IMMUNOHISTOLOGIC STUDIES OF SYNOVIOCYTES AND SYNOVIAL EXUDATE CELLS*

By ERIC R. HURD, T. DOUGLAS KINSELLA,‡ AND MORRIS ZIFF

(*From the Rheumatic Diseases Unit, Department of Internal Medicine, The University of Texas Southwestern Medical School, Dallas, Texas 75235*)

There has been much interest in recent years in the significance of cytoplasmic inclusions in synovial fluid leukocytes. Parker and Schmid (1) have shown that complexes containing heat-aggregated IgG and rheumatoid factor are phagocytosed and appear in polymorphonuclear cells as cytoplasmic inclusions. Hollander and Rawson et al. (2, 3) have demonstrated that such inclusions containing IgG and IgM are found in the polymorphonuclear leukocytes of rheumatoid synovial effusions and have suggested that these inclusions cause release of lysosomal enzymes with resulting inflammation. The presence of immune complexes in the synovial fluid which may contribute to the development of these complexes has been demonstrated in two laboratories. Hannestad (4) has demonstrated a precipitin reaction between high titer rheumatoid sera and certain rheumatoid synovial fluids, indicating the presence of aggregated IgG in these fluids; and Winchester et al. (5) have demonstrated the presence of 7–30S complexes containing mainly IgG, which formed precipitates with added IgM rheumatoid factor.

The present studies have investigated the presence of immune complexes in rheumatoid synovial fluid by incubating the fluid with normal polymorphonuclear cells and subsequently examining these cells for the presence of inclusions. This work has been previously described elsewhere (6). These studies have also sought to determine whether immune complexes present in the fluid are phagocytosed by the phagocytic lining cells of the rheumatoid synovial membrane. To accomplish this, synovial tissue has been digested with trypsin and the separated synovial lining cells stained for immunoglobulins by the fluorescent antibody technique. This work has also been reported elsewhere (7).

Materials and Methods

Phagocytosis of Inclusions from Synovial Fluid.—Normal polymorphonuclear cells were obtained from the blood of either normal persons or trauma patients. Red cells were sedimented with 6% dextran. The washed buffy coat cells were then combined with cell-free synovial fluid

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‡ Present address: Rheumatic Diseases Unit, Queen's University, Kingston, Ontario, Canada.

and incubated for 1 hr at 37°C with gentle shaking. After incubation, the cells were centrifuged, washed, resuspended in saline, and centrifuged onto glass slides in a Shandon cytocentrifuge (Shandon Scientific Co., Inc., Sewickley, Pa.)

Addition of IgM Rheumatoid Factor or Waldenström IgM to Rheumatoid Factor–Negative Fluids.—Factor–negative synovial fluids which had previously failed to produce inclusions were divided into two portions. To one, chromatographically purified IgM rheumatoid factor was added to a concentration of 1 mg/ml, and to the other, an equal amount of Waldenström IgM with no rheumatoid factor activity was added. Normal buffy coat leukocytes were then added to each fluid and the mixture incubated for 1 hr at 37°C. The cells were then centrifuged, washed, and slides prepared in the cytocentrifuge.

Immunofluorescent Staining of Polymorphonuclear Cells.—Cells were stained with various fluoresceinated goat antisera directed against human β_1C, IgG, and IgM, and examined for the presence of leukocyte inclusions. In all preparations where inclusions were present, sequential staining with fluoresceinated and rhodaminated antibodies was carried out. Control preparations were made in which cells not previously incubated in synovial fluid were stained sequentially in the same manner.

Tests for Rheumatoid Factor.—The Hyland latex test and sensitized sheep cell agglutination (SSCA)[1] test were used for determination of rheumatoid factor in synovial fluid and serum.

Synovial Membrane Digestion and Immunofluorescent Staining Technique.—Synovial tissue was obtained at arthrotomy and by needle biopsy using the Parker-Pearson needle. Within 2 hr after surgery, synovial specimens, appropriately washed and trimmed, were placed in fresh medium (normal saline, 10 ml; fetal calf serum, 1.5 ml; $NaHCO_3$ 7.5%, 1 ml; penicillin 200,000 units/ml, 1 ml; and ethylenediaminetetraacetate [EDTA] in a final concentration of 0.4%) and minced. The minced synovium was digested with trypsin at a final trypsin concentration of 0.25%. After digestion, gross particles were sedimented by gravity and 4–8 drop aliquots of the supernatants were transferred to a cytocentrifuge and spun at 700 rpm. Slide preparations were air-dried and stained by the fluorescent antibody technique. Combined immunofluorescent staining, using antisera conjugated with rhodamine and fluorescein, was utilized. The antisera employed included rabbit and goat anti–human IgG and goat anti-IgA, IgM, and the β_1C-β_1A component of complement.

RESULTS

Phagocytosis of Inclusions from Rheumatoid Factor–Positive Synovial Fluids.—In Fig. 1 are shown the large inclusions present in normal polymorphonuclear leukocytes incubated with the synovial fluid of a seropositive patient with rheumatoid arthritis (RA). Such inclusions were stained sequentially with fluoresceinated anti-β_1C and rhodaminated anti-IgG, fluoresceinated anti-β_1C and rhodaminated anti-IgM, and fluoresceinated anti-IgG and rhodaminated anti-IgM. The combined staining patterns demonstrated the presence of IgG with β_1C, IgM with β_1C, and IgG with IgM. These results suggest that the inclusions represented a complex of IgG, IgM, and complement components.

Table I summarizes the results obtained with fluids of 19 patients with adult RA. The majority of the 13 RA synovial fluids with sensitized sheep cell titers of 1/14 or greater yielded immunoglobulin-containing inclusions when incubated

[1] *Abbreviations used in this paper:* RA, rheumatoid arthritis; SSCA, sensitized sheep cell agglutination.

with normal buffy coat cells. In contrast, no inclusions were seen with factor-negative fluids. It is of interest that all eight of the IgM-positive inclusions observed also stained for IgG, and five of these also stained for β_1C, suggesting strongly that IgM inclusions are present as IgG–IgM–complement complexes.

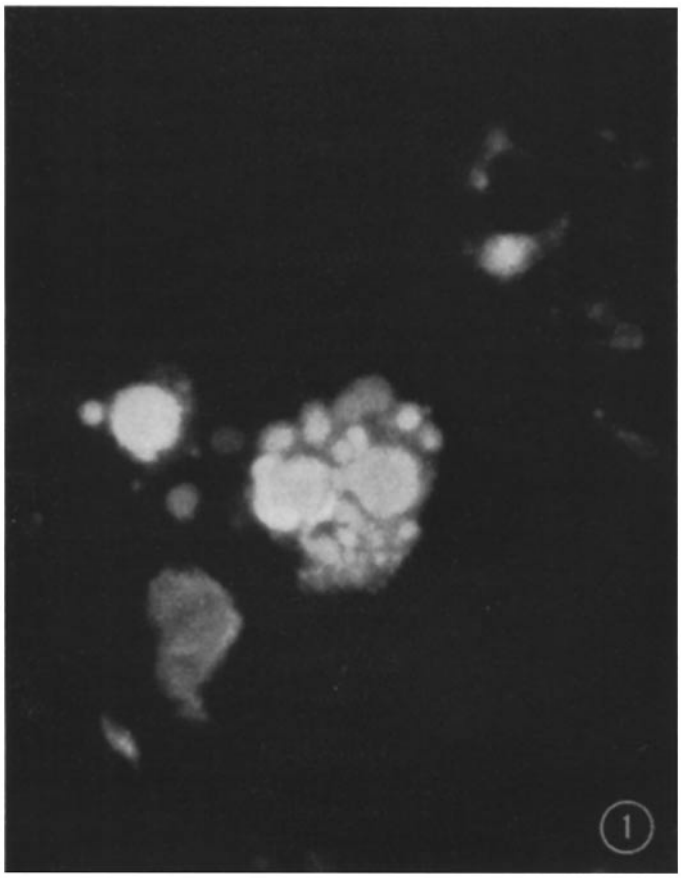

FIG. 1. Immunofluorescent staining of a normal leukocyte after incubation with rheumatoid factor-positive synovial fluid. Large inclusions are seen staining positively for β_1C. These also stained positively for IgG and IgM. × 950. Reprinted by copyright permission from *Arthritis Rheum.* 1970. **13**:724.

Synovial Fluids from Miscellaneous Arthritides.—Table II shows the results obtained with fluids from 22 patients with a group of miscellaneous arthritides. None of these synovial fluids gave positive rheumatoid factor tests by either latex or SSCA tests. In contrast to the results shown in Table I, it can be seen

TABLE I

Relation of SSCA Titer of Synovial Fluid to Inclusions Phagocytosed from Rheumatoid Effusions

Fluorescent antibody stain	Reciprocal SSCA titer Adult RA	
	≥14	<14
IgG	9/13	0/6 $P \leq 0.01$
IgM	8/13	0/6 $P \leq 0.025$
β_1C	6/13	1/6 $P > 0.05$

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TABLE II

Inclusions Phagocytosed from Nonrheumatoid Synovial Fluids

Fluorescent antibody staining	Inclusions (No. positive/No. tested)
β_1C	4/22
IgG	3/22
IgM	1/22

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TABLE III

Production of Inclusions upon Addition of Rheumatoid Factor: Comparison of Factor-Negative Rheumatoid and Nonrheumatoid Fluids

Fluorescent antibody staining	Adult rheumatoid effusions	Nonrheumatoid effusions
IgG	5/9	3/16 $P \leq 0.025$
IgM	5/9	3/16 $P \leq 0.025$
β_1C	4/9	1/16 $P \leq 0.025$

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that only four of 22 fluids produced β_1C-containing inclusions, three IgG-containing inclusions, and only one IgM-containing inclusions. These findings indicate that small amounts of material containing IgG and complement may be phagocytosed from nonrheumatoid inflammatory synovial effusions.

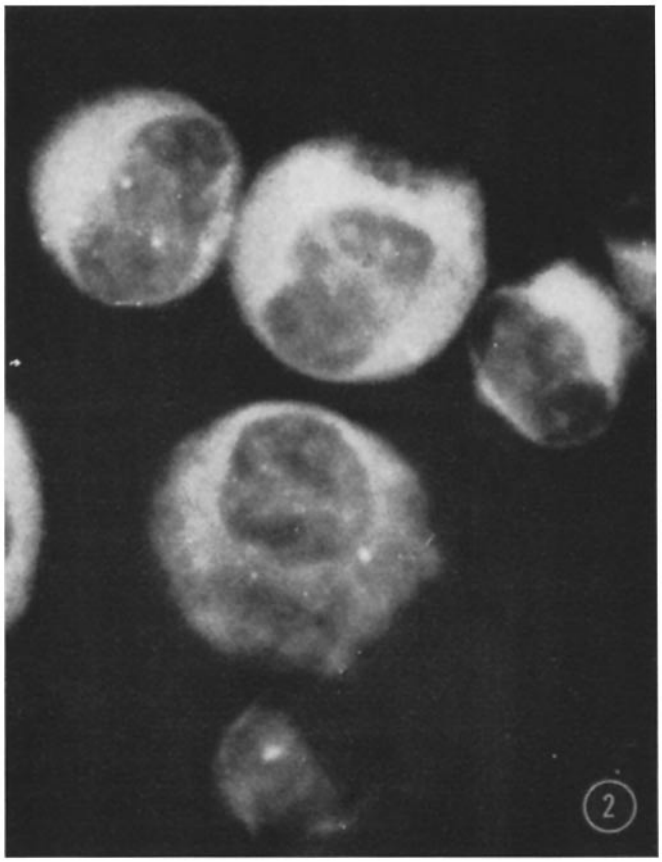

FIG. 2. Seronegative adult RA. A cluster of synovial A and C type cells shows diffuse cytoplasmic staining for β_1C. These cells also stained positively for IgG in a similar distribution. × 950.

Effect of Addition of Rheumatoid Factor to Factor-Negative Fluids.—Of the nine previously negative adult rheumatoid fluids tested, five developed IgG and IgM-containing inclusions, and four developed β_1C-containing inclusions after addition of purified rheumatoid factor to the fluid (Table III). Addition of the same amount of a Waldenström IgM, which had no rheumatoid factor activity,

produced no significant inclusions. After addition of rheumatoid factor to a group of 16 miscellaneous control fluids (see Table III), inclusions were obtained from only three of 16 of these fluids. These stained for IgG and IgM, but only one was positive for β_1C. These findings provide evidence for the presence of

TABLE IV

Diffuse Fluorescent Staining of Cytoplasm of Type A and Type C Lining Cells in Various Diagnostic Groups

Diagnosis	No. of patients tested	No. of patients positive*				
		IgG	IgM	RF‡	β_1C	IgG—β_1C§
Ruptured meniscus	3	0	0	0	0	0
Degenerative joint disease	4	1	0	0	0	0
Miscellaneous arthritides‖	6	0	0	0	0	0
Juvenile RA (RF−)	7	5	0	0	5	4(6)¶
Adult RA (RF−)	5	5	0	0	5	4(4)**
RA (RF+)	5	5	0	0	5	5(5)

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* Values represent number of patients showing greater than 2+ staining of over 50% of types A and C lining cells.

‡ RF = rheumatoid factor.

§ Represents sequential staining with rhodamine-conjugated anti-IgG and fluorescein-conjugated anti-β_1C.

‖ Reiter's syndrome, 1; gonococcal arthritis, 3; pseudogout, 1; nail-patella syndrome, 1.

¶ Only 6 patients tested, 4 positive.

** Only 4 patients tested, all positive.

TABLE V

*Components of Cytoplasmic Inclusions in Phagocytic Lining Cells in Seropositive RA**

No. of Patients	No. positive for inclusions containing					
	IgG	IgM	RF‡	β_1C	IgG-β_1C§	IgG-IgM‖ (No. positive/No. tested)
5	4	4	3	2	2	3/3

* All other diagnostic groups negative for inclusions.

‡ RF = rheumatoid factor.

§ Sequential staining with rhodamine-conjugated anti-IgG and fluorescein-conjugated anti-β_1C.

‖ Sequential staining with rhodamine-conjugated anti-IgG and fluorescein-conjugated anti-IgM.

aggregated IgG in the majority of factor-negative rheumatoid effusions and some inflammatory nonrheumatoid effusions.

Presence of Immunoglobulin Complexes in Phagocytic Lining Cells of Rheumatoid Synovial Membrane.—When synovial cell preparations were stained directly

with conjugated antisera to IgG, IgM, and the β_1C component of complement (in some cases staining for anti-C1q was also carried out), two patterns of cytoplasmic fluorescence, restricted to the phagocytic type A and C lining cells, were noted. The first of these was a diffuse staining which, on occasion, took on a

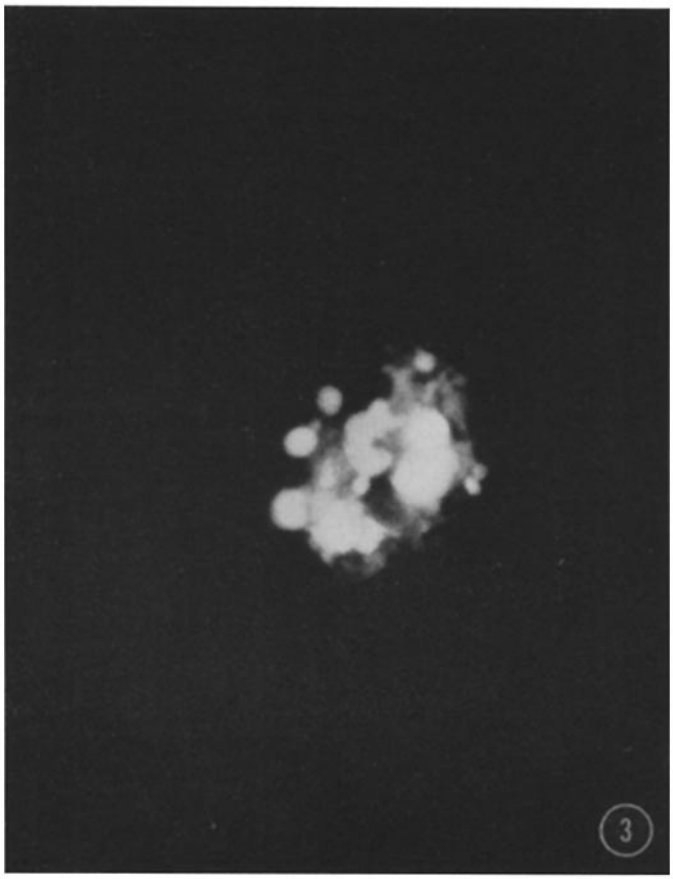

FIG. 3. Seropositive adult RA. A single synovial C type cell shows discrete inclusions in the cytoplasm. These inclusions showed positive combined staining for IgG and IgM. Similar inclusions also stained positively for IgG and β_1C and IgM and β_1C. × 950.

finely granular appearance. The second was characterized by the presence of comparatively large and discrete cytoplasmic inclusions.

Diffuse Staining Pattern.—Applying the criterion that at least 50% of the phagocytic cells show 2+ or greater cytoplasmic fluorescence, all of the seropositive patients, all of the adult seronegative patients, and five of the seven

seronegative juvenile RA patients showed diffuse staining for IgG (Fig. 2 and Table IV). Similar staining was rarely observed in the cells of other arthritides. When present in nonrheumatoid cells, this staining pattern was weak and involved lessthan 10% of the phagocytic cells.

Diffuse staining for the β_1C component of complement was found in 15 of the 17 RA patients tested; however, diffuse staining for IgM or for rheumatoid factor was not observed in any of the groups studied. When sequential staining with rhodamine-conjugated anti-IgG and fluorescein-conjugated anti-β_1C was carried out, 13 of 15 patients with RA showed simultaneous localization of both IgG and β_1C within phagocytic lining cells. No patients in any of the other disease groups showed this phenomenon. These findings suggest that a complex made up of IgG and complement components is diffusely present in the cytoplasm of the phagocytic synovial cells in both seronegative and seropositive patients.

Demonstration of Discrete Inclusions in Synovial Lining Cells.—The second pattern of immunofluorescence, characterized by the presence of discrete inclusions, was observed only in the lining cells of seropositive rheumatoid patients (Table V). In these cells, discrete and usually large inclusions were observed within the cytoplasm of the type A and type C lining cells (Fig. 3). These were present in four of the five rheumatoid factor-positive patients studied. These inclusions stained positively for IgG and for IgM in the case of four of the five seropositive patients studied; inclusions containing both IgG and IgM, as demonstrated by combined staining, were found in all of three patients studied this way; and inclusions staining positively for both IgG and β_1C were present in two of four patients studied. Thus in seropositive rheumatoid patients, single and combined staining produced evidence for the presence of inclusions staining positively for IgG, IgM, and β_1C in the phagocytic lining cells.

DISCUSSION

The demonstration that normal polymorphonuclear cells phagocytose inclusions, from rheumatoid factor–positive synovial fluids which stain positively for IgG, IgM, and β_1C, suggests that complexes of these immunoglobulins exist in such fluids. Of interest, however, is the observation that the addition of IgM rheumatoid factor to some factor-negative rheumatoid fluids and to a few nonrheumatoid inflammatory fluids yielded inclusions with a similar staining pattern. This would indicate that an altered form of IgG is present in factor-negative fluids, which is available for reaction with IgM rheumatoid factor.

Hannestad (4) has shown that rheumatoid synovial effusions develop precipitin lines with rheumatoid factor–containing sera, and Winchester et al. (5) have demonstrated the presence in rheumatoid synovial effusions of IgG complexes which bind with complement. The present observation provides evidence that an aggregated form of IgG, which is capable of reacting with IgM rheumatoid

factor, is present in factor-negative rheumatoid synovial fluids and some nonrheumatoid fluids. It is possible, at least in the rheumatoid patients, that this complex is identical with the IgG complex formed by IgG rheumatoid factor, which has been described by Winchester et al.

Examination of the synovial lining cells of rheumatoid synovial tissue by the immunofluorescent staining technique has shown that the phagocytic synovial cells contain immunoglobulins. In seronegative patients, the cytoplasm of these cells showed a diffuse positive staining for IgG and the β_1C component of complement. This suggests the presence in these cells of one or more IgG complexes, combined with complement, which have originated in the synovial fluid. The fact that the staining pattern had a smooth to granular appearance suggests that these complexes are either soluble or partially degraded, once having been phagocytosed. It should be pointed out that the IgG complexes described by Winchester et al. appear to be soluble in character.

In phagocytic synovial lining cells from seropositive rheumatoid patients, discrete inclusions stainable for IgG, IgM, and β_1C were demonstrable. This suggests that complexes made up of these components are present in the synovial effusions of these patients and that, like the IgG complexes discussed above, these are phagocytosed in vivo by the synovial lining cells. It may well be that the phagocytosis of these complexes serves as a trigger for some aspects of the rheumatoid inflammatory reaction since the entry of such immune complexes into the lysosomes of the synovial lining cells may lead to injury of these cells.

SUMMARY

Inclusions were demonstrated in normal polymorphonuclear cells incubated with rheumatoid factor–positive synovial fluid. These stained positively for IgG, IgM, and the β_1C component of complement. When normal polymorphonuclear cells were incubated with factor-negative rheumatoid fluid, inclusions were not obtained. However, after addition of IgM rheumatoid factor to such fluids, discrete inclusions were observed with similar staining properties. In a minority of nonrheumatoid fluids, inclusions were also obtained after addition of IgM rheumatoid factor. The phagocytosis of inclusions from synovial fluids after addition of IgM rheumatoid factor suggests the presence of aggregated IgG in these fluids.

Immunofluorescent staining of phagocytic synovial lining cells, isolated from synovial tissue by trypsin digestion, demonstrated a diffuse staining pattern for IgG and the β_1C component of complement in cells from both seropositive and seronegative patients. In seropositive patients, discrete inclusions were also observed which stained positively for IgG, IgM, and β_1C. These findings provide evidence of in vivo phagocytosis of immune complexes from the synovial fluid.

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