Phagocytic Function of Polymorphonuclear Leukocytes in Rheumatic Diseases

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ABSTRACT Phagocytosis of yeast particles by peripheral blood and synovial fluid neutrophils was compared in the sera and synovial fluids from 16 osteoarthritis, 23 rheumatoid arthritis, and 12 miscellaneous arthritis patients. Phagocytosis by normal peripheral blood neutrophils was decreased equally and significantly in all synovial fluids. All synovial fluid neutrophils demonstrated decreased phagocytic capacity in all media. Rheumatoid arthritis synovial fluid neutrophils showed significantly less phagocytosis than miscellaneous arthritis synovial fluid neutrophils. Normal peripheral blood neutrophils which in vitro had previously ingested monosodium urate crystals or oil red O, subsequently exhibited a normal yeast phagocytic capacity. Normal peripheral blood neutrophils, which had ingested preformed immunoglobulin G-rheumatoid factor complexes exhibited significantly less yeast phagocytic capacity than control cells or cells preincubated with the individual complex components. There was a significant correlation between the log of the reciprocal of the rheumatoid factor titer in sera used to produce complexes and the phagocytic capacity exhibited by test neutrophils. Ingestion of immunoglobulin G-rheumatoid factor complexes may be important in the production of the cellular phagocytic defect which this study has demonstrated in rheumatoid arthritis synovial fluid neutrophils.

INTRODUCTION

Phagocytosis of immunoglobulin G-rheumatoid factor (IgG-RF)[1] complexes in rheumatoid arthritis (RA) (1) and monosodium urate crystals in gout (2) is an essential element of the inflammatory processes in these diseases. The effect of immune complex ingestion on polymorphonuclear leukocytes must also be considered in view of the reported increased prevalence of intra-articular infection in patients with RA (3). In an effort to examine the functional consequences of these phenomena we have utilized an in vitro yeast phagocytic system (4) to delineate the cellular (polymorphonuclear leukocyte) and humoral (supporting media) aspects of phagocytosis in various arthritides and the cellular aspects of phagocytosis in an experimental cell system.

METHODS

Serum and/or synovial fluid was obtained from 25 normal control subjects and from 16 osteoarthritis (OA), 23 RA, and 12 miscellaneous inflammatory arthritis (MA) patients. The MA group consisted of one pseudogout, one septic arthritis, four psoriatic arthritis, and six gout patients. Fluid was obtained in 10-ml glass tubes containing no additives or 143 U.S.P. U of sodium heparin depending on whether samples were to be used for cellular or humoral phagocytosis studies. Fluids from all of the patients were utilized in the humoral studies. In 13 of the patients both cellular and humoral studies were performed. The clinical and laboratory data concerning these patients is shown in Table I. The RA and MA patients shown here were similar in age, sex, duration of disease, and peripheral blood and synovial fluid neutrophil count. Medications also were similar in that some patients in both groups received aspirin and prednisone. Differences in medications were also apparent in that the gout patients were taking colchicine and allopurinol and the septic arthritis patient was receiving penicillin.

Rheumatoid factor (RF) titers of all serum and synovial fluid samples were determined utilizing the Hyland quantitative macroscopic tube test (RA-test, Hyland Laboratories, Costa Mesa, Calif.). The RF titer of the sample being tested was defined as the reciprocal of the highest dilution causing clumping of the IgG-coated latex particles (5). All sera used for experimental studies were tested

An abstract with preliminary data was published in 1972 *Arthritis Rheum.* **15:** 130.

Received for publication 30 November 1972 and in revised form 22 February 1973.

[1] *Abbreviations used in this paper:* IgG, immunoglobulin G; MA, miscellaneous arthritis; OA, osteoarthritis; RA, rheumatoid arthritis; RF, rheumatoid factor.

TABLE I

*Clinical and Laboratory Data on Patients for Both Cellular and Humoral Studies**

No.	Sex/age	Daily oral medication	Duration of disease	Total leukocytes $\times 10^3$ / Percent neutrophils		Rheumatoid factor titer		Percent synovial fluid cells with inclusions	Mean no. yeast ingested by synovial fluid neutrophils
				Peripheral blood	Synovial fluid	Peripheral blood	Synovial fluid		
1	M/49	4.8 g aspirin	5 yr	10.8/83	14.4/75	160	160	30	2.1
2	M/51	6.0 g aspirin 7.5 mg prednisone	8 yr	7.8/87	23.4/93	Negative	160	10	1.3
3	M/68	4.8 g aspirin 5.0 mg prednisone	27 yr	13.5/82	8.3/60	80	80	5	1.1
4	M/65	3.9 g aspirin 5.0 mg prednisone	9 yr	7.6/75	24.0/68	320	80	10	1.7
5	F/58	3.9 g aspirin	10 yr	8.2/60	13.2/78	640	640	100	1.3
6	F/56	4.8 g aspirin	3 yr	6.2/65	29.0/77	320	5120	100	1.3
7	F/35	None	3 days	7.8/60	8.4/82	Negative	40	50	.9
8	M/73	1.0 mg colchicine 200 mg allopurinal	10 yr	14.6/80	23.4/84	Negative	Negative	95‡	2.7
9	F/65	200 mg allopurinal	4 days	11.6/84	7.2/95	Negative	40	5‡	3.3
10	M/50	1.8 g aspirin	4 days	13.6/60	2.1/89	Negative	Negative	1‡	2.4
11	F/55	3.6 g aspirin 5.0 mg prednisone	15 yr	10.3/75	40.5/77	Negative	Negative	1	2.9
12	M/58	4.5 g aspirin	2 yr	13.0/67	17.0/88	Negative	Negative	40	2.0
13	F/30	1 × 10^7 U penicillin	2 days	10.7/69	47.6/93	Negative	40	10	2.5

* Diagnosis: first seven patients RA; patients 8 and 9 gout; patient 10 pseudogout; patients 11 and 12 psoriatic arthritis; and patient 13 septic arthritis.
‡ Crystals.

separately utilizing the methods described by Singer and Plotz (6), and RF titers identical to those recorded utilizing the Hyland test were obtained. All assays of phagocytosis were started within 1 h of obtaining the synovial fluid or peripheral blood from the patients. Neutrophils were separated and assays performed utilizing the methods described by Miller and Nilsson (4) with the modification that final counting of yeast particles per 100 consecutive neutrophils was performed with the observer blinded as to the source of the test materials. In this assay, baker's yeast (1×10^9/ml of Earle's solution) was opsonized with a 1/10 dilution of the patient's serum or synovial fluid. This suspension was then incubated for 30 min on a rotating wheel at 37°C with an equal volume of washed buffy coat or synovial fluid neutrophils (5×10^6/ml of Earle's solution). Tubes were then centrifuged and the sediment smeared for Wright's staining and counting. Final counts were reported as the mean number of yeast particles per neutrophil seen in the first 100 neutrophils encountered on each smear.

Aggregated IgG was prepared from Cohn Fraction II and complexed with RF as described by Zucker-Franklin (7) with the modification that 2 ml of fresh normal sera was added to the tubes in which the complexes were formed. The methods of Schumacher and Phelps (8) were utilized in the preparation of synthetic monosodium urate crystals and in the fixation and electron microscopic examination of control cells and cells incubated with IgG-RF complexes. Oil red O was dissolved in paraffin and particles 0.5–5 μm in diameter were prepared according to the method of Stossel, Pollard, Mason, and Volan (9). In all experimental cell studies, normal peripheral blood type O neutrophils were separated as before and incubated for 30 min with the preformed complexes or monosodium urate crystals at 37°C. These cells were then washed twice in normal saline. Viability testing was done by adding 1 drop of 1% trypan blue vital dye to 1 drop of cell suspension and examining the neutrophils for dye exclusion. The data from all studies were analyzed using Duncan's new multiple range test for analysis of variance (10). All significant differences exhibited P values of 0.01 or less.

RESULTS

As shown in Fig. 1, the phagocytic capacity of normal peripheral blood neutrophils in the synovial fluids from 16 OA, 23 RA, and 12 MA patients did not differ, how-

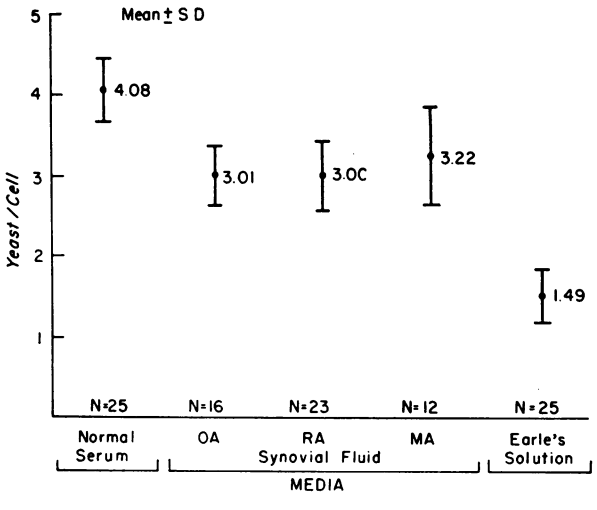

FIGURE 1 Yeast phagocytosis by normal peripheral blood neutrophils in media from various sources. A humoral defect in phagocytosis is demonstrated in OA, RA, and MA synovial fluids.

ever, this level of phagocytosis was significantly lower than that observed in normal serum and significantly higher than that seen in Earle's solution. In additional studies normal peripheral blood neutrophils exhibited normal phagocytosis in the sera of 11 of the RA and 6 of the MA patients. Fig. 2 depicts yeast phagocytosis in normal serum by neutrophils from the peripheral blood of normal subjects and from the peripheral blood and synovial fluid of the seven RA and six MA patients described in Table I. There were no significant differences in phagocytic capacity among the peripheral blood neutrophils. Phagocytosis by both RA and MA synovial fluid neutrophils was significantly decreased and that of the RA patients was significantly less than that of the MA patients. As shown in Table I the RA patients' synovial fluids exhibited more cells with inclusions and tended to have higher titers of RF than the synovial fluids of the MA patients. There were, however, no significant correlations when the RA patients' RF titers, numbers of neutrophils with inclusions, and synovial fluid neutrophils' phagocytic capacities were compared on an individual basis within the RA group.

Normal peripheral blood neutrophils were incubated on eight occasions with IgG-RF complexes formed from the serum of a patient with a high titer (80,000) of RF. These test neutrophils then ingested 1.2±0.19 (mean ±SEM) yeast particles per cell which was significantly ($P < 0.01$) less than the 3.5±0.17 yeast particles per cell ingested by the control neutrophils incubated under the same conditions without the complexes. Significant differences were not noted when combinations of the complex components were incubated separately with the control cells. Experiments in which cells had preingested

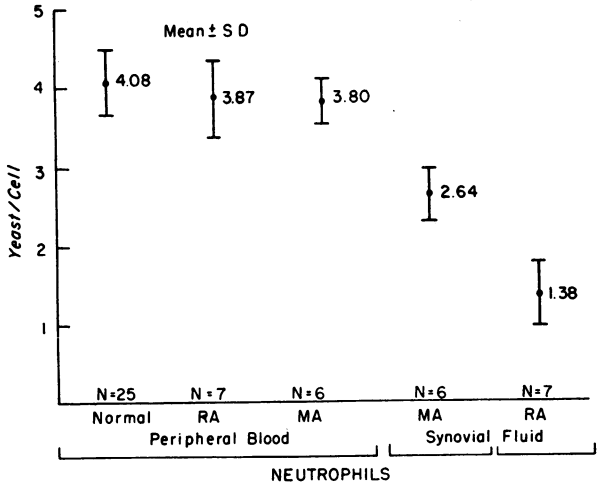

FIGURE 2 Yeast phagocytosis in normal serum by neutrophils from various sources. A cellular defect in phagocytosis is demonstrated in MA and RA synovial fluid neutrophils. This defect is significantly more pronounced in RA synovial fluid neutrophils.

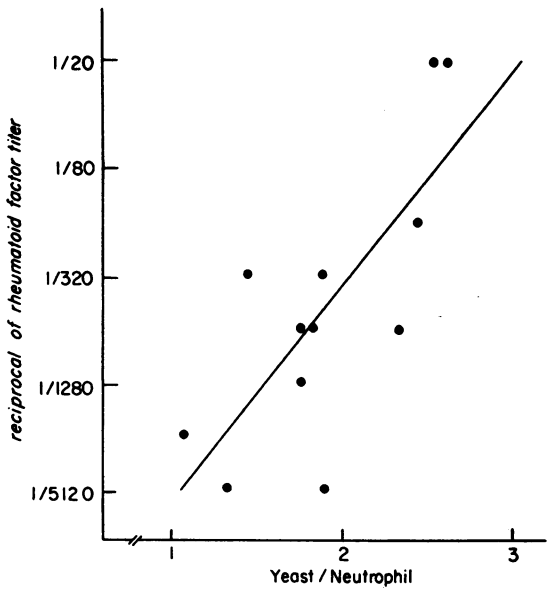

FIGURE 3 Effect of variations in rheumatoid factor on yeast phagocytosis. As the amount of rheumatoid factor in the sera used to form IgG-RF complexes decreases, there is an increase in the number of yeast particles ingested by neutrophils from normal controls which had previously been incubated with the complexes.

complexes showed 1–78% (mean of 50%) of the cells with medium to large inclusion bodies on examination of wet smears. These same percentages of cells showed vacuoles in the cytoplasm when the cells were examined after being stained with Wright's stain. Equal numbers (> 90%) of the test and control cells were viable when tested with 1% trypan blue. Many of the cells incubated with IgG-RF complexes exhibited large numbers of phagosomes containing finely granular material when examined by electron microscopy. These inclusions were not seen in control cells incubated without the complexes. Fig. 3 shows the data obtained when the sera from a separate group of 12 patients with classical RA (receiving no medications at the time of the study) were used as sources for RF. These patients' titers ranged from 20 to 5,120. There was a significant ($P < 0.01$) correlation ($r = 0.756$) between the log of the reciprocal of the RF titer in the test sera and the yeast phagocytosis of the test neutrophils. The number of cytoplasmic vacuoles seen in the cells stained with Wright's stain or inclusions seen in those studied unstained by wet smear examination did not show a reproducible correlation with either the RF titer of the serum employed or the phagocytic capacity of the test neutrophils.

Normal peripheral blood neutrophils preincubated on eight occasions for 30 min with monosodium urate crystals in Earle's solution or in Earle's solution alone ingested 3.0±0.15 and 3.1±0.2 yeast particles per cell, respectively, in normal serum. When these cells were

examined with polarized light microscopy, 50% were found to contain one or more of the negatively birefringent monosodium urate crystals. Crystals were also seen in 50% of these neutrophils on examination of Wright's stained smears of these preparations. Differential counting of cells which had phagocytized crystals and those which had not, demonstrated that both cell groups phagocytized a mean of three yeast particles per cell. When normal peripheral blood neutrophils incubated with paraffin oil red O particles were examined under wet smear, 40% of the neutrophils were found to have ingested these particles. However, when these neutrophils were examined under Wright's stain, only 1–10% of the white cells were seen to have intracytoplasmic vacuoles. Cells prepared in this way were again studied with the yeast phagocytic system. In three different trials there was no difference between the control cells' uptake of the yeast particles and that exhibited by the neutrophils which had previously ingested the paraffin oil red O particles.

DISCUSSION

These studies confirm the work of Bodel and Hollingsworth (11) which demonstrated with a staphylococcal system decreased phagocytosis by both peripheral blood and synovial fluid neutrophils in RA synovial fluid. The yeast system employed here also has detected a humoral phagocytic defect in the synovial fluid of patients with other types of arthritis and a cellular phagocytic defect more marked in RA than MA patients' synovial fluid neutrophils. These phagocytic defects along with the chemotactic defect described by Mowat and Baum (12) may be factors in the clinically observed (3) increased incidence of septic arthritis in patients with RA.

Experimental cell systems were utilized to eliminate some of the clinical and immunochemical variables inherent in patient studies and to examine more closely the effects of IgG-RF complex or monosodium urate crystal phagocytosis on polymorphonuclear leukocyte function. These studies have shown that ingestion by neutrophils of monosodium urate crystals does not interfere with subsequent yeast phagocytosis. This finding suggests that in patients with gout there is a different mechanism of interaction between the ingested particle and the neutrophil. The absence of subsequent lowering of yeast phagocytosis after neutrophil ingestion of paraffin oil red O, which produces spatially similar intracytoplasmic inclusions (9) to those produced by the complexes (7), again suggests that the IgG-RF complexes react in a specific way in the ingesting neutrophils to prevent subsequent yeast phagocytosis. Trypan blue staining demonstrated no increased incidence of cell death in the test neutrophils as a cause for this phenomenon. The fairly constant correlation between the RF titers of the sera employed in producing the complexes and the phagocytosis lowering effect on the test neutrophils suggests the existence of a dose-response relationship in this experimental system. Further studies are necessary, however, to define more clearly the mechanism by which IgG-RF complexes may be important in the production of the decreased phagocytic capacity shown in this study to be present in the synovial fluid polymorphonuclear leukocytes of patients with rheumatoid arthritis.

ACKNOWLEDGMENTS

The authors gratefully acknowledge early assistance provided by Dr. Michael Miller and Mr. Kenneth Myers. In addition we acknowledge with appreciation the excellent technical assistance provided by Mrs. Karen Stott and the statistical analysis provided by Leonard Rhyne, Ph.D.

This work was supported by Veterans Administration Training Grant TR-39, National Institutes of Health grants RR-336 and 5404, Arthritis Foundation Clinical Research Center Funds, and grants from Lilly Research Laboratories and the Barsumian Fund.

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