IMMUNOREACTANTS IN RHEUMATOID SYNOVIAL EFFUSIONS*

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The inclusion of rehumatoid arthritis in this symposium implies that it is a disease whose pathogenesis is, at least in part, related to immune complexes. This paper will review some of the evidence supporting this concept. In addition, data will be presented showing that the synovial membrane produces significant amounts of IgG which may be involved in local immune complex formation. Finally, it will attempt to dispel the myth that the rheumatoid joint is just an inert sac involved by a chronic, indolent process.

The rheumatoid synovium differs from normal in several particulars. It is usually thickened by edema. The lining is hypertrophied and redundant and forms many villus projections. Microscopic examination of these villi shows hypertrophy and hyperplasia of the lining cells and a great increase in small blood vessels. The normally delicate fibrillar stroma is infiltrated with cellular elements, predominantly mononuclear, often collected into dense nodular aggregates having the appearance of lymphoid follicles (Fig. 1). This resemblance to lymph nodes prompted analysis of the function of these cells. Immunofluorescent studies show that the lymphocytes and plasma cells in the synovial villus make or store immunoglobulins and anti-gamma globulins (rheumatoid factors) (1, 2). In vitro experiments employing radio-labeled amino acids indicate that the immunoglobulin-synthesizing capacity of the rheumatoid synovium is similar to that of human lymph nodes or splenic tissues (3). In recognition of this synthetic potential, a study was designed to measure the IgG in synovial fluid which is made by the intact rheumatoid synovium, and to estimate the amount produced daily in a single knee joint (4).

Five patients with classical rheumatoid arthritis, two with degenerative joint disease, and two with Reiter's syndrome, were studied. Radio-labeled albumin (^{131}I) and labeled IgG (^{125}I) were administered together intravenously and allowed to equilibrate with the synovial fluid proteins. Serial-specific activities of the albumin and IgG were determined in plasma and synovial fluid. Equilibration was assumed to have occurred when the ratio of the synovial fluid to plasma-specific activity was constant. The methods of protein preparation, radio labeling, isotope counting and testing to insure homogeneity,

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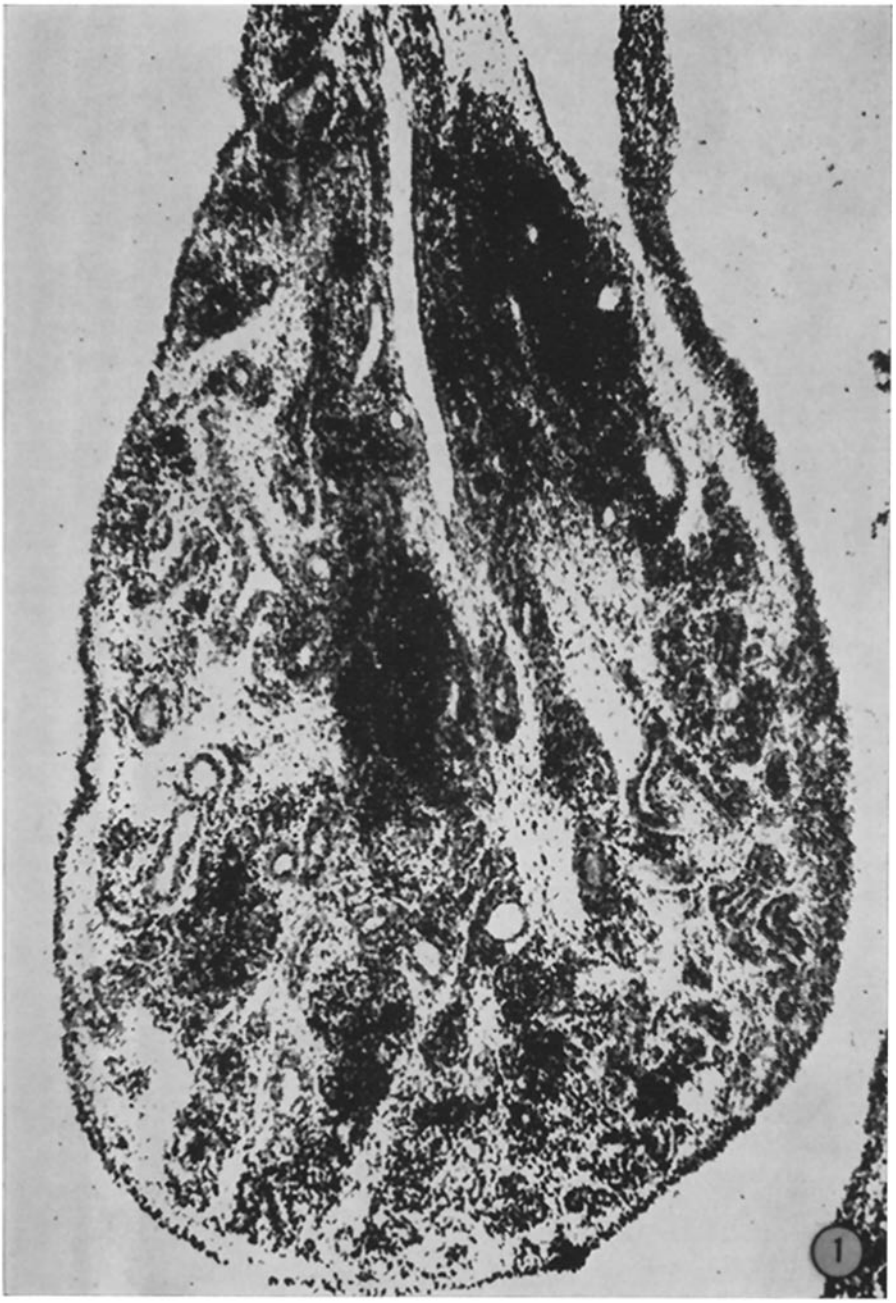
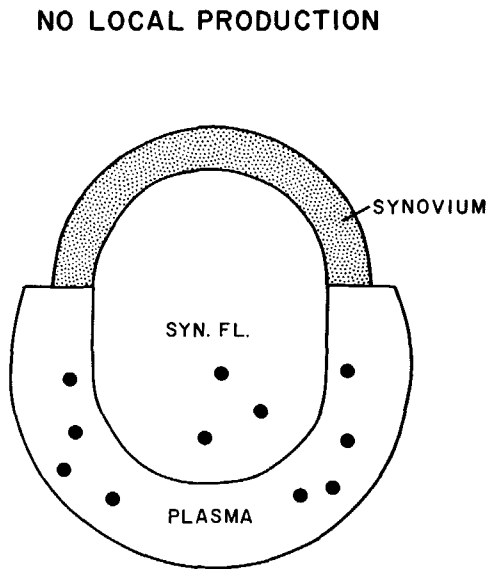
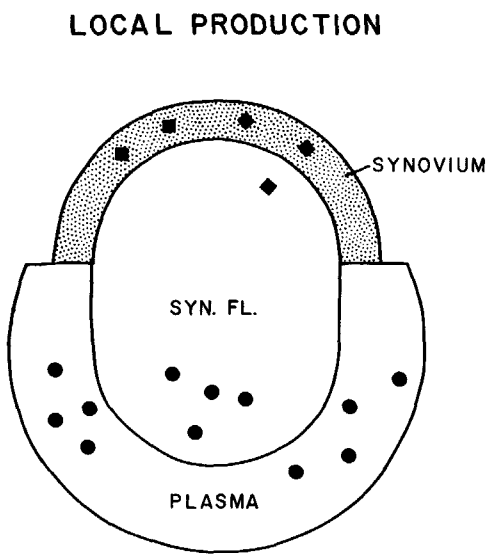


FIG. 1. Section through a rheumatoid synovial villus. The subsynovium shows congestion and edema and is infiltrated with mononuclear cells. In some areas the local collections of these cells resemble lymphoid follicles. × 100.



		PLASMA	SYN. FL.
Protein	mg/ml	80	40
Radioactivity	cpm/ml	800	400
Specific Activity	cpm/mg	10	10

FIG. 2 A



		PLASMA	SYN. FL.
Protein	mg/ml	80	40
Radioactivity	cpm/ml	800	320
Specific Activity	cpm/mg	10	8

FIG. 2 B

FIG. 2. Schematic representation of the model used for determining IgG production in the rheumatoid synovium. (A) Expected synovial fluid and plasma-specific activities in the absence of local production. (B) Expected synovial fluid and plasma-specific activities if there were production of protein in the synovium.

sterility, and lack of pyrogenicity have been described (5). Protein concentrations were measured by radial immunodiffusion. The theoretical basis for these experiments is demonstrated in Fig. 2. If the synovial fluid protein studied is derived from the plasma, and there is no local production of protein by the synovium, then the specific activity of the protein in synovial fluid and plasma should be identical. This would be true regardless of the actual protein concentrations in the two compartments (Fig. 2 A). On the other hand, if there is

TABLE I

Patient	DX*	RF	VOL	Pl Alb	SF Alb	$\frac{\text{SF Alb SA}}{\text{Pl Alb SA}}$	Pl IgG	SF IgG	$\frac{\text{SF IgG SA}}{\text{Pl IgG SA}}$
						%			%
D. M.	RA	1024	30	38.3	19.6	109	23.7	12.5	85
C. G.	RA	0	30	30.4	15.4	98	19.7	7.8	88
L. V.	RA	64	10	32.2	15.3	129	12.5	9.8	81
R. S.	RA	32	50	28	21	100	15.9	14.1	76
E. P.	RA	128	50	32.3	22.5	105	11.6	9.0	84
R. G.	R	0	75	36	31	110	8.3	5.7	100
F. S.	R	0	20	47	32	99	27.0	17.0	101
M. J.	DJD	0	50	29.5	15.3	103	14.4	6.5	102
H. B.	DJD	0	30	27.2	15.4	104	19.6	9.0	99.5

* DX: RA, rheumatoid arthritis; R, Reiter's syndrome; DJD, degenerative joint disease.
RF, rheumatoid factor (reciprocal of bentonite flocculation titer).
VOL, volume of synovial effusion.
Pl Alb, mg albumin/ml plasma.
SF Alb, mg albumin/ml synovial fluid.
$\frac{\text{SF Alb SA}}{\text{Pl Alb SA}}$, ratio of specific activity of synovial fluid albumin to plasma albumin.
Pl IgG, mg IgG/ml plasma.
SF IgG, mg IgG/ml synovial fluid.
$\frac{\text{SF IgG SA}}{\text{Pl IgG SA}}$, ratio of specific activity of synovial fluid IgG to plasma IgG.

local production of protein, then the synovial fluid protein would be derived from two sources; labeled protein entering from the plasma and unlabeled protein from the synovium. In this instance the specific activity of the synovial fluid protein would be consistently less than the plasma protein. This difference would be the measure of the contribution from local production. In the example shown (Fig. 2 B) 20% of the protein would be derived from local production.

The ratio of the specific activity of synovial fluid albumin to plasma albumin was approximately equal (100%) in all of the nine patients studied 1 wk after injection and remained so for the next 3–5 wk. In the case of IgG the

specific activity of synovial fluid IgG increased for the first few days after injection. Within 1 wk the synovial fluid IgG specific activity was identical to the plasma IgG-specific activity in both patients with osteoarthritis and in the two subjects with Reiter's syndrome. Thereafter the IgG-specific activities declined at the same rates so that the ratio of synovial fluid to plasma remained about 100%. These findings were interpreted to show that there was no local production of IgG by the synovium in these subjects. In contrast, the ratio of the specific activities of synovial fluid IgG to plasma IgG was always less than 100% in the five rheumatoid subjects. It was between 76 and 88% (Table I) and remained constant for each of the patients throughout the investigation (5–10 wk). The difference between the ratio obtained and 100% is the measure of unlabeled IgG produced by the synovium. Therefore, 12–24% of the IgG in the synovial fluid of these five rheumatoid subjects was produced locally. Since the dilution of the labeled IgG in the synovial fluid by local production of unlabeled IgG is constant, it is possible to estimate the amount of IgG produced by a single knee joint in 1 day. This was done in the following manner. It has been shown that the half-life of IgG in the rheumatoid knee joint is 0.7–1.5 days (5). In other words, one-third to one-half of the synovial fluid IgG is replaced daily. The amount of protein that turns over per day in the articular cavity would then be equal to the amount of protein in the joint (volume of the effusion $\times$ the protein concentration) $\times$ 0.33–0.50. The amount of protein turned over daily multiplied by the fraction produced locally should equal the amount produced in the synovium per day. Using these calculations the knee joints of these five rheumatoid subjects produced IgG in amounts ranging from 5 to as much as 100 mg daily.

At this time the antibody nature of the IgG can only be assumed. However, the finding of greater concentrations of some antibodies in synovial fluid than in the accompanying serum lends support to the idea of local antibody production. Antinuclear antibodies are sometimes found in fluids when absent from the serum (6, 7). Lysates from cells of joint effusions have antinuclear activity when it cannot be detected in the fluid from which the leukocytes were derived (8). Tests for rheumatoid factors are occasionally positive in synovial effusions when they are negative in the serum (9, 10). Hannestad and Mellbye reported that the ratio of synovial fluid γM-type rheumatoid factor to serum rheumatoid factor was greater than the ratio of two other γM antibodies in 1 of 13 rheumatoid patients studied (11). The IgG anti–γ-globulin present in complex form in some rheumatoid effusions is seldom found in the serum, but when present it is in lesser concentrations than in the fluid (12).

Present concepts of the pathogenesis of rheumatoid inflammation are derived from studies of joint effusions. There is reason to believe that antigen(s) combining with antibody(s) activates the complement sequence, generating a variety of biologically active materials, including some with potent chemotac-

tic properties. These bring polymorphonuclear leukocytes into the articular cavity, where they are attracted to and ingest the immune complexes. After phagocytosis the neutrophils discharge from their lysosomal granules a variety of hydrolytic enzymes. It is these substances that appear to play a central role in the proliferative and destructive changes characteristic of rheumatoid arthritis. The evidence supporting this pathogenetic concept has been presented in detail (13) but can be summarized as follows. Despite relatively normal serum concentrations of hemolytic complement, there is a disproportionate lowering of complement, compared to other joint fluid proteins in rheumatoid synovial fluids (14, 15). Individual complement components are depleted and a number of by-products of the complement sequence are found in these effusions (16–18). As outlined by Ruddy (19) these results are most consistent with immune activation of the complement sequence, presumably by antigen–antibody complexes. Three-quarters of rheumatoid synovial fluids contain a factor resembling soluble complexes. When these fluids are perfused into intact guinea pig lungs they cause the release of histamine (20). Complexes can be precipitated from some rheumatoid joint fluids by an IgM rheumatoid factor (21). Similar complexes have been detected by ultracentrifugal analysis or by direct precipitation with isolated C1q (22, 23). As shown by Winchester and associates (24) these complexes are composed of IgG and 7S anti-IgG (24). Cryoprecipitable complexes containing varying proportions of IgG, IgM, DNA, and antinuclear factors have been detected regularly in fluids from seropositive rheumatoid patients but not in companion serum samples (25, 26). A variety of potentially important immunoreactants are found in the cells from rheumatoid synovial effusions. 19S and 7S immunoglobulins, anti-gamma globulins, and components of the complement system have been detected in the cytoplasm of synovial fluid leukocytes and in the phagocytic cells lining the synovial membrane (27–31). Their relationship to one another and to rheumatoid joint inflammation has been analyzed by Ziff (32).

One characteristic of rheumatoid arthritis is the remarkable accumulation of white blood cells, predominantly polymorphonuclear, in the articular cavity. Leukocyte counts of 50,000/mm^3 are not unusual. This means that there are 50 million cells/cc. Thus, a knee joint with a small 10 cc effusion actually contains as many as 500 million white cells. These cells have been shown to have a half-life of 3–4 hr in the rheumatoid joint (33). Therefore, in the example outlined, each day over one billion white cells enter the articular cavity to participate in the inflammatory response.

The knowledge that small molecular weight proteins derived from C3 and C5 have chemotactic activity (34), the evidence for activation of the complement system in rheumatoid effusions, and the finding of breakdown (conversion) products of C3 in some rheumatoid joint fluids (Fig. 3) suggested that

synovial fluids should be analyzed for chemotactic activity (35). It is not surprising, therefore, that chemotactic activity was found in a relatively high percentage (70%) of rheumatoid fluids. This activity was demonstrated in two distinct substances. All but one of the positive fluids studied showed a high molecular weight chemotactic factor. A combination of physical-chemical techniques indicated that the activity was attributable to the trimolecular

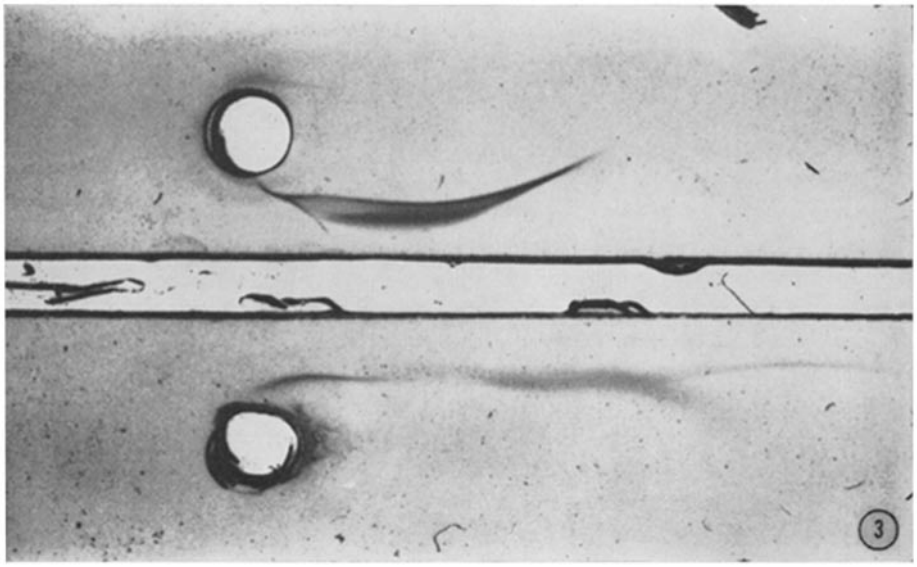


FIG. 3. Immunoelectrophoretic pattern of fresh plasma (ethylenediaminetetraacetate [EDTA]) from a patient with rheumatoid arthritis (top well) and an EDTA synovial fluid from the same subject (bottom well). The antiserum to human C3 detects a partial conversion of C3 to C3i in the synovial fluid plus a more anodal precipitin arc. These are not seen in the fresh plasma obtained at the same time.

complex of C $\overline{567}$. Two-thirds of the fluids also showed a lightweight chemotactic factor which was identified as a cleavage product of C5 (C5a). In addition, approximately one-half of the rheumatoid fluids contained an enzyme capable of producing a chemotactically active cleavage product, similar to the lightweight chemotactic factor, when incubated with isolated human C5. On the basis of substrate specificity and susceptibility to inhibitors, the C5-cleaving enzyme is very similar to an enzyme extractable from lysosomal granules of human granulocytes.

Thus, in the rheumatoid joint conditions are ideally suited for the perpetuation of articular inflammation. The ease with which plasma proteins pass

through an inflamed synovium makes it likely that the substrate C5 is being continually delivered into the joint space. The presence of the C5 cleaving enzyme would ensure the rapid production of C5a, intensifying the already acute inflammatory response by accelerating the delivery of more and more neutrophils from the circulation. Like other lysosomal enzymes, the C5-cleaving enzyme is released during phagocytosis. The presence of immune complexes in rheumatoid synovial fluids would set the stage for phagocytosis and the release of the C5-cleaving enzyme.

At this point it should be clear that the articular cavity in patients with rheumatoid arthrititis contains abundant quantities of immunoreactants. It is therefore reasonable to propose that this disease be added to the list of human "immune complex diseases." As in so many of the other immune complex diseases, information about the manner in which such complexes produce tissue injury is becoming quite definitive, but knowledge of the antigens participating in their formation remains limited. While there is as yet no evidence to support the thesis, it is a reasonable assumption that the responsible antigen resides in the synovial membrane. Hopefully, the antigen, or antigens, which initiates the rheumatoid inflammatory process will soon be identified, thus opening the way to preventing or manipulating this disabling disease.

SUMMARY

Measurements were made of the IgG in synovial fluid which is synthesized by the intact rheumatoid synovium. In five rheumatoid subjects 12–24% of the IgG present in their synovial fluid was derived from local production. No evidence for local production was found in patients with degenerative arthritis or Reiter's syndrome. It was estimated that as much as 95 mg of IgG was produced by the synovium of a single knee joint daily. Rheumatoid inflammation is associated with the presence of large numbers of white blood cells, predominantly polymorphonuclear, in the articular cavity. Each day as many as one billion white cells enter the articular cavity to participate in this inflammatory response. Factors causing the directed migration of polymorphonuclear leukocytes were found in the majority of rheumatoid effusions studied. The chemotactic activity is, in large part, related to the fifth (C5) and sixth (C6) components of human complement. Physical-chemical techniques indicate that the activity is attributable to C $\overline{567}$ and C5a, a cleavage product of C5. In addition to the presence of preformed chemotactic factors, more than half of the rheumatoid fluids contain an enzyme capable of generating chemotactic activity from the fifth component (C5) of human complement. This information supports the concept that rheumatoid joint inflammation is an example of immune complex disease in which a significant proportion of the immunoreactants are derived from local production.

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