Enhanced bacterial phagocytosis by peripheral blood monocytes in rheumatoid arthritis

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SUMMARY To assess the functional state of peripheral blood monocytes in rheumatoid arthritis, we have measured phagocytosis of *Staphylococcus aureus* and *Proteus mirabilis* in 48 patients and 28 controls. Using radiolabelled bacteria preopsonised in normal human serum we have demonstrated significantly enhanced uptake of both organisms by patients' monocytes: (Median % uptake *Staph. aureus*: patients = 35.8; controls = 19.3; $p < 0.001$. Median % uptake *P. mirabilis*: patients = 32.3; controls = 19.8; $p < 0.01$.) These results indicate that patients' monocytes exist in an activated state, which may be important in the pathogenesis of rheumatoid arthritis.

Cells of the mononuclear phagocyte series (macrophages MO) are thought important in the pathogenesis of chronic inflammatory diseases including rheumatoid arthritis.¹ In addition to secreting proteolytic enzymes capable of cartilage destruction² MO also secrete factors capable of stimulating other cells to release inflammatory and proteolytic substances.³ In association with enzyme secretion other MO activities, including adherence, spreading, bacterial phagocytosis, and bacterial and tumour cell killing, are increased on stimulation.⁴ Type A synovial cells, which exhibit macrophage-like features,⁵ also secrete proteolytic enzymes.⁶ Previous workers who examined the phagocytic capacity of polymorphonuclear leucocytes (PMN) from peripheral blood and synovial cavity⁷ demonstrated reduced phagocytosis by synovial fluid PMN of RA patients and reduced phagocytosis by normal peripheral blood PMN incubated in RA synovial fluid. Studies of MO phagocytosis in RA patients have revealed conflicting results. Using different techniques 3 authors found no difference between MO phagocytic activity (PA) in uncomplicated RA patients and controls,⁸⁻¹⁰ but Hoch and Schur found enhanced phagocytosis of IgG coated sheep red blood cells by RA patients' monocytes.¹¹ A pilot study in our laboratory indicated enhanced bacterial phagocytosis and killing by RA MO,¹² so we extended our study to confirm this work.

Materials and methods

Forty-eight patients with classical or definite RA were studied, and 28 normal volunteers served as controls. Ten patients and 11 controls were tested on more than one occasion, making a total of 58 patients and 52 controls. The female:male ratio of patients was 3:1 and of controls 1.7:1. The median age for patients was 54 years and for controls 41. Clinical details of the patients are shown in Table 1. The physician's assessment of disease activity was made by considering the combination of morning stiffness,

Table 1 Details of RA patients

	<i>n</i>	<i>Range</i>	<i>Median</i>
Age (years)	48	24-74	54
Duration of disease	48	1-40	11.5
Haemoglobin (g/dl)	48	8.1-16.8	11.4
ESR	48	3-132	56
<i>Rheumatoid factor</i>			
Currently < 1:32	18		
Currently > 1:32	19		
<i>Treatment</i>			
Analgesics only	3		
NSAID	29		
NSAID + 2nd line	6		
NSAID + steroid/cytotoxic	10		
<i>Disease activity (physician's assessment)</i>			
Quiescent	4		
Moderate	18		
Severe	26		

NSAID = Nonsteroidal anti-inflammatory drugs. 2nd line = gold, penicillamine, or levamisole

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articular index (Ritchie), and ESR, and a global assessment was arrived at. All experiments were carried out on hospital inpatients, and blood samples were taken at the same time each day for phagocytic experiments.

Phagocytic activity (PA) was measured by a modification of the method of Peterson *et al.*¹³ Briefly *Staphylococcus aureus* (NCTC 51330) and *Proteus mirabilis* (laboratory strain) maintained in laboratory culture were radiolabelled by inoculation into Mueller-Hinton broth (Oxoid Ltd, Basingstoke, England) containing 20 μ l 3 H-thymidine (25 μ Ci/mmol specific activity, Radiochemical Centre, Amersham, England.) After overnight culture at 37°C the bacteria were washed and resuspended at 1×10^9 colony forming units/ml by a spectrophotometric method. Bacteria (1×10^8 /ml) were then opsonised for 15 minutes in normal human serum in Hanks's balanced salt solution with 0.1% gelatin (gel HBSS) at a serum concentration previously shown to give optimal PA (*Staph. aureus* 20%, *P. mirabilis* 50%). Opsonised bacteria were spun and resuspended to the initial concentration in gel HBSS.

Peripheral blood monocytes were isolated from heparinised venous samples by a modification of the method of Boyum.¹⁴ 10 ml of blood was mixed with 3 ml of 6% dextran and gravity sedimented for 45–60 min. Leucocyte rich plasma was layered on to Ficoll Hypaque (Pharmacia Ltd, Uppsala, Sweden) and centrifuged at 200 g for 10 minutes. The mononuclear cell layer was carefully removed, washed, counted, and resuspended to a final concentration of 5×10^6 /ml. Mononuclear preparations stained by Wright's and nonspecific esterase (Sigma, Poole) contained 25–35% monocytes on morphological criteria, and the proportion of monocytes was similar in patients and controls.

Opsonised radiolabelled bacteria (0.1 ml) and mononuclear cells (0.1 ml) were added to duplicate polypropylene vials and incubated with shaking for 15 minutes at 37°C. Thereafter phagocytosis was terminated in one set of vials by the addition of phosphate buffered saline (PBS) at 4°C. Duplicate vials were assessed for total bacterial radioactivity by the addition of 3 ml scintillation fluid (Aquasol LKB Instruments Ltd, London, England) and counted on a liquid scintillation counter (A). The first set of vials meanwhile had the nonphagocytosed bacteria removed by differential centrifugation (160 g) and washing in PBS (4°C) 3 times. The final pellet of cells containing phagocytosed bacteria was counted after the addition of scintillation fluid as above (B). The uptake of bacteria was then calculated as:

$$\% \text{ Uptake test} = \frac{\text{cpm B}}{\text{cpm A}} \times 100\%$$

A further set of tubes containing only bacteria were similarly handled, giving a reading representing nonspecific bacterial aggregation and adherence to the tube (*blank*). PA was expressed as:

$$\text{PA} = \% \text{ uptake test} - \% \text{ uptake blank.}$$

In some experiments phagocytosis of *Staph. aureus* was measured after the addition of lysostaphin.¹⁵ The result of some experiments using *Staph. aureus* rich in protein A gave unexpectedly high blank uptakes, and results where blank values were less than 20% were considered separately. Statistical analysis was performed by appropriate nonparametric tests.¹⁶

Results

Phagocytosis of *Staph. aureus* and *P. mirabilis* by MO of RA patients and controls is shown in Figs 1 and 2.

Fig. 1 Uptake of *Staph. aureus* opsonised in 20% normal human serum by monocytes from RA patients and controls.

Fig. 2 Uptake of *P. mirabilis* opsonised in 50% normal human serum by monocytes from RA patients and controls.

Highly significant increases in PA were seen in RA patients, and the results were not altered by the exclusion of *Staph. aureus* tests with blanks greater than 20%. Since the sex ratios of patients and controls were different, MO PA was compared in men and women, but no significant differences were found (median % phagocytosis *Staph. aureus*: men = 21.5%, women = 28.9% $p = \text{NS}$, Mann-Whitney U test). A significant correlation was, however, seen between PA and age (Fig. 3), with increasing values with age. In view of some disparity in age between the patient and control groups a comparison of PA in the 30–60 age group was specifically made. The patients' PA remained elevated compared with that of controls (median % phagocytosis *Staph. aureus*: patients (39) = 35.9%, controls (36) = 20.0%, $p < 0.005$),

Fig. 3 Percentage uptake of *Staph. aureus* opsonised in 20% normal human serum correlated with age of patients or control.

Table 2 Phagocytosis versus disease activity and treatment

	<i>n</i>	% Phagocytosis <i>P. mirabilis</i> (median)	Significance (Mann-Whitney)
Activity			
Moderate	19	40.8	NS
Severe	25	32.0	
Treatment			
NSAID	30	39.4	vs. NSAID: $p = 0.06$
NSAID + 2nd line	6	23.2	
NSAID + steroid/ cytotoxic	10	16.1	

Mann-Whitney U test. PA of *Staph. aureus* and *P. mirabilis* were also significantly correlated ($r_s = 0.56$, $p < 0.001$, Spearman rank correlation). The addition of lysostaphin resulted in a small reduction in PA values (mean reduction = $6.6\% \pm 1.7$, 1 SD, $n = 9$), and the reduction in PA values was similar for patients and controls.

The patients' PA was then correlated with clinical parameters, but no correlation was seen with duration of disease, haemoglobin, ESR (Spearman rank), or seropositivity for rheumatoid factor (Mann-Whitney U test). Comparison with treatment and the physicians' assessment of disease activity (Table 2) showed non significant reductions of phagocytosis in severe disease and in the steroid/cytotoxic drug treated group.

Discussion

Contrary to 3 previous reports^{8–10} we have shown enhanced phagocytosis by peripheral blood mono-

cytes from RA patients. In one study⁹ microscopical assessment of latex particle phagocytosis was used and in another⁸ microscopical assessment of the uptake of yeast opsonised by immunoglobulin and/or complement. In the latter study the numbers were small, but patients with RA complicated by cutaneous vasculitis had reduced complement mediated phagocytosis. Hurst and Nuki¹⁷ showed reduced PA in patients with systemic lupus erythematosus (SLE), a disease frequently associated with hypercomplementaemia and circulating soluble immune complexes. In another study serum from patients with SLE and RA depressed the phagocytosis of yeast by normal monocytes,¹⁸ and Svensson and colleagues suggested that immune complexes in the sera of SLE patients interfered with the opsonisation process.¹⁹

Microscopical assays are difficult to perform and subject to interobserver error, as it may be difficult to decide if a yeast cell is on the cell surface or truly phagocytosed. As previously reported,¹⁵ the addition of lysostaphin showed that 90% of bacterial uptake was the result of ingestion rather than adherence to the cell surface by our technique. Furthermore the results seen with staphylococci were paralleled by those with proteus, an organism which is much less likely to exhibit nonspecific adherence. In another study¹⁰ a nonsignificant increase in the uptake of killed ¹²⁵I labelled *Staph. albus* and *shigella* by RA monocytes was recorded. However, the uptake of live ³H-thymidine labelled *Staph. albus* did not differ from normal in 6 RA patients. No details of opsonisation or measurement of phagocytosis are given in that study, and it is difficult to make a comparison with the present work.

Increased binding and phagocytosis of IgG labelled sheep red blood cells has been demonstrated in RA patients,¹¹ and in addition it was noted that steroid treated patients had significantly lower uptake than patients on other treatment. By contrast the phagocytosis of radiolabelled aggregated IgG by RA MO did not differ from the uptake by control MOs. As in our own study, no correlation was demonstrable between clinical indices of RA activity and PA. We have demonstrated the importance of matching ages between patients and controls, as this may affect the results. In addition the finding that RA patients with vasculitis have low PA and patients without extra-articular complications have normal or enhanced PA⁸ suggests that a general RA population will include a wide range of PA, which could mask any differences from normal which would otherwise emerge. Enhanced PA appears to be a cellular phenomenon, as our own study and that of Hurst and Nuki¹⁷ used normal serum as an opsonin, but we cannot exclude the possibility of activation mediated by the patient's own serum prior to cell separation.

Further studies to examine the effects of RA serum on RA and control cells are in progress. As our results and those of Hoch and Schur¹¹ suggest that steroids may influence MO phagocytic activity, the test could be used to evaluate potential antirheumatic drugs. Since enhanced PA is also associated with activation of other MO functions, including bacterial killing²⁰ and the secretion of proteolytic enzymes,⁴ it would appear that some RA patients have a population of cells inappropriately stimulated and capable of releasing proteolytic enzymes which could degrade cartilage. This activation may be of importance in the pathogenesis of rheumatoid arthritis.

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References

- 1 Denman A M. The macrophage, inflammation and rheumatoid arthritis. In: Panayi G S, Johnson P M, eds. *Immunopathogenesis of rheumatoid arthritis*. Surrey: Reedbrooks, 1979: 123.
- 2 Muirhead K D. Lysosomal enzymes in synovial membrane in rheumatoid arthritis: relationship to joint damage. *Ann Rheum Dis* 1972; **31**: 265-71.
- 3 Dayer J M, Robson D R, Krane S M. Prostaglandin production by rheumatoid synovial cells: stimulation by a factor from human mononuclear cells. *J Exp Med* 1977; **145**: 1399-404.
- 4 North R J. The concept of the activated macrophage. *J Immunol* 1978; **121**: 806-9.
- 5 Theofilopoulos A N, Carson D A, Tavassoli M, et al. Evidence for the presence of receptor for C3 and IgG Fc on human synovial cells. *Arthritis Rheum* 1980; **23**: 1-9.
- 6 Fell H B, Jubb R W. The effect of synovial tissue on the breakdown of articular cartilage in organ culture. *Arthritis Rheum* 1977; **20**: 1359-71.
- 7 Turner R A, Schumacher H R, Myers A R. Phagocytic function of polymorphonuclear leukocytes in rheumatic diseases. *J Clin Invest* 1973; **52**: 1632-5.
- 8 Hurst N P, Nuki G. Evidence for defect of complement-mediated phagocytosis by monocytes from patients with rheumatoid arthritis and cutaneous vasculitis. *Br Med J* 1981; **282**: 2081-2.
- 9 Wynne K M, Dieppe P A, Scott J, Huskisson E C. Cellular phagocytic studies in rheumatoid arthritis patients treated with levamisole. *Ann Rheum Dis* 1981; **40**: 382-7.
- 10 Bar-Eli M, Ehrenfeld M, Litvin Y, Gallily R. Monocyte function in rheumatoid arthritis. *Scand J Rheumatol* 1980; **9**: 17-23.
- 11 Hoch S, Schur P H. Monocyte receptor function in patients with rheumatoid arthritis. *Arthritis Rheum* 1981; **24**: 1268-77.
- 12 Gemmell C G, Steven M M, Sturrock R D. Phagocytosis and killing of bacteria by monocytes of patients with rheumatoid arthritis. In: Easman C S F, Gaya H, eds. *Proceedings of the second international symposium on infection and the immunocompromised host*. London: Academic Press, in press.
- 13 Peterson P K, Verhoef J, Sabbath L D, Quie P G. Kinetics of phagocytosis and bacterial killing by human polymorphonuclear leukocytes and monocytes. *J Infect Dis* 1977; **136**: 502-9.
- 14 Boyum A. Separation of leukocytes from blood and bone marrow. *Scand J Clin Lab Invest* 1968; **21**: suppl 97.
- 15 Verhoef J, Peterson P K, Quie P G. Kinetics of staphylococcal opsonization, attachment, ingestion and killing by human polymorphonuclear leukocytes: a quantitative assay using [³H] thymidine labelled bacteria. *J Immunol Methods* 1977; **14**: 303-11.

- 16 Siegel S. *Non parametric statistics for the behavioural sciences*. Tokyo: McGraw-Hill, Kogakusha, 1956.
- 17 Hurst N P, Nuki G. Abnormalities of monocyte C3b and Fc receptor phagocytic function in SLE. *Arthritis Rheum* 1982; **25**: S74.
- 18 Svensson B. Occurrence of deficient monocyte yeast cell phagocytosis in presence of rheumatic sera. *Scand J Rheumatol* 1980; **31** (suppl): 21-7.
- 19 Svensson B, Norberg R, Torskensson R. Effects of cryoglobulins and aggregated IgG on in vitro monocyte phagocytosis. *Scand J Rheumatol* 1978; **31** (suppl): 57-65.
- 20 Steven M M, Sturrock R D, Gemmell C G. Bacterial killing by mononuclear phagocytes in rheumatoid arthritis. Presented to the Combined Meeting of the Medical Research Society and the Scottish Society for Experimental Medicine. Edinburgh. *Clin Sci* 1982; **63**: 31-2 (abstr).