Immunoglobulin phagocytosis by granulocytes from sera and synovial fluids in various rheumatoid and nonrheumatoid diseases

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Cats, A., Lefeber, G. J. M., and Klein, F. (1975). *Annals of the Rheumatic Diseases*, 34, 146. **Immunoglobulin phagocytosis by granulocytes from sera and synovial fluids in various rheumatoid and nonrheumatoid diseases.** (1) The phagocytosis of human IgG, IgM, and C3 by granulocytes from various rheumatoid and nonrheumatoid sera and synovial fluids (SF) was investigated by *direct* examination of the patient's leucocytes and by *indirect* testing by incubation of normal donor leucocytes with various sera and SF. (2) In rheumatoid arthritis (RA) phagocytosis of IgM, IgG, and C3 was common from sera and SF. There was a strong correlation of IgM and C3 phagocytosis with the occurrence of rheumatoid factor. The phagocytosed IgM is probably rheumatoid factor. In SF both the direct and indirect test method yielded equally positive results; in serum the direct test was negative throughout. (3) In systemic lupus erythematosus there was phagocytosis of IgG, IgM, and C3 from serum (indirect test), IgM not being correlated with the latex-fixation test and probably of antinuclear antibody nature. Phagocytosis decreased after treatment of the disease. Sera from many other rheumatic diseases frequently gave weak IgG phagocytosis, but rarely did IgM or C3. (4) IgG, and sometimes C3, was frequently taken up from IgG myeloma sera (indirect test). IgM and IgG were taken up from Waldenström's macroglobulinaemia sera, independent of IgM concentration. It is possible that an aggregation tendency of particular paraproteins determines Ig uptake from these sera. (5) IgG was taken up from half of the studied sera of infectious diseases in the indirect test, including two cases with Hodgkin's disease as well. Three sera from patients with untreated trypanosomiasis were positive for IgG as well as for IgM. (6) Normal healthy control sera remained negative, even after prolonged preservation or frequent freezing and thawing: only among very old sera were a few positive observations recorded.

Immunoglobulin phagocytosis appears to be a common phenomenon in a number of conditions. It seems probable that soluble immune complexes, or in other cases non-immune aggregates, may cause phagocytosis.

Endocytosis of immunoglobulins (Ig) by granulocytes in rheumatoid arthritis (RA) has been observed by many authors* (for a recent review see Zvaifler, 1973). IgG, IgM, and complement (C3) have been found in synovial fluid granulocytes and seen to be taken up by leucocytes from normal donor blood after incubation with synovial fluid (SF) from seropositive

RA patients, but not, or to a much lesser extent, from cases of seronegative RA and other rheumatic diseases. Less is known about phagocytosis of immunoglobulins in peripheral blood leucocytes and about the uptake of immunoglobulins and complement from pathological sera by normal donor leucocytes.

This report deals with the results of an extensive study of immunoglobulin and complement phagocytosis from SF and from serum *in vivo* (direct test)

* The term phagocytosis is currently in use to describe this process, and will be used in this article.

as well as *in vitro* by fresh normal donor granulocytes (indirect test). Phagocytosis from SF and serum of patients with RA are compared with similar findings in other rheumatic and unrelated diseases. It will be shown that immunoglobulins can be phagocytosed from sera and SF in a great variety of clinical conditions but not from sera of healthy controls.

Material and methods

PATIENTS

All patients diagnosed as RA fulfilled at least 5 A.R.A. criteria (Ropes, Bennett, Cob, Jacox, and Jessar, 1959). The patients with psoriatic arthritis had psoriasis in addition to at least 4 A.R.A. criteria.

The diagnosis systemic lupus erythematosus was made according to the criteria proposed by Cohen, Reynolds, Franklin, Kulka, Ropes, Shulman, and Wallace (1971). The patients with juvenile RA fulfilled the Taplow criteria (Ansell and Bywaters, 1959). Patients with Reiter's syndrome had the complete triad. The diagnosis of crystal synovitis was established by showing urate or pyrophosphate crystals in the synovial fluid. Patients with paraproteinaemia had characteristic bone marrow cytology as well as a paraprotein in the serum.

Of the patients with an infectious disease, three had trypanosomiasis, six septicaemia, two subacute endocarditis, and nine pulmonary infection (2 of viral origin and 2 empyema).

METHODS

Sera and plasma

These were obtained by venepuncture and kept at -20°C . Synovial fluids from knee joints were collected from 140 patients with various rheumatic diseases (see results) by aspiration of the joint under sterile conditions.

Ragocyte and crystal examination

A portion of synovial fluid was heparinized and a preparation of leucocytes was obtained directly from this portion by single centrifugation without washing. This preparation was examined unfixed by light-microscopy at 10×100 magnification for ragocytes and by polarized light for crystals. Ragocytes were graded in the same way as fluorescent inclusions (see below).

Direct test for Ig inclusions

Leucocytes from a further 10 ml synovial fluid containing 0.01 mol/l EDTA, or buffy coat leucocytes from citrate plasma, were concentrated by 5 minutes' centrifugation in an ordinary table centrifuge. The sediment was then washed three times with 0.15 mol/l NaCl buffered to pH 6.8 containing 5% bovine serum albumin, 0.5% EDTA and 10^2 units of penicilline g/ml. Smears were made and fixation was carried out with 96% alcohol containing 5% acetic acid at -20°C for 15 min. After fixation the preparations were washed in phosphate buffered saline (PBS) of pH 7.8 at 4°C and kept at -20°C , wrapped in cellophane before use. Specific conjugate was then added and the preparations incubated for 30 min in a moist chamber at room temperature. The conjugates used in this study were FITC-labelled antisera against human IgG, IgM, and C3, prepared in goats (Miles Laboratories, Kankakee,

Ill., U.S.A.). The anti-IgG and IgM conjugates were checked for specificity on various myeloma and Waldenström's bone marrow preparations (Hijmans, Schuit, and Klein, 1969). The conjugated anti-Ig sera were used in dilutions of 1:5 to 1:10. After incubation free conjugate was removed by washing for 12 min in PBS at room temperature. The preparations were immersed in a drop of 90% buffered glycerol and examined under a cover slip by a Zeiss microscope adapted for fluorescent microscopy with an Osram lamp HBO 200W and filters BG38 and KP490.

Indirect test for Ig inclusions

For the indirect tests the buffy coat of a sufficient amount of normal human O Rh + donor blood was washed three times with saline. 10–15 drops of the washed buffy coat were incubated at 37°C with 0.4 ml serum or synovial fluid for 1.5 h, then centrifuged and washed three times with albumin buffer as described earlier. Smears were made and prepared for fluorescent microscopy as described above. Fig. 1 gives a flow sheet of these procedures.

Fluorescence was expressed semiquantitatively in four grades:

- : no fluorescence (doubtful considered as negative);
- + : few fluorescent cells with small inclusions (Fig. 2);
- ++ : more fluorescent cells with stronger fluorescence and small inclusions (Fig. 3);
- +++ : many fluorescent cells with strong fluorescence and large inclusions (Fig. 4).

When EDTA was added to synovial fluid or serum in a concentration greater than 0.01 mol/l, phagocytosis was inhibited, giving rise to extracellular fluorescence of inclusion-like structures. Since phagocytosis took place in heparinized plasma as in serum, it is evident that fibrinogen does not interfere with immunoglobulin phagocytosis.

Latex fixation test (LFT)

This was performed according to Valkenburg (1963) after previous heating of the synovial fluids at 56°C for 30 min to inactivate inhibitors. It appeared that the final concentration of 0.01 mol/l EDTA present in the synovial fluid did not interfere with the test.

Antinuclear antibodies (ANA)

These were detected in unfixed blood smears by the immunofluorescence procedure described before, using conjugates against human IgG (Miles) or total human serum (Philips Duphar).

DNase treatment

(0.05 ml of a 0.07% DNase (Miles) solution in phosphate buffer of pH 6.8 containing 0.017 mol/l magnesium sulphate was added to 0.3 ml serum and incubated for 1 h at 37°C . Sera were then kept at 4°C for 24 h when a further 0.05 ml DNase solution was added for another incubation. Controls without DNase were included. Leucocytes were then added to serum for phagocytosis as described.

Results

A RAGOCYTES IN SYNOVIAL FLUID

Independent of the diagnosis, ragocytes were observed among the SF leucocytes of patients with

FIG. 1 *Scheme of procedures used in testing for Ig inclusions*

FIG. 2 *Few fluorescent cells with small inclusions. 4 × 50*

FIG. 3 *More fluorescent cells with stronger fluorescence and small inclusions. 4 × 50*

FIG. 4 *Many fluorescent cells with strong fluorescence and large inclusions. 4 × 50*

and without RA (Table I). The leucocytes in rheumatoid factor positive SF contained more and usually larger inclusions than leucocytes from SF in which rheumatoid factor was absent. The difference was significant ($0.01 > P > 0.005$; $\chi^2_{(3)} = 16.992$). These findings are in agreement with the observations of

Zucker-Franklin (1966), who also found the number of cells showing cytoplasmic abnormalities and the number of cytoplasmic inclusions per cell to be significantly greater in granulocytes from synovial effusions of patients with classical RA than in other arthritides and osteoarthritis. It is evident, however

Table I *Immunoglobulins, complement inclusions, and ragocytes in synovial fluid leucocytes in rheumatic diseases (direct test)*

	Total no.	Ragocytes*				IgG				IgM				C3			
		+++	++	+	-	+++	++	+	-	+++	++	+	-	+++	++	+	-
Sero + SF + RA	44	14 3 ND	11	13	3	25	15	2	2	15	12	11	4	9	14	13	7
Sero + SF - RA	10	3	2	4	1	1	2	1	6			1	9			1	9
Sero - SF - RA	23	1 1 ND	6	11	4	1 ND	1	11	10	3 ND		3	17	1 ND		1	21
Psoriasis Sero - SF - RA	9			5	4			7	2				9				9
Ankylosing spondylitis Reiter's syn- drome	9	1 1 ND	2	4	1		1	5	3		1		8				9
Juvenile RA sero -	8	1	1	5	1			4	4			1	7			2	5
Crystal synovitis	15	1	1	4	9	3 ND		5	7	3 ND			12	1 ND		1	12
Osteo- arthritis	15	2 3 ND		3	7			8	7			1	13	2 ND		3	12
Septic arthritis	2	1		1				2					2	1 ND			1
Others	5	1 1 ND	1	1	1			1	4				5	1 ND		1	4
Total	140																

*+, - = degree of phagocytosis.
ND = not done.

that notwithstanding the correlation with rheumatoid factor, ragocytes can be found in a considerable number of seronegative patients, even those without RA, and that the ragocyte has little, if any, diagnostic value.

B PHAGOCYTOSIS OF IMMUNOGLOBULINS FROM SYNOVIAL FLUID

Direct test

IgG inclusions in SF leucocytes were observed by immunofluorescence in 42 of the 44 patients with rheumatoid factor activity in the SF. In this connexion it should be noted that rheumatoid factor was found in the SF of only 44 out of 54 seropositive patients. In six of the ten patients in whom rheumatoid factor was present only in the serum, IgG was absent in the SF leucocytes. The SF leucocytes of about 50% of both rheumatoid and nonrheumatoid patients with no rheumatoid factor activity either in their blood or SF contained smaller numbers of IgG inclusions (see Table I).

Approximately 90% of the seropositive rheumatoids with a positive LFT in the SF had IgM inclusions

in their SF leucocytes, whereas rheumatoid and nonrheumatoid patients with a negative LFT in their SF had positive inclusions only rarely. The association between rheumatoid factor in the SF, irrespective of serum titre and IgM inclusions, was significant ($P < 0.0005$, $\chi^2_{(1)} = 28.673$).

C3 inclusions (grade + or more) were rarely seen in the SF leucocytes in the absence of IgM inclusions, although dubious C3 inclusions were present in about 50% of all patients with one + IgG. (It has already been noted that all dubious inclusions were graded as negative results.) It was a common phenomenon that fluorescence of IgM and C3 was much weaker in all preparations than IgG fluorescence. The association between IgM and C3 in inclusions was significant ($P < 0.0005$; $\chi^2_{(1)} = 32.942$).

Indirect test

No appreciable difference was observed between inclusions in the patients' own synovial leucocytes and the inclusions in normal donor leucocytes after incubation in synovial fluids (Table II). This is in contrast to what was found for peripheral blood leucocytes (see below).

Table II Comparison of IgG, IgM, and C3 inclusions in SF leucocytes and in donor leucocytes after incubation with SF

Patient	Inclusions in leucocytes of synovial fluid: direct test			Inclusions in donor leucocytes after incubation with synovial fluid: indirect test			Latex titre
	IgG	IgM	C3	IgG	IgM	C3	
1	+++*	+++	+++	+++	++	+	≥ 10 240
2	++	++	+	+++	+++	++	≥ 10 240
3	+++	+++	++	+++	++	+	≥ 10 240
4	++++	+++	+++	+++	+++	+++	≥ 10 240
5	+	—	+	+	—	—	≥ 10 240
6	++	++	++	++	++	++	≥ 10 240
7	+++	+++	+++	++	+	+	≥ 10 240
8	+++	++	+	+++	++	++	22 560
9	+++	+++	?	+++	+++	++	≥ 10 240
10	++	+	—	+	+	—	2 560
11	+++	+++	++	+++	+++	+++	≥ 10 240
12	—	—	—	+++	+++	+	5 120
13	+++	++	++	+++	+++	++	≥ 10 240
14	++++	++	++	+++	+++	++	≥ 10 240

* Degree of phagocytosis.

C PHAGOCYTOSIS OF IMMUNOGLOBULINS FROM SERUM

Direct test

No IgG inclusions were found in the buffy coat leucocytes of patients with seropositive RA, contrary to what has been reported by Vaughan, Barnett, Sobel, and Jacox (1968). Resuspending peripheral blood leucocytes from RA patients in their own serum produced some inclusions. This phenomenon was augmented after the serum had been previously frozen and thawed (Table III). In 2 of 15 preparations from patients with systemic lupus erythematosus, IgG inclusions were found in the direct test. IgM and C3 were not investigated in the direct test with serum.

Indirect test

Normal donor leucocytes after incubation with sera from 30 seropositive RA patients all contained IgG inclusions; in 24 samples IgM inclusions were found (Table IV). C3 inclusions were found in all of

Table III IgG inclusions in leucocytes of RA patients before and after reincubation with autologous serum

Patient	Direct test	Incubation with own serum after freezing	Incubation with own nonfrozen serum
1	—	++ or +++	+
2	—	++	+
3	—	+	+
4	—	++	—
5	—	++	+
6	—	+	+
7	—	+	—
8	—	+	+

Table IV IgG, IgM, and C3 inclusions in donor leucocytes after incubation with different sera (indirect test)

	Total no.	IgG				IgM				C3			
		+++	++	+	—	+++	++	+	—	+++	++	+	—
RA sero +	30	6	15	9		1	3	20	5	16	ND		
RA sero —	23	2	11	5	5	7	1	1	14	3	5	8	
SLE	14	9	3	2		3	3	7	1	5	5	2	2
IgG myeloma	19	7	4	4	4		2		17	2	3	9	5
Waldenström's macroglobulinaemia	9	1	2	4	2	1	2	2	4	1		1	7
Infectious diseases	20	1	4	5	10		2	1	17	1	3	3	13
Healthy controls	19				19				19				19

SLE = systemic lupus erythematosus.

the 14 cases investigated. In the sera of 23 patients with seronegative RA treated in the same way, IgG inclusions in the leucocytes were found in 18 samples. C3 and IgM inclusions were looked for in 16 of these seronegative RA sera, and were found in 8 and 2 samples respectively. Two seronegative patients whose sera produced only IgG inclusions showed a positive antinuclear antibody. Donor leucocytes, incubated with sera from 14 patients with systemic lupus erythematosus, only two of which were latex positive, contained IgG in all cases, IgM in 13 cases, and C3 inclusions in 12 cases. It proved to be possible to absorb out rheumatoid factor activity almost completely by repeating the described phagocytosis procedure 3 to 4 times with different batches of leucocytes before performing the latex test in the absorbed serum. This was confirmed for 4 different sera. The same was shown to be true for antinuclear antibody. Treatment of LE sera with DNase somewhat diminished IgM phagocytosis, but there was no influence on the uptake of IgG.

Donor leucocytes incubated with 19 IgG myeloma sera contained IgG inclusions in 15 cases and C3 inclusions in 14 cases. 3 cases were completely negative. In 3 cases a discordance was found for IgG and C3. In only 2 cases, both with a high number of leucocytes with larger IgG and C3 inclusions, IgM was present as well: one of these had a positive LFT and antinuclear antibody. Two other samples with a strongly positive LFT produced no IgM inclusions, nor did another sample with a positive antinuclear antibody only.

Donor leucocytes incubated with sera of 9 patients with Waldenström's macroglobulinaemia had IgG as well as IgM inclusions in 5 cases. Only 2 of these clearly showed complement inclusions. Two IgM paraprotein serum samples produced no inclusions at all. 20 sera of patients with different bacterial, viral, and spirochaetal infectious diseases gave IgG inclusions in 10 cases. Three of these samples gave a positive fluorescence for complement. From three trypanosomiasis sera IgM as well as IgG was phagocytosed. Two of the patients whose sera produced IgG and complement were suffering from Hodgkin's disease as well as from a severe infection.

In leucocytes treated with recently obtained sera from 19 healthy controls no immunoglobulins or complement inclusions were found. Control experiments, in which 7 of these sera were frozen and thawed 8 times before incubation with leucocytes, gave completely negative results. Only 4 of 18 control sera obtained five years earlier produced IgG inclusions, IgM being always negative. At least one of these positive cases had a history of previous infection and probably contained antigen-antibody complexes.

Sera of seropositive RA patients heated for half an hour at 56°C showed larger (but not an increased number) of IgG inclusions and the same or sometimes

fewer complement inclusions after incubation with the patient's own, or with O+ donor, leucocytes.

Discussion

RHEUMATOID ARTHRITIS

This study confirms previous observations (see Kinsella, Baum, and Ziff, 1970; Zvaifler, 1973), that the phagocytosis of IgM from SF is a frequent phenomenon in seropositive RA. It appears that IgM inclusions are found in SF leucocytes almost exclusively if rheumatoid factor is present in the SF. The converse was shown by Hollander, McCarty, Astorga, and Castro-Murillo (1965); after washing and disrupting SF leucocytes they found a positive LFT in the extracts.

The rheumatoid factor character of phagocytosed IgM was further substantiated by absorption experiments with phagocytes.

Because clear-cut fluorescence of C3 correlated strongly with the presence of IgM in the inclusions, it appears that IgM enhances C3 uptake, although classical rheumatoid factors may not always be complement-fixing antibodies (Klein and Van Zwet, 1968) and may actually inhibit complement binding (Heimer, Levin, and Kahn, 1963). Zvaifler and Schur (1968), however, could show complement binding by rheumatoid factor under certain conditions. It has been suggested by Ruddy and Austin (1970) that only moderate activation of the complement system is produced by IgG anti-IgG complexes. The presence of IgM-rheumatoid factor may greatly augment the effect of these immune complexes, leading to a marked increase of complement binding. Tesar and Schmid (1970) consider that rheumatoid factor blocks complement-binding sites on IgG complexes and substitutes its own instead, leading to a net increase in complement fixation. It is also possible, however, to interpret our findings as a parallelism between IgM phagocytosis and independent complement fixation, by the mere presence of more IgG complexes in such cases.

IgG was taken up from SFs not only in seropositive RA, but also in seronegative RA and other joint diseases, although the amount was then usually low.

Because SF leucocytes tended to show few IgG inclusions in inflammatory and degenerative joint diseases, it does not seem justifiable to ascribe chronic inflammation exclusively to the excessive phagocytosis of immunoglobulins by leucocytes. It is still possible that in some cases such phagocytosis has an enhancing effect on the inflammatory reaction in the joint.

Since SFs were kept at room temperature or 4°C before fixation of the slides, aggregation of IgG might have occurred *in vitro*, a possible explanation for the occurrence of small amounts of IgG and C3 inclusions in the leucocytes, in particular of non-

rheumatoids; but this is improbable since the direct and indirect tests gave almost identical results.

Phagocytosis is independent of the leucocyte source; O + donor leucocytes had the same phagocytosing ability as the patient's own cells after incubation in SF. This led to the investigation of immunoglobulin phagocytosis from the sera of patients with RA as well as with other diseases by the indirect test method. IgG was found to be taken up from all seropositive RA and the majority of seronegative RA sera. IgM and complement phagocytosis was mainly confined to positive RA sera just as has been found in the SFs. In this connexion it should be noted that IgG complexes in RA sera and joint fluids have been shown with a different immunochemical technique by Hannestad (1968) and by Winchester, Kunkel, and Agnello (1971).

IgG and IgM inclusions were only rarely observed in circulating leucocytes of RA patients in the direct test. Vaughan and others (1968), examining blood leucocytes of RA patients, found immunoglobulin inclusions in a considerable number of them. This could not be confirmed by us under the conditions used in their experiments. Previous freezing at -20°C or heating at 56°C for 1 hour increased the yield of IgG inclusions in RA sera, although more IgG remained extracellular after inactivation.

Again these observations pose the question whether the observed phagocytosis is due to complexes of immunoglobulins or whether some or even most of the IgG phagocytosis might be due to aggregates of a nonimmune nature. This is a crucial question to which our experiments cannot give a definite answer. One might even suppose that the aggregation of existing immune complexes is favoured by freezing and thawing, since normal sera did not show Ig phagocytosis even after frequent freezing and thawing. An alternative hypothesis is that pathological IgG is more prone to aggregate formation than its normal counterpart.

In our experience phagocytosis of artificially produced aggregates from IgG preparations produced inclusions which were morphologically indistinguishable from those obtained by incubation with sera, whereas nonaggregated IgG was not phagocytosed (unpublished observations).

In the experiments where sera were heated at 56°C before incubation it appeared that complement phagocytosis was not decreased, suggesting stabilization by fixation to the complex.

IMMUNOGLOBULIN PHAGOCYTOSIS IN SYSTEMIC LUPUS ERYTHEMATOSIS (SLE)

Incubation of leucocytes with serum from SLE patients in the indirect test produced IgG, IgM, and C3 inclusions in nearly all the 14 cases, and IgG in 2 of 14 direct tests. In a few cases it was noted that after successful therapy fewer Ig inclusions were found

than before treatment. There is evidence that circulating immune complexes are a common phenomenon in SLE (Cochrane and Koffler, 1973). Our observations support the hypothesis that circulating complexes are involved. Surprisingly we found IgM inclusions in donor leucocytes treated with sera of SLE patients although these sera had a negative LFT. Because in SLE circulating DNA-anti-DNA-complexes occur, a few SLE sera were treated with DNase. After such treatment somewhat fewer IgM inclusions were found in the leucocytes in the indirect test, but IgG inclusions did not change. It is known that in SLE sera some of the antinuclear antibodies are IgM, but IgG is predominant (Goodman, Fahey and Malmgren, 1960). It is probable that at least part of the phagocytosed IgM is taken up as an ANA-DNA-complex, although the results with DNase are not clear enough to be conclusive. They are confirmed, however, by the results of absorption experiments with phagocytes.

IMMUNOGLOBULIN PHAGOCYTOSIS IN PARAPROTEINAEMIA

More difficult to understand is the high incidence of leucocyte inclusions in the indirect test with sera of patients with multiple myeloma. No viral or other antigen is known to provoke an IgG paraprotein response and therefore circulating immune complexes do not seem probable. Polymerization of IgG paraproteins of low molecular weight resulting in protein aggregates has been described, in particular in γG3 myelomas (Capra and Kunkel, 1970). Our study suggests that circulating IgG aggregates would be present in a high number of cases with multiple myeloma, but this remains to be investigated.

An alternative explanation again might be that abnormal IgG, even in the native state, might be phagocytosed. The phagocytosis of IgM as well as of IgG and C3 in Waldenström's macroglobulinaemia remains unexplained.

INFECTIOUS DISEASES

Sera from patients with infectious diseases did not always show the phagocytosis phenomenon in the indirect test. In 3 cases of trypanosomiasis and in 2 patients who were also suffering from a myeloproliferative disease (Hodgkin's disease), phagocytosis was apparent. At the time of investigation all patients, except the trypanosomiasis cases, were already under treatment. Further studies are necessary to establish whether the negative results are due to treatment. It may be assumed that at that time no circulating complexes were present. In the cases of Hodgkin's disease some analogy may exist with the paraproteinaemias since both diseases result from malignant processes in the lymphoid system. In the untreated trypanosomiasis cases phagocytosis of

circulating immune complexes might be a reasonable explanation.

General conclusions

From our study it appears that Ig inclusions are by no means limited to the synovial fluid granulocytes of seropositive RA patients. In particular, IgG phagocytosis can often be found in seronegative RA and in other rheumatic diseases, even noninflammatory conditions such as osteoarthritis. Important differences from seropositive RA do exist, but these are of a quantitative rather than of a qualitative nature.

Normal leucocytes also take up immunoglobulins from pathological sera of very different origins, such as RA, SLE, paraproteinaemia, and some infectious diseases, but not from normal sera. *In vivo* inclusions are found in SF leucocytes but not in circulating blood leucocytes, possibly because of rapid clearance by mononuclear phagocytes in the liver, spleen, and lung of circulating Ig complexes or of the leucocytes that phagocytose such complexes. The occurrence of

intravascular Ig complex phagocytosis is also not yet established. These observations raise the question of whether immunoglobulins are taken up as an antigen-antibody complex or as an aggregate of different origin.

It has already been emphasized that the experiments reported here provide no information as to the nature of the phagocytosed Ig. In whatever form this Ig may be, it seems certain that immunoglobulins from normal healthy persons are not taken up under the conditions used in these experiments, although they may be easily phagocytosed after heat denaturation. Immunoglobulin phagocytosis cannot be regarded as an artefact, produced by the experimental conditions only. In some cases (SLE, infectious diseases) phagocytosis in the form of immune complexes seems probable; in others, like the paraproteinaemias, a satisfactory explanation has yet to be provided.

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