consumption,[2] large 19S IgM-containing circulating immune complexes,[3] and deposition of complement-containing immune complexes in small arterioles.[4] The episodic nature of clinical manifestations of vasculitis in rheumatoid arthritis suggests the possibility of intermittent saturation of immune clearance mechanisms. The cells thought to be responsible for clearance of circulating immune complexes are the mononuclear phagocytes of the spleen and liver.[5] As part of an investigation of mononuclear phagocyte function in rheumatoid arthritis we performed in-vitro measurements of monocyte phagocytosis in patients with uncomplicated rheumatoid arthritis, patients with rheumatoid cutaneous vasculitis, and normal controls.

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## Subjects and methods

Mononuclear cells were separated from 10 ml of venous blood using a density gradient,[6] washed, and resuspended in Hanks's balanced salt solution plus 0·1% gelatin. The concentration of monocytes present was determined using a rapid Coulter sizing technique[7] validated by comparison with differential cell counts performed with non-specific esterase stains.[8]

Heat-killed yeast were preopsonised in bulk, resuspended to $10^7$/ml, and stored in aliquots in liquid nitrogen. Direct immunofluorescence verified that *Candida albicans* opsonised in fresh human serum was coated with complement (C3) and immunoglobulins (IgG, IgA, and IgM) while *Saccharomyces cerevisiae* in human serum was coated only with complement (C3). To provide yeast coated with IgG alone *C albicans* was opsonised in pooled human IgG.

Phagocytosis was measured by a modification of the method of Leijh *et al.*[9] Equal volumes (150 µl) of mononuclear cells and yeast particles were dispensed into six wells (400 µl), 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 and diluted in counting fluid (2% acetic acid and gentian violet), and numbers of extracellular yeast remaining were counted in haemacytometers. The rate of phagocytosis was determined by the fall in yeast concentration. The method was validated using 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

$$K = \frac{1}{t \times [M\varphi]} \times \ln \frac{No}{N_t} \text{ ml/min/monocyte}$$

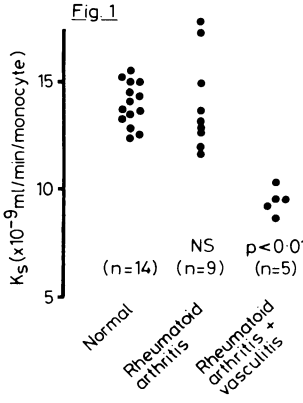
by measuring changes in the numbers of yeast (N) over a 20-minute interval (t) in the presence of a known monocyte concentration [Mφ]. The rate constant was measured initially using C *albicans* opsonised

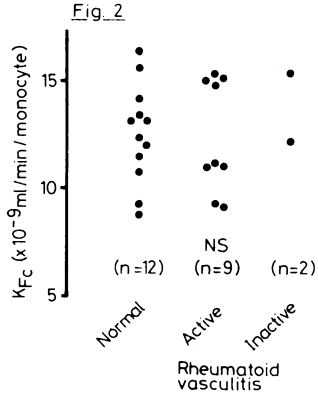
in human serum ($K_s$) from 14 normal controls and 14 patients with classical rheumatoid arthritis,[10] five of whom had active cutaneous vasculitis. Subsequently the rate constant was measured for Fc-mediated uptake ($K_{Fc}$) using *C albicans* opsonised in pooled human IgG and for C3-mediated uptake ($K_c$) using *S cerevisiae* opsonised in human serum from a group of 12 normal controls and nine patients with cutaneous vasculitis. Serum and EDTA plasma drawn at the time of the phagocytic assay were stored in liquid nitrogen for subsequent measurement of serum C3 and C4 concentrations (radial immunodiffusion; Seward Laboratories Immunostics), Clq binding activity,[11] and anticomplementary activity.[12]

Cytocentrifuge smears and washed cell suspensions of mononuclear cells were examined for the presence of intracytoplasmic immune complexes and membrane-associated immune complexes respectively by direct immunofluorescence using fluorescein conjugated antigammaglobulins and anti-β1C/β1A (Nordic Diagnostics).

## Results

In the initial study using *C albicans* opsonised with both C3 and immunoglobulins there was no difference in the rate constant $K_s$ between normal controls and patients with uncomplicated rheumatoid arthritis, but $K_s$ was significantly reduced ($p < 0.01$, rank sum test) in the patients with active vasculitis (fig 1). In subsequent studies, in which the rate constants for C3-mediated uptake ($K_c$) and Fc-mediated uptake ($K_{Fc}$) were measured separately, no change was found in $K_{Fc}$ in the patients with vasculitis compared with the normal controls (fig 2) but $K_c$ was significantly reduced ($p < 0.01$, rank sum test) (fig 3). In three subjects whose vasculitis was judged to be clinically inactive $K_c$ was in the normal range. All patients in whom $K_c$ was low had low serum C3 concentrations, while the three subjects with inactive vasculitis and normal $K_c$ had normal serum C3 concentrations (table). There was no clear correlation between a reduction in $K_c$ and results of immune complex assays. All patients had high Clq binding activity both during and after vasculitic episodes and all had raised anticomplementary activity during vasculitis (table). Two of the three patients with inactive vasculitis had persistently raised anticomplementary activity.



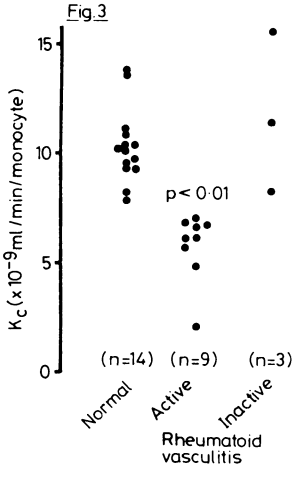




FIG 1—Monocyte phagocytic rate constant ($K_s$) in normal subjects and patients with rheumatoid arthritis and vasculitis.

FIG 2—Rate constant ($K_{Fc}$) for Fc-mediated phagocytosis in normal subjects and patients with active and inactive vasculitis.

FIG 3—Rate constant ($K_c$) for complement-mediated phagocytosis in normal subjects and patients with active and inactive vasculitis.

*Complement-mediated phagocytosis ($K_c$), serum complement (C3 and C4) concentrations, and results of immune complex assays in patients with rheumatoid arthritis and rheumatoid cutaneous vasculitis*

| Patients | $K_c$ (ml/min/monocyte) | C3 (mg/100 ml) | C4 (mg/100 ml) | Clq binding activity (%) | Anticomplementary activity titre |
|---|---|---|---|---|---|
| Uncomplicated rheumatoid arthritis | Normal | >123 | >33 | 0-90 | 0 |
| Active vasculitis (n = 9) | Low | 64-115 | 10-26 | 72-99 | 2-16 |
| Inactive vasculitis (n = 3) | Normal | >123 | 21-37 | 58-99 | 0-2 |

No intracytoplasmic or membrane-associated immune complexes were found in monocytes from four patients with active vasculitis and low $K_c$.

## Discussion

These studies appear to show for the first time selective functional depression of complement-mediated monocyte phagocytosis in patients with rheumatoid vasculitis. The synchronous depression of $K_c$ and serum C3 concentration in the presence of high Clq binding activity and anticomplementary activity might suggest that saturation of complement-mediated clearance mechanisms is associated with the appearance of clinical vasculitis in rheumatoid arthritis. A similar defect limited to complement-mediated clearance of coated red cells has recently been shown in vivo in patients with primary biliary cirrhosis,[13] a disease that is associated with circulating complexes containing IgM and IgG and with increased complement catabolism.[14] Large complexes containing 19S complement-fixing IgM are also found in rheumatoid vasculitis, but the relation is not constant and the appearance of vasculitis unpredictable.[3] The finding that complement-receptor-mediated phagocytosis is depressed in these patients while Fc-receptor function is unchanged might suggest that these complexes are binding preferentially to complement receptors and that binding of IgG Fc ligands is sterically inhibited by the presence of IgM rheumatoid factor. However, attempts to show the presence of surface-attached or intracytoplasmic immune complexes in monocytes from these patients by direct immunofluorescence have been unsuccessful.

An alternative explanation for these findings may be that rheumatoid vasculitis is associated with a change in the composition of the peripheral monocyte population and that the defect in complement-mediated phagocytosis is unrelated to the binding of immune complexes to these cells. Previous work[15] has shown the presence of circulating functionally immature monocyte precursors in patients with rheumatoid arthritis and systemic lupus erythematosus. We are currently exploring the relative maturation of complement and Fc-receptor function in monocytes from patients with these diseases.

Whatever the basis for the underlying defect of complement-mediated phagocytosis it could have direct relevance to the pathogenesis of rheumatoid vasculitis.

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# Hypercoagulation in glomerulonephritis

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## Abstract

**The clotting values of 50 patients with glomerulonephritis were examined. Three different coagulation groups were recognised: those with normal clotting values (group 1); those with high concentrations of factor VIII but otherwise normal clotting results (group 2); and patients who showed the presence of an activator of the intrinsic coagulation pathway, indicated by the presence of a short activated partial thromboplastin time or the ability of patients' plasma to shorten control clotting time in mixing studies (group 3). Patients in group 2 either had a uniform rise in all three components of the factor VIII molecule or a disproportionately higher concentration of factor-VIII-related antigen. In contrast, the level of VIII clotting activity in patients in group 3 was always higher than concentrations of either VIIIAg or VIIIWF. A significantly high incidence of thrombotic complications was observed in patients in group 3 but in none of the patients in either group 1 or group 2. Impaired renal function was more common in patients in groups 2 and 3, with higher mean serum creatinine concentrations in those in group 3.**

**Patients with glomerulonephritis who have a short partial thromboplastin time with kaolin or who shorten control clotting time form a subgroup in whom hypercoagulation could adversely affect the course of their disease. The value of antiplatelet or anticoagulant treatment in these patients needs to be explored.**

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## Introduction

The role of the coagulation process in the pathogenesis of glomerulonephritis and its complications have become increasingly recognised. Experimental evidence and findings on pathological examination of kidneys from patients with glomerulonephritis suggest that local intravascular coagulation plays an important role in the progression of the renal disease.[1] In these patients the presence of a systemic hypercoagulable state may contribute to the deterioration of renal function and may be associated with a high incidence of thrombotic complications. In patients with established nephrotic syndrome a high incidence of thromboembolic phenomena has been reported.[2] The observed high concentrations of factors V and VIII[3] and low concentrations of antithrombin III[4] are thought to be important in the pathogenesis of this complication. The clotting values of other patients with glomerulonephritis have, however, received little attention in recent reports. This paper reports an investigation of the clotting values of 50 patients with glomerulonephritis and establishes the incidence of a coagulation accelerating factor recognisable in vitro and its possible influence on the clinical course of these patients.

## Patients and methods

Fifty patients (24 men, 26 women) aged from 12 to 78 years (mean $37 \pm 16$) were examined. In all patients the diagnosis of glomerulonephritis was confirmed by renal biopsy. Two additional groups served as controls: (*a*) 20 healthy individuals (medical and laboratory personnel), and (*b*) 70 age- and sex-matched inpatients with various medical disorders. These inpatients included 20 patients with renal disease not characterised by glomerulonephritis but with a comparable degree of renal impairment as the study group and 50 with various disorders such as ischaemic heart disease, cerebrovascular accidents, chronic bronchitis, collagen disease, malignant lymphoma, and thyroid disease. None of the control patients were known to have a haemorrhagic tendency.

Blood was collected in 1/10 volume 3·8% trisodium citrate. Platelet-poor plasma was obtained by centrifugation at 2000 *g* for 15 minutes. Prothrombin, thrombin, and Stypven times were measured by standard methods. Partial thromboplastin time with kaolin (PTTK) was measured by incubating 0·1 ml of plasma with 0·05 ml kaolin 50 mg/ml and 0·05 ml Platelin (General Diagnostics) for 3 minutes