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Research Article

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Response of the Rheumatoid Synovial Membrane to Exogenous Immunization

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A B S T R A C T The secondary immune response to tetanus toxoid in 14 patients with rheumatoid arthritis (RA) has been studied in suspension cultures of peripheral blood lymphocytes (PBL) and synovial membrane obtained at synovectomy. Sequential cultures of PBL from three normal subjects established the optimal time of antibody response at 5 days. At this time, the antitetanus antibody produced was predominantly IgG, comprising half of this immunoglobulin fraction. Rheumatoid synovium synthesized 5–9 times more IgG than PBL, expressed as per cent of total protein synthesis, but only negligible amounts of tetanus antibody. The same results were observed in synovial cultures following repeated immunization and after the additional intra-articular injection of tetanus antigen. This marked limitation of the synovium to respond to exogenous antigen in spite of its large immunoglobulin production was considered consistent with a prior commitment of the synovial lymphoid infiltrate to other antigen.

INTRODUCTION

Microscopic examination of rheumatoid synovial tissue reveals a chronic inflammatory infiltrate consisting primarily of lymphocytes, plasma cells, and macrophages. Immunofluorescent studies (2, 3) have shown that many of these infiltrating cells contain the immunoglobulins IgG and IgM as well as rheumatoid factor. The local synthesis of these immunoglobulins within the rheumatoid synovium has been demonstrated by an in vitro culture technique and the proportion of newly synthesized protein representing γ-globulin has been shown to be similar to that produced by normal human spleen and lymph nodes (4). The antigenic specificity of the im

munoglobulin produced by the rheumatoid synovium is unknown. It could have antibody characteristics reflecting the general immune status of the patient, or it could represent specific antibody directed against antigens localized within the inflamed joint. To distinguish between these two possibilities, a comparison has been made of the immunoglobulin and specific antibody synthesized in vitro by rheumatoid synovial tissue with that produced simultaneously by peripheral blood lymphocytes (PBL) obtained from patients immunized with tetanus toxoid.

In recent experiments performed in this laboratory (5), a nonspecific synovitis was produced by streptolysin S in rabbits undergoing a secondary response to bovine serum albumin (BSA) and the synthesis of specific antibody by the synovial membrane measured in vitro. The percentage of γ-globulin representing specific antibody formed by the synovium of these rabbits was similar to that produced by PBL cultured simultaneously. However, in the present investigation, the rheumatoid synovium was found to be markedly limited in its response to tetanus antigen in comparison with the PBL.

METHODS

Patients. Synovial tissues and peripheral blood specimens were obtained from 16 adult patients with classical rheumatoid arthritis (RA) as defined by the criteria of the American Rheumatism Association (6) and from three otherwise normal patients undergoing knee cartilage revision procedures. In addition, the synovium from the knee of a patient with acute Reiter's syndrome was obtained by repeated needle punch biopsies 6 days after immunization, and cultured in a similar manner. Sequential PBL cultures derived from three normal individuals were also studied.

Tetanus immunization. To determine the optimal time prior to synovectomy for immunization of patients with RA, sequential bleedings of 50 ml were obtained from three normal subjects at the time intervals indicated in Fig. 1 following secondary immunization with 0.5 ml of alum phosphate adsorbed tetanus toxoid (Parke, Davis & Co., Detroit, Mich.). The same volume of tetanus toxoid was injected intramuscularly in 14 patients with RA at 2- to 14-day intervals prior to synovectomy. The nonrheumatoid patients were

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immunized 6 days before surgery; three of the rheumatoid patients had received one, and three others, two tetanus toxoid injections during the 6 months prior to the immunization given before synovectomy. In the remaining eight patients, whether or not previous tetanus immunization had occurred could not be determined. 2 of the 14 patients, L. C. and M. M., were given an intraarticular injection of one-one thousandth and one-five hundredth of the challenging antigen dose into a metacarpophalangeal (MCP) and a knee joint, respectively, in addition to the usual 0.5 ml of tetanus toxoid intramuscularly prior to synovectomy. In the case of L. C., one injected MCP and four noninjected MCP joints were operated upon, thus permitting a comparison of the response to direct intra-articular antigenic stimulation with the response elicited by antigen given systemically. Synovial and PBL cultures from two patients who were not immunized prior to surgery were also studied.

Tissue incubation. Synovial tissue was cultured as described previously (4), with the following modifications. To each gram of minced synovial membrane, there were added 3 ml of Eagle's medium (7) lacking arginine, leucine, lysine, valine, and serum, but supplemented with 25 μg of hydrocortisone per ml and 3 μCi of an amino acid-^{14}C labeling mixture (Schwarz BioResearch, Orangeburg, N. Y.) containing arginine-^{14}C (166 mCi/mmole), leucine (103 mCi/mmole), lysine (100 mCi/mmole), and valine (80 mCi/mmole).

Peripheral blood lymphocyte incubation. Venous blood, heparinized and diluted by an additional one-third volume of 6% dextran (mol wt 200,000) in saline, was permitted to sediment for 1.5 hr at 37°C. The leukocyte-rich supernatant was sampled for a cell count, then centrifuged at 150 g for 10 min at 4°C. The unwashed cell pellet was resuspended in medium to a final concentration of 10×10^6 cells per ml and 1 μCi of the amino acid-^{14}C labeling mixture added per ml. The incubation was conducted in a bubble tube in the same manner as that of the synovial suspension (4).

Processing of culture suspernatants. At the end of the 6-hr incubation period, the cell or tissue suspension was frozen, thawed once, and spun at 105,000 g for 1 hr in a Spinco Model L ultracentrifuge at 4°C. The supernatants were divided into two equal portions which were processed as diagrammed in Fig. 2. One portion was dialyzed against
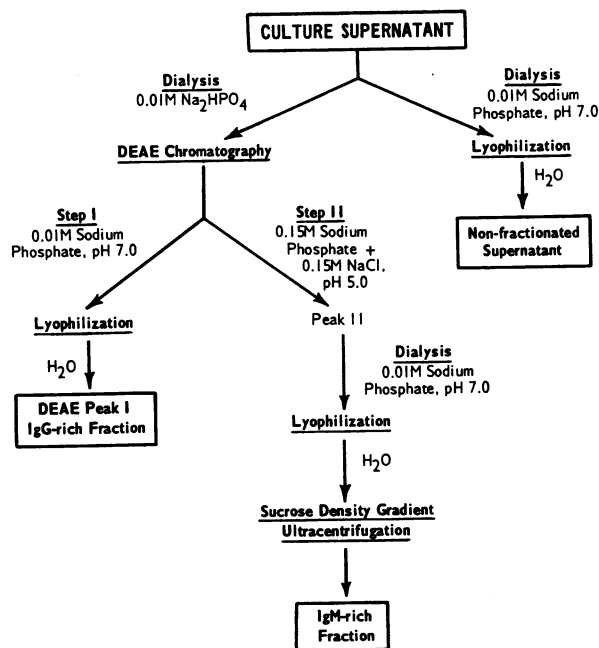
FIGURE 2 Processing of culture supernatants.

0.01 M Na$_2$HPO$_4$, and chromatographed on diethylaminoethyl cellulose (DEAE, capacity, 1 mEq/g), which had been adjusted to pH 7.0. Elution was performed first with 0.01 M sodium phosphate buffer pH 7.0, to yield an IgG-rich fraction (peak I), and then with 0.15 M sodium phosphate, pH 5.0, containing 0.15 M NaCl, to remove most of the residual protein including other immunoglobulins (peak II). The first peak was lyophilized and reconstituted with water to a volume equal to 10% of the original volume of column effluent. To obtain more information about the antibody content of the IgM fraction, peak II of both the PBL and synovium derived from two patients with RA was dialyzed, lyophilized, and the concentrated sample subjected to sucrose density gradient ultracentrifugation (8). Those fractions previously shown by similar separations to contain IgM were pooled and dialyzed.

The remaining equal portion of each supernatant was dialyzed against 0.01 M sodium phosphate buffer, pH 7.0, lyophilized, and reconstituted to one-half of its original volume for comparison of the recovery of radioactivity of total protein and specific antibody with that of the above fractionated portions.

Preparation of antisera. Specific goat antisera to IgG and IgA, and rabbit antiserum to IgM were prepared as previously described (4). Human anti-tetanus antibody was secured from a normal subject following two injections of 0.5 ml of tetanus toxoid or purchased from The Cutter Laboratories, Berkeley, Calif. This antiserum, when permitted to react with a nonalum-adsorbed tetanus toxoid preparation, kindly furnished by the Research Division of Parke, Davis & Co. Laboratories, Detroit, Mich., produced a single precipitin line by Ouchterlony analysis (9).

Immunoglobulin and specific antibody determinations. Before specific precipitation of antibody-^{14}C, all supernatant fractions were subjected to two prior immune precipitations with BSA : anti-BSA at equivalence in order to remove nonspecific radioactivity (10). The appropriate nonradioactive
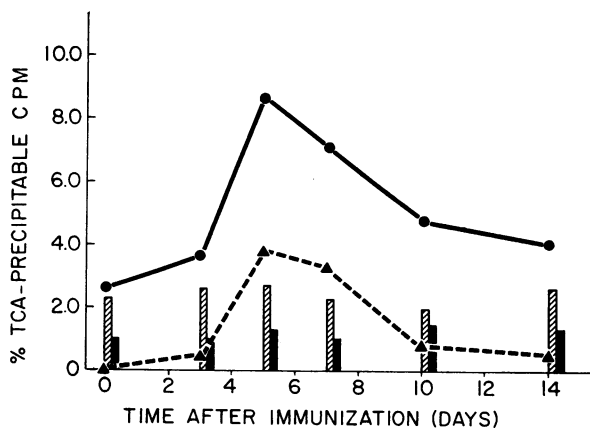
FIGURE 1 Total IgG (●) and tetanus antibody (▲) synthesis by normal peripheral blood lymphocytes. Each point represents the average of the unfractionated supernatants of three cultures. IgA (hatched bar) and IgM (solid bar) synthesis at the same time periods are presented in the bar graph.
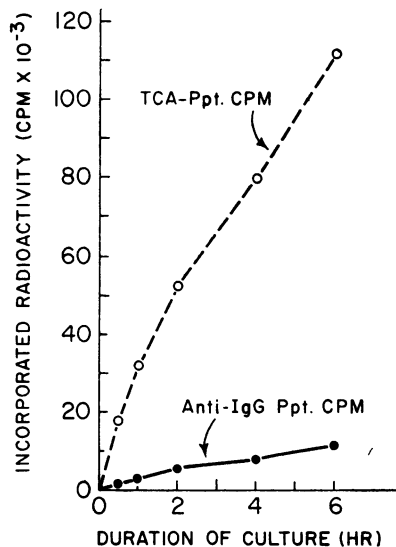
FIGURE 3 ^{14}C-labeled amino acid incorporation into soluble protein and IgG by rheumatoid synovial membrane. Ppt. = precipitable.

immunoglobulin (IgA, IgG, or IgM) and specific antisera were added at slight antibody excess to aliquots of the above supernatant to obtain 2 mg of antigen-antibody precipitate. After incubation at 37°C for 1 hr, then overnight at 4°C, each tube was centrifuged at 17,000 rpm in a Sorvall SS-1 centrifuge (Ivan Sorvall, Inc., Norwalk, Conn.). The supernatant was checked by capillary precipitation with additional antibody to demonstrate that complete precipitation had occurred. The immune precipitates were then washed and radioactivity determined as described previously (4). Tetanus: anti-tetanus precipitations were performed in a similar manner. Protein-bound radioactivity was determined by precipitation with 10% trichloroacetic acid.

Radioimmunoelectrophoresis. Samples of concentrated culture supernatant, to which a small amount of normal human serum containing tetanus antibody as carrier had been added, were subjected to immunoelectrophoresis in agar (11) and developed against anti-whole human serum in one trough and against tetanus antigen in the other. Following repeated washing, slides were dried and exposed to Polaroid Land Film, Type 57, ASA 3000, for 5 wk before development.

RESULTS

Tetanus antibody response in PBL. In three normal subjects challenged with tetanus toxoid, the following evidence indicated that the tetanus antibody was present predominantly in the IgG fraction. Precipitation of nonfractionated supernatants of PBL incubations with anti-human IgG followed by anti-tetanus: tetanus precipitation showed that all ^{14}C-labeled tetanus antibody had been removed by the anti-IgG; testing of the sucrose density gradient fractions of the supernatants which contained IgM failed to demonstrate ^{14}C-labeled tetanus antibody; and radioimmunoelectrophoresis of the supernatant solutions produced a radioactive precipitin line with tetanus

antigen in the slowly moving IgG region, but not in the areas occupied by IgA or IgM. Tetanus antibody–[14]C determinations on DEAE peak I fractions gave similar results to those shown in Fig. 1 for the unfractionated supernatant samples, further supporting the predominant IgG character of the newly synthesized tetanus antibody.

As seen in Fig. 1, a progressive rise and subsequent decline in IgG synthesis, expressed as per cent of TCA-precipitable protein, was observed with the maximum response occurring on day 5. The levels of IgA and IgM synthesis showed little daily variation. The change in the level of ^{14}C-labeled tetanus antibody paralleled that of IgG-^{14}C, comprising an average of 51% of the newly synthesized IgG at the peak response. At the time of maximum response, a 3.6-fold increase in the mean level of IgG-^{14}C was observed in comparison with the pre-immunization values. From these results, it was apparent that patients with RA should be immunized at 5–7 days prior to synovectomy in order to obtain an optimal tetanus antibody response in PBL.

Continuity of protein synthesis in synovium and PBL. Individual samples of an incubating synovial tissue suspension from one patient were removed at hourly intervals and the culture supernatants measured for total protein and IgG production (Fig. 3). An approximately linear incorporation of radioactive amino acids into both protein and IgG was observed, suggesting that the availability of label was uniform during the time of incubation and that the metabolic activity of the tissue remained intact. Similar findings were obtained for cultures of PBL.

Comparison of DEAE-fractionated supernatants of PBL and synovium with the unfractionated portion. The two protein peaks from DEAE-cellulose chromatography of the supernatants of both the PBL and synovial tissue incubations contained approximately 60% of the applied radioactivity. Peak I contained an average of 28% of the recovered protein-^{14}C of the PBL and 37% of the recovered synovial protein-[14]C. Comparison of unfractionated supernatants with DEAE peak I fractions from PBL showed the latter to contain more than 70% of the IgG and the only demonstrable ^{14}C-labeled tetanus antibody in the normal subjects. In the two cultures of rheumatoid synovium and PBL respectively, in which the DEAE fractions were compared with the unfractionated supernatants, only whole supernatant and DEAE peak I of PBL contained measurable amounts of anti-tetanus radioactivity. This limitation of tetanus antibody to DEAE peak I permitted the use of this fraction for comparison of the per cent of the IgG representing specific antibody in PBL and synovial cultures.

Comparison of immunoglobulin synthesis by rheumatoid synovial membrane with that of PBL. Table I compares the incorporation of ^{14}C-labeled amino acids into

TABLE I

Comparison of Immunoglobulin and Tetanus Antibody Synthesis by PBL and Synovium

Grouping of patients by time of synovectomy after tetanus challenge (number)		Per cent of TCA-precipitable cpm in unfractionated supernatants as				Tetanus antibody as % of IgG*
		IgG	IgA	IgM	Total	
Not challenged (2)	PBL	2.4	2.0	1.2	5.6	6.9
	Synovium	15.2	6.5	1.5	23.2	0.4
2.5–3 days (3)	PBL	4.0	3.4	1.4	8.8	2.7
	Synovium	30.7	2.9	1.5	35.1	0.3
4–6 days (10)	PBL	3.1	3.2	1.6	7.9	21.2 ±6.7‡
	Synovium	16.2	3.2	2.0	22.9	0.8 ±0.6
14 days (1)	PBL	4.1	4.3	1.7	10.1	5.7
	Synovium	36.8	2.4	2.0	41.2	0.1
Normals (3) 5–7 days	PBL	9.2	2.7	1.2	13.1	51.0

* Derived from DEAE peak I IgG.
‡ SD.

immunoglobulins and tetanus antibody in synovial tissue and PBL cultures obtained from 14 RA patients 2.5–14 days following injection of tetanus antigen. Results of similar studies in two nonchallenged patients are also shown. In addition, the distribution of radioactivity in the immunoglobulins synthesized by the PBL of three normal subjects challenged 5–7 days prior to culture is also presented. These results demonstrate a 5- to 9-fold greater production of IgG by synovium, expressed as a percentage of total synthesized protein, than by PBL in both tetanus-challenged and nonchallenged patients. In spite of this active synthesis of IgG there was negligible tetanus antibody synthesis by synovium at the time periods studied. In striking contrast, however, was the significant synthesis of tetanus antibody in the PBL cultures of those patients immunized 4–6 days before synovectomy. Thus, although the protein synthesized by the rheumatoid synovium to this particular group contained 5.2 times more IgG than that produced by PBL, the per cent of this IgG representing specific antibody was 24 times greater in the PBL cultures.

Table II presents the synthesis of specific antibody as the fraction of TCA-precipitable radioactivity, and as the fraction of the IgG present in DEAE peak I from the PBL and synovial cultures of each of the 10 patients challenged 4–6 days prior to synovectomy. Anti-tetanus antibody synthesis was detected in the PBL cultures of each of these patients. In this group, the proportion of IgG-^{14}C representing specific antibody to tetanus varied from 13.9 to 34.2% (mean, 21.2%). In synovial tissue,

however, only 0–2.0% (mean, 0.8%) of the IgG synthesized represented specific tetanus antibody.

Of considerable interest is the observation that only minimal synthesis of ^{14}C-labeled tetanus antibody occurred in PBL at days 3 or 14 after antigenic challenge, indicating the limited period of time during which an immune response can be demonstrated in peripheral blood leukocytes.

Comparison of IgG and tetanus antibody synthesis by synovia and PBL of nonrheumatoid patients. Table III presents the results obtained from cultures of synovial membrane from four patients with diseases other than rheumatoid arthritis. The knee joint of the patient with Reiter's syndrome was repeatedly biopsied with a Parker-Pearson (12) needle until approximately 100 mg of synovial tissue had been obtained. This was cultured in a similar manner to that used for the rheumatoid synovia. The Reiter's synovial tissue produced about one-third as much IgG as the average rheumatoid synovium, 7.4% compared to 20.1% of the TCA-precipitable radioactivity in the unfractionated culture supernatants. Only 3.3% of this IgG coprecipitated as specific tetanus antibody compared to an average of 0.8% in the rheumatoid synovial cultures (Table II). The Reiter's patient's PBL was not cultured. The synovia and PBL of three otherwise normal patients undergoing knee cartilage revision procedures following trauma were also studied. Two of the three showed tetanus antibody production by peripheral blood lymphocytes, but none of the three synovia produced detectable tetanus antibody. The amount of IgG produced by these synovia was very small: an av-

TABLE II

Comparison of IgG and Tetanus Antibody Synthesis by PBL and Synovial Tissue Derived from Patients Challenged with Tetanus Toxoid 4–6 days Prior to Culture*

Patient		% TCA-precipitable cpm as		% IgG as tetanus antibody
		IgG	Tetanus antibody	
E. L.	PBL	7.1	0.99	13.9
	Synovium	79.0	0.15	0.25
G. W.	PBL	13.0	2.46	18.9
	Synovium	46.0	0.11	0.23
M. W.‡	PBL	3.6	1.23	34.2
	Synovium	38.9	0.29	0.74
M. G.	PBL	9.1	1.26	13.8
	Synovium	65.2	0.39	0.62
M. P.	PBL	3.9	0.75	19.2
	Synovium	41.6	0.74	1.77
W. H.	PBL	8.6	2.72	31.6
	Synovium	54.3	0.86	1.59
J. P.	PBL	11.3	2.35	20.7
	Synovium	53.0	0.35	0.66
V. M.‡	PBL	5.7	1.5	26.3
	Synovium	44.7	0.9	2.0
L. C.	PBL	5.1	1.2	23.5
	Synovium§	43.7	0.29	0.66
	Synovium‖	31.3	0.00	0.00
M. M.	PBL	3.7	0.61	16.5
	Synovium‖	89.4	0.29	0.32
Mean	PBL	7.1	1.5	21.2
	Synovium	54.9	0.4	0.8

* All precipitations shown above were done on peak I DEAE.
‡ M. W. and V. M. received three tetanus toxoid injections during the 4 months prior to surgery.
§ Pooled synovia from four metacarpophalangeal joints which were not injected intra-articularly.
‖ These synovia were derived from joints injected intra-articularly with 15 and 30 μg of tetanus toxoid 4 and 6 days prior to surgery.

erage of *2.7%* of TCA-precipitable radioactivity in DEAE peak I compared to 54.9% for the average of the rheumatoid synovia (Table II), essentially a reflection of their lack of lymphocytic infiltration.

Intra-articular tetanus toxoid injection. Two patients, L. C. and M. M., received a supplementary intra-articular injection of 15 and 30 μg, respectively, of tetanus toxoid 4 and 6 days prior to synovectomy, in addition to the usual intramuscular injection. In these patients, tetanus antibody comprised 23.5 and 16.5%, respectively,

of the IgG-^{14}C of PBL cultures (Table II). However, only negligible amounts (0.0 and 0.3%, respectively) of the IgG-^{14}C synthesized by the synovial tissue obtained from the injected joints represented specific antibody. In L. C., only 0.66% of the IgG-^{14}C produced by the non-injected metacarpophalangeal synovial tissue was measured as "specific" tetanus antibody, about the same as that from the synovial cultures from the two nonchallenged patients who produced 0.4 and 0.5%, respectively (Table I). It is apparent that no increase in specific antibody synthesis resulted from intra-articular injection of tetanus antigen.

DISCUSSION

Although cells comprising the chronic inflammatory infiltrate of the rheumatoid synovial membrane synthesize a significant proportion of their soluble protein as immunoglobulin (4), the antibody specificity of this γ-globulin relative to the over-all immune status of the patient is unknown. The administration of a secondary antigenic challenge with tetanus toxoid at varying times prior to synovectomy has permitted evaluation of the amount of specific antibody synthesized by the rheumatoid synovium, and comparison of this response with that of the PBL which were shown to be responding actively at the time of surgery.

When two distinctively different tissues such as synovium and PBL are compared in their ability to incorporate ^{14}C-labeled amino acids, the data derived do not indicate an absolute level of protein or specific antibody synthesis. The pool size of the amino acids used for labeling may well be different in the two culture systems, resulting in different specific radioactivities for the incorporated amino acids. This would cause variation in the amount of label incorporated into similar quantities of newly synthesized protein. However, a comparison of the relative proportion of total protein synthesis within each tissue representing immunoglobulin or specific antibody would seem justifiable since the specific radioactivity of newly synthesized total protein and antibody may be assumed to vary in parallel within a given culture.

The present study (Fig. 1) is the first to establish the time of maximum antibody synthesis by PBL in the anamnestic response in man. It has demonstrated a clearly defined period of specific antibody synthesis with an optimum on the 5th day. However, the choice of the best time for study of an immune response by synovial tissue to permit a meaningful comparison with the response of PBL was less obvious. The period from 2.5 to 6 days following injection of antigen was chosen for the following reasons. The secondary immune response of man is similar to that of the rabbit in that maximum

TABLE III

Comparison of IgG and Tetanus Antibody Synthesis by PBL and Synovial Tissue
Derived from Nonrheumatoid Patients

Patient	Diagnosis	% TCA-precipitable cpm as IgG in DEAE peak I		Tetanus antibody as % of IgG
J. W.	Reiter's syndrome	Synovium	7.4*	3.3*
S. H.	Knee cartilage revision	Synovium	1.9	0.0
		PBL	6.5	17.7
T. C.	Knee cartilage revision	Synovium	3.8	0.0
		PBL	19.0	17.9
W. H.	Knee cartilage revision	Synovium	2.6	0.0
		PBL	1.6	0.0

* Unfractionated supernatant used rather than DEAE peak I.

serum antibody titers to most antigens are present on the 5th day (13) after immunization. In vitro cultures of rabbit spleen and lymph node cells have shown the peak secondary antibody response to be attained by the 3rd day and to continue for several days (14, 15). Extrapolating the observations in rabbit lymphoid tissues to man would suggest that the cells of the human PBL responsible for antibody production are derived from lymphoid organs which undergo an anamnestic response about 2 days earlier. Presumably synovial synthesis of tetanus antibody, if dependent upon arrival of actively responding lymphocytes, would be under way by the time the peak response had passed in the PBL. This would assume that the synovial membrane exchanged lymphocytes freely with the recirculating lymphocyte pool (16). Pertinent to this question are studies in rabbits by Jasin and Ziff (5) who produced chronic synovitis with streptolysin S in animals previously immunized with BSA and challenged 3 days before synovectomy. They found 28% of the large amount of immunoglobulin being synthesized by the rabbit synovium to be specific anti-BSA at the time that 43% of the immunoglobulin of the PBL represented this antibody. This result suggested a rapid exchange of circulating blood lymphocytes with the chronic lymphoid infiltrate in the rabbit synovium even by the 3rd day following immunization. By analogy, one would predict, therefore, that an immune response to an exogenous antigen in the PBL should also be present in the RA synovium at this time.

The amounts of IgA and IgM produced by PBL and synovium (Table I) were quite variable, but since neither type of immunoglobulin was observed to participate in the tetanus antibody response, only the changes involving IgG will be emphasized. Synovium synthesized from 5 to 9 times as much IgG as PBL when calculated as the per cent of total TCA-precipitable cpm (Table I), yet made only negligible amounts of tetanus antibody at

periods varying from 2.5 to 14 days after challenge. On the other hand, in the ten RA patients receiving tetanus antigen 4–6 days prior to synovectomy, tetanus antibody averaged 21% of the IgG synthesized by the PBL. Thus, the RA synovium was deficient in its response to an exogenous antigen.

Not only did the synovium fail to synthesize tetanus antibody at the time when the PBL were producing considerable amounts of this antibody, but it also failed to do so immediately prior to this time period and subsequently to it when antibody synthesis was not occurring in the PBL. The ratio of γ-globulin synthesized to TCA-precipitable protein synthesized has been found to be similar in RA synovium to that of human spleen and lymph nodes (4). Since in studies cited above (14), lymph nodes demonstrated maximum antibody synthesis by the 3rd day after antigenic challenge, this time period was carefully evaluated in the present study. When 2.5 and 3 days were allowed between injection of tetanus antigen and synovectomy, negligible antibody was formed in both PBL and synovial tissue cultures. Similar results were observed in the PBL and synovial cultures from the patient studied 14 days after tetanus injection.

At least two possibilities could account for the deficient synovial response to tetanus antigen. The lymphocytes of the synovium may not exchange freely with the recirculating lymphocyte pool (16) so that those lymphoid cells of the synovium which produce immunoglobulin do so in response to local antigens or to some earlier immunological commitment. On the other hand, the recirculating lymphocyte pool, although capable of exchange with the cells present in the synovial infiltrate, may have contained adequate numbers of immune cells for too brief a period of time to contribute significantly to the chronic lymphoid infiltrate. It should be noted, however, that two of the 10 optimally immunized patients shown in Table II had been immunized with teta-

nus antigen three times during the 4 months prior to surgery. It would be expected that this repeated challenge would have provided large enough numbers of circulating immune cells to be sufficient for adequate equilibration with the RA synovium. Yet, in these patients, too, synovial synthesis of tetanus antibody was either negligible or absent.

Loewi (17) has also studied experimental immune inflammation in the synovial membrane. He found that immunized guinea pigs, when injected with specific antigen intra-articularly, rsponded with a chronic mononuclear inflammatory reaction, suggesting that active immunization within the joint was required for persistence of the local synovitis. If local penetration of the joint by antigen were necessary to activate antibody synthesis, and this did not occur in adequate amount following the usual intramuscular injection of the antigen, it would be possible to explain the lack of specific antibody synthesis which was observed. For this reason, in two patients, the challenging dose was supplemented with a small amount of antigen administered intra-articularly 4 and 5 days before surgery. In one of these, L. C., a number of joints were subjected to synovectomy, permitting a comparison of the effect of intra-articular injections of antigen with that of systemic immunization alone (Table II). Essentially no tetanus antibody was formed by any of these synovial cultures, although the PBL of both patients produced specific antibody. Thus, even intra-articularly administered antigen did not stimulate synovial antibody synthesis in systemically immunized patients. Since synovial membrane did not synthesize antibody either before, during, or after the time when the PBL were synthesizing such antibody, and since the synovium of immunized patients did not respond to locally injected antigen, it would appear likely that the γ-globulin synthesized in rheumatoid synovitis represents a commitment to some other antigen. It is possible that such an antigen might be localized to the joint. The recent studies of experimental pyelonephritis produced by *E. coli* by Lehmann, Smith, Miller, Barnett, and Sanford (18) provide an excellent example of such local synthesis of antibody to a known antigen by a chronic lymphoid infiltrate similar to that found in the rheumatoid synovium.

Unfortunately, synovectomy is rarely performed in patients with inflammatory arthritis other than rheumatoid arthritis. In the four synovial cultures from non-rheumatoid patients presented in Table III, only one from a patient with Reiter's syndrome could be considered truly comparable with rheumatoid synovial tissue. It is of interest that this Reiter's tissue also showed a low percentage (3.3%) of the IgG produced in culture to be anti-tetanus antibody. It is interesting, although of questionable significance, that this was a higher percentage of IgG representing antibody than any of the rheumatoid synovia studied (average 0.8%).

Although the PBL cultured from 10 of the patients were secured at an optimum time for antibody synthesis, the amounts of newly synthesized IgG and tetanus antibody measured were only 30–40% of that observed in the three normal control subjects. However, the small number of the normal subjects studied and the difference in age of the two groups does not permit a conclusion relative to this apparent difference.

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