Immunoglobulin G_3 Subclass Production by Rheumatoid Synovial Tissue Cultures

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ABSTRACT The cellular infiltrate in the deeper layers of the rheumatoid synovium produces a substantial amount of immunoglobulin (Ig)G. Culture supernatants of synovial tissues from 31 patients with rheumatoid arthritis (RA) undergoing joint replacement or synovectomy have been analyzed for the subclass of IgG present. IgG_3 was measured by separation with Staphylococcal Protein A chromatography, precipitation with specific anti-IgG_3 antibody, and differential separation of IgG_3 heavy chains using polyacrylamide gel electrophoresis. IgG from RA synovial cultures contained an average of 41% IgG_3 (range, 8–97%) compared with 12% IgG_3 (range, 6–17%) in the serum IgG of the same patients. A group of non-RA control lymphoid tissues (four lymph nodes and five tonsils) produced 23% of total IgG as the IgG_3 subclass (range, 16–35%). An average of only 9% of the synovial IgG showed aggregation compatible with IgG-rheumatoid factor (IgG-RF). Purified IgG from some of the RA synovial culture supernatants also showed significant restriction when separated by isoelectric focusing. This restriction and the enrichment for the IgG_3 subclass in the IgG from RA synovial cultures suggest that either an antigen in the inflamed joint is selectively stimulating an antibody in this subclass, or that significant differences in the catabolic rate of this subclass are found in cultures of synovial tissue when compared with that occurring in intact patients.

INTRODUCTION

Immunofluorescent studies (1, 2) have shown the infiltrating lymphoid cells in the rheumatoid synovium to contain IgG and IgM, some of which has rheumatoid factor (RF)[1] activity with an unexpectedly large number of synovial plasma cells staining for the IgG_3 subclass and lambda light chain (3). This increase in IgG_3 subclass (3) stimulated the present investigation. Unusual increases in different IgG subclasses in immune diseases with identified antigens also stimulated study of IgG subclass production by the rheumatoid synovium. For example, in 15 of 15 patients with idiopathic thrombocytopenic purpura, the antiplatelet antibodies were of the IgG_3 subclass (4) and in five of six patients with anti-Factor VIII antibodies, the IgG_4 subclass predominated (5, 6).

The amount of IgG made by cultured rheumatoid arthritis (RA) synovium is similar to that produced by human spleen and lymph nodes (7). However, the RA synovial infiltrate does not respond to exogenous antigen (tetanus toxoid) given before surgery, even when patients are injected repeatedly (8). The antigenic specificity of most of the immunoglobulin produced by the RA synovium is unknown. Part of this study used ^{14}C-labeled IgG produced by cultured RA synovia to determine the proportion that was IgG-RF. The IgG_3 subclass composition of the IgG in RA synovial culture supernatants was also measured and compared with the level of IgG_3 in the serum of the same patients. Enrichment of RA synovial IgG for the IgG_3 subclass, if confirmed, might suggest stimulation by an antigen shared by different patients with a restricted local immune response in the inflamed synovium.

METHODS

Patients. Synovial tissues were cultured from 31 adult patients with RA as defined by the American Rheumatism

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[1] *Abbreviations used in this paper*: PAGE, polyacrylamide gel electrophoresis; RA, rheumatoid arthritis; RF, rheumatoid factor.

Association (9). Four lymph nodes and five tonsils, all obtained at the time of elective surgery in patients without RA, and one spleen obtained after an automobile accident from a patient with seronegative RA were also cultured in a similar manner.

Tissue incubation and supernatant fractionation. Washed synovial tissue, lymph node, tonsil, or spleen fragments were cultured as described previously (7), with the following modifications. The Eagle's medium (10) lacked amino acids, but contained 5% fetal calf serum. The pH was maintained at 7.2 with 0.03 M *N*-2-hydroxethyl-piperazine-*N'*-2-ethanesulfonic acid (Hepes). The medium contained 5 μc/ml of a labeling mixture (ICN Chemical, Inc., Irvine, Calif.) containing 15 essential ^{14}C-labeled amino acids (135–330 mCi/mmol). The tissue was cultured in petri dishes in 95% air–5% CO_2 at 37°C for 18 h. After incubation, following centrifugation at 100,000 *g*, the amount of IgG in each culture supernatant was determined using radial immunodiffusion in Ultra Low Level IgG plates (Kallestad Laboratories, Chaska, Minn.) according to the method of Mancini et al. (11). The first nine synovial tissue culture supernatants were processed using DEAE-cellulose chromatography (7). The remaining 22 cultures were processed using an anti-human IgG immunoadsorbent prepared as described below.

Immunoadsorbent column for synovial IgG. Antiserum to human IgG was prepared in rabbits. Two Sepharose-4B columns, one containing human IgM-kappa, 15 mg/ml of gel, and the other pooled human IgG, 20 mg/ml of gel, were prepared (12). The rabbit antiserum was passed through the IgM column and the eluate then absorbed on the IgG column that had been previously equilibrated with 1 M NaCl, 0.1 M sodium borate, pH 8.5. After washing to remove proteins not specifically bound (13), the anti-IgG was eluted with 0.1 M glycine-HCl, pH 3.0, and neutralized with 0.5 M phosphate buffer, pH 7. This rabbit anti-IgG, shown to be specific for the IgG heavy chain, was attached to Sepharose-4B (12) with the following modifications. Only 40 mg of CNBr/ml of gel was used, and the coupling step was performed overnight in 0.1 M phosphate, pH 7.0, with 9.4 mg of rabbit anti-IgG/ml of activated gel, then unreacted groups were blocked with 1 M ethanolamine in the NaCl-borate buffer. These anti-human IgG columns (0.9 × 2 cm) bound a maximum of 6.1 mg of IgG/ml of gel.

Isolation of culture supernatant IgG. Supernatants from the RA synovial, lymph node, tonsil, and spleen cultures were dialyzed against 0.1 M phosphate, pH 7.0, to remove most unincorporated radioactivity, then passed through the anti-IgG immunoadsorbent that had been previously equilibrated with the NaCl-borate buffer. After washing with the same buffer, the bound IgG was eluted in 1-ml fractions with 0.1 M glycine-HCl, pH 3.0, and neutralized with 0.3 ml of 0.5 M phosphate, pH 7.0. An aliquot of each fraction was counted in 6 ml of a scintillation mixture containing 500 ml of toluene, 6.86 g PPO, 0.14 g dimethyl-POPOP and 500 ml Triton X-100.

Protein A fractionation of IgG from culture supernatant. IgG eluted from the DEAE-cellulose column or from the IgG-immunoabsorbent was then passed through a column of Sepharose 4B-Staphylococcal Protein A (14) (Pharmacia Fine Chemicals, Uppsala, Sweden) (0.9 × 3 cm) that had been equilibrated with 0.1 M phosphate buffer, pH 7.0. After washing, the bound IgG was eluted in 1-ml fractions with 0.1 M glycine-HCl, pH 3.0, and neutralized as above. An aliquot of each fraction was then counted in the liquid scintillation counter.

Immune precipitation of the IgG from culture supernatant. To define the light chain and IgG_3 subclass composition of the IgG in the culture supernatants, tube precipitations with the following antisera were performed: goat antisera against human IgG heavy chain, and both kappa and lambda light chains (Meloy Laboratories, Springfield, Va.), and rabbit antisera against human IgG_3 subclass (Nordic Immunological Laboratories, Tilburg, Netherlands). These antisera were shown to be monospecific using immunoelectrophoresis against purified IgG myeloma proteins of known subclass type. All tube precipitations were done in antibody excess. Aliquots were added to a sufficient amount of the appropriate myeloma protein to give a total of 30 μg of IgG per tube, and 200 μg of specific antibody added. Antigen-antibody precipitates were washed twice with 0.9% NaCl, dissolved in 0.5 ml of 1.0 M NaOH, and counted in Dimilume-30 (Packard Instrument Co., Inc., Downers Grove, Ill.).

Slab-gel electrophoresis and isoelectric focusing. IgG fractions were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) using a modification of the method described by Tegtmeyer et al. (15). Acrylamide stock solutions (5 and 20%) were deionized using 5 g of Bio-Rad-Ag 501-X8 resin per 100 ml stock solution (16). The stacking and separation gels were prepared and electrophoresis was performed in a slab gel apparatus (Bio-Rad Laboratories, Richmond, Calif.). After injecting each sample (1–20 μl) under the borate buffer in the individual wells, the samples were stacked at 4 mA per slab gel for 45 min, and then run at 15 mA per slab gel for 3 h. The gels were acid-fixed, stained (17), photographed, and vacuum dried (Bio-Rad model 224 Slab Gel Dryer). Isoelectric focusing was performed by the method of Marine Colloids, Inc., Rockland, Maine (18).

Fluorography of ^{14}C-labeled IgG. After SDS-PAGE, ^{14}C-labeled IgG was identified by fluorography (19). The acid-fixed gels were soaked in water for 30 min, then in 10 vol of 1 M sodium salicylate, pH 7.0, for an additional 30 min, and vacuum-dried before fluorography on Kodak XR-5 film for 4 wk at −70°C. Film was developed in an automatic Kodak X-ray Processor (Eastman Kodak Co., Rochester, N. Y.)

Iodination of myeloma protein markers. DEAE-Cellulose purified IgG_1 and IgG_3 myeloma proteins were ^{125}I-labeled using the lactoperoxidase method (20).

Sephadex G-200 chromatography. Affinity purified IgG from culture supernatants was chromatographed on a Sephadex G-200 column (1.5 × 85 cm) to determine the amount of self-association of the IgG present. The Sephadex G-200 column was equilibrated and eluted with 0.2 M borate buffer containing 0.15 M NaCl, pH 8.1. A 1.1-ml sample containing 40,000 cpm of ^{14}C-labeled IgG from culture supernatant, 4 mg of an IgG_1 myeloma protein and 5 mg of an IgM macroglobulinemia protein was fractionated. Absorbance of each 2-ml fraction was determined at 280 nm, and an aliquot of each fraction counted in Dimilume-30.

RESULTS

Recovery of total IgG and IgG subclasses

Comparison of DEAE-cellulose chromatography with the IgG immunoadsorbent. Analysis of the IgG recovered from the immunoadsorbent showed that an

TABLE I

Clinical Data, Weight of Tissue Cultured, and Fraction of Total Soluble Protein Representing IgG*

Patient	Age, race, sex	RA latex titer	Major drugs‡	Wet wt. of tissue cultured (g)	cpm adsorbed to Anti-IgG immunoadsorbent	Percent total recovered as IgG
J.B.	37 WF	1:640	Pred.	2.5	95,090	3
J.D.	63 WF	1:80	Gold	2.5	60,410	9
B.E.	78 WF	1:320	None	3.0	47,450	6
M.F.	47 WF	1:160	Pred.	2.5	161,300	17
M.H.	63 WF	Neg	None	1.8	6,650	1
M.Ho.	42 WF	1:1,280	Pen.	2.5	18,510	4
L.J.	77 WF	1:2,560	Gold	2.5	280,850	12
M.J.	58 WF	1:320	None	2.1	30,600	5
A.K.	71 WF	1:640	Pen.	0.3	119,650	20
B.K.	40 WM	1:640	None	0.3	235,750	41
F.L.	56 WF	1:2,560	Pred.	2.5	40,360	4
A.M.	46 WF	1:160	Pred.	2.0	598,060	30
L.P.	36 WF	1:160	Pred.	5.0	35,300	3
M.M.	73 WF	1:640	None	2.5	12,480	2
M.P.	47 AIF	1:160	Gold	2.4	565,940	28
E.R.	63 WF	1:320	None	2.5	85,610	7
S.S.	39 WF	1:320	Pen., Pred.	0.5	24,470	4
B.T.	71 WF	1:640	None	2.5	36,920	2
A.W.	63 WF	1:2,560	Pred., Gold	2.0	12,440	2
K.W.	36 WF	Neg	None	0.9	5,840	1
L.W.	30 WF	Neg	None	2.4	86,840	4
J.Y.	61 WF	1:160	Pen., Pred.	2.3	103,460	11

* Total soluble protein was determined by precipitation with 5% trichloroacetic acid and counting insoluble radioactivity.

‡ Most patients were on aspirin or other nonsteroidal anti-inflammatory drugs. Pen., D-penicillamine, Pred., prednisone < 10 mg/d, Gold, gold thiomalate, usually 50 mg/mo. W, white, AI, American Indian, F, female, M, male.

average of 9.5% of the total soluble protein synthesized was IgG (Table I). There was a considerable variation in specific radioactivity (counts per minute per milligram) of the isolated IgG, ranging from 10,000 to 392,000 cpm/mg with a mean of 90,000 cpm/mg. Before application to the immunoadsorbent, IgG in the culture supernatant was measured by radial immunodiffusion. There was a mean of 0.32 mg of IgG per ml of original culture supernatant (range, 0.04–0.63). The acid eluate from the anti-IgG immunoadsorbent contained a mean of 0.31 mg of IgG per ml of original culture supernatant (range, 0.15–0.64) indicating minimal losses of synovial IgG with this isolation procedure.

The first nine synovial culture supernatants were fractionated to obtain an IgG-rich fraction by DEAE-cellulose chromatography. This IgG-rich fraction contained no IgA or IgM, but it provided only ~50% recovery of the total IgG present in the original culture supernatant. When several synovial cultures were found to have high levels of IgG_3, there was concern that selective recovery of the IgG_3 with loss of a substantial amount of IgG_1 and IgG_2 was occurring with this method. At that point, it was decided to use a rabbit anti-human IgG immunoadsorbent to process the next 22 RA synovial culture supernatants. This method resulted in the recovery of >90% of the total IgG in the culture supernatant. However, as can be seen from Table II, there is wide variation in the amount of IgG_3 from one synovial culture to another by both methods. From 14 to 97% of the IgG recovered by DEAE-cellulose chromatography was IgG_3 and from 8 to 64% of the IgG recovered from the immunoadsorbent was IgG_3. Therefore, ^{125}I-labeled myeloma proteins of the various IgG-subclasses were prepared, and recoveries assessed by each method. Two important findings resulted from these studies. With DEAE-cellulose chromatography, although total IgG recoveries were lower, the losses of IgG_1 and IgG_3 were the same. Both proteins remained undenatured and reactive with specific antiserum to the appropriate subclass. Although the recoveries of total IgG was substantially higher with the anti-IgG immunoadsorbent, there was mild denaturation of recovered IgG_3. Al-

TABLE II

Fractionation of IgG from RA Synovial Culture Supernatant on Protein A Column: Determination of Light Chain Type

Culture supernatant	Unbound cpm	Percent total recovered	Total IgG precipitated by, Anti-IgG_3	Anti-lambda*
J.B.	11,800	27		54
S.B.‡	127	92		
J.D.	1,900	9		
N.D.‡	3,018	97	93	90
B.E.	5,270	27		40
T.E.‡	227	48		
M.F.	16,800	26		53
G.H.‡	7,624	95	92	94
M.H.	1,360	43		
M.Ho.	1,450	38		
V.H.‡	102	48		
L.J.	21,560	36		56
M.J.	2,730	24		
A.K.	7,110	31		43
B.K.-1‡	6,418	97	74	93
B.K.-2	7,140	17		33
K.K.‡	386	14		
F.L.	2,960	26		23
S.L.‡	2,477	49		
A.M.	16,310	13		37
M.M.	1,880	57		
H.P.‡	2,615	94	98	94
L.P.	2,270	20		
M.P.	14,770	8		54
E.R.	5,390	26		
S.S.	2,490	36		
B.T.	2,430	20		
A.W.	1,510	32		
K.W.	1,020	64		
L.W.	2,040	20		40
J.Y.	15,080	48		
Mean±SE		41±5.3		57±6.6

* Percent IgG composed of lambda light chains was calculated as the counts per minute precipitated by anti-lambda antiserum/(counts per minute precipitated by anti-lambda antiserum + counts per minute precipitated by anti-kappa antiserum on the supernatant of the same sample).

‡ The IgG of the initial nine RA synovial supernatants was purified using DEAE-cellulose chromatography (see text). The remaining samples were prepared using the anti-IgG immunoadsorbent.

though it remained soluble, only ~20% of the IgG_3 was now reactive with the specific antiserum obtained from Nordic Laboratories. However, the acid-eluted IgG_3 myeloma proteins continued to be completely precipitated by rabbit antiserum to human IgG, and were not adsorbed by the protein A column. With these results, it was felt that combination of the results obtained by the two separation procedures was justified, and these are shown in Table II and Fig. 1.

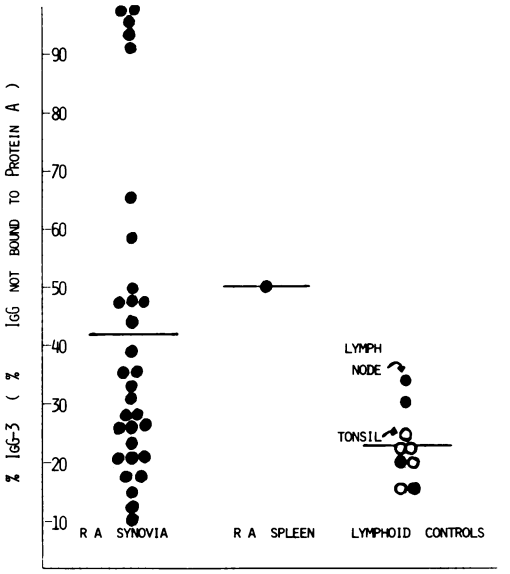

FIGURE 1 Comparison of the percentage of IgG represented by IgG_3 released in tissue culture by RA synovia to the percent released by non-RA lymph nodes and tonsils and by the spleen of a patient with seronegative RA. Tissue culture supernatants of all tissues were adsorbed on an anti-IgG immunoadsorbent, and the ^{14}C-labeled IgG eluted with acid buffer. The purified IgG was then passed over a protein A column and the radioactivity in the IgG fraction (IgG_3) not bound to the protein A determined.

Fractionation of synovial IgG using protein A. IgG isolated on the anti-IgG immunoadsorbent was chromatographed on a protein A column (14) (Table II). The proportion of IgG_3 (unbound counts per minute) present in the synovial IgG varied from 8 to 97% (mean 41%). Recovery of ^{14}C-labeled IgG from the Protein A column varied from 80 to 95%. Only 13 of the 31 supernatants were studied for light chain types because of insufficient ^{14}C-labeled material in the other samples.

Characterization of synovial IgG by SDS-PAGE and fluorography. IgG fractions from rheumatoid synovial culture, B.T., that was acid-eluted from the immunoadsorbent column, and a portion further fractionated on the protein A column were checked for purity by SDS-PAGE. The heavy chains of purified IgG from normal serum and from rheumatoid synovial culture supernatant migrated as broad bands (IgG_1, IgG_2, and IgG_4) and fainter, slower migrating narrow bands (IgG_3). The applied samples showed only trace amounts of proteins other than IgG to be present. When this IgG purified from the immunoadsorbent was chromatographed on protein A, the unbound frac-

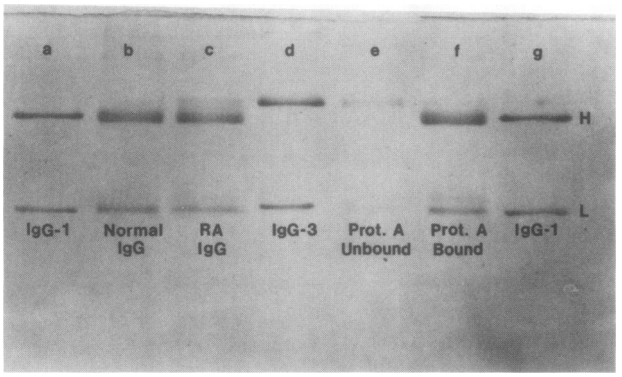

FIGURE 2 SDS-PAGE analysis of IgG from synovial culture, B. T., isolated on the anti-IgG immunoadsorbent and further fractionated on protein A. Samples were placed at the top of the slab gel. Myeloma proteins served as markers for the heavy (H) and light (L) chains. (a and g) IgG_1 marker, (b) IgG from normal serum, (c) IgG from RA synovial culture before protein A, (d) IgG_3 marker, (e) IgG_3 from RA synovial culture from fraction not bound to protein A, (f) IgG_1, $-_2$ and $-_4$ of RA synovial culture eluted by acid buffer from protein A.

tion (Fig. 2e) comigrated with the IgG_3 marker (Fig. 2d), whereas the bound fraction eluted from the protein A (Fig. 2f) comigrated with an IgG_1 marker (Fig. 2g). These results demonstrate that the synovial IgG was isolated in almost pure form from the culture supernatants by the immunoadsorbent column, and that the protein A column bound only the IgG_1, IgG_2, and IgG_4 subclasses, but not the IgG_3 subclass.

It was possible that a highly labeled non-IgG protein might be present in the fraction not bound by protein A that would increase the apparent percentage of the IgG_3 present. Therefore, ^{14}C-labeled IgG derived from a rheumatoid culture supernatant, M.F., was chromatographed on protein A and the fractions were analyzed by SDS-PAGE followed by fluorography to detect radioactive, but otherwise, invisible, protein bands. The bound fraction from protein A (Fig. 3b) comigrated with an IgG_1 marker (Fig. 3a and 3c), while the unbound fraction (Fig. 3e) comigrated with an IgG_3 marker (Fig. 3d and 3f) as would be expected due to the higher molecular weight of the heavy chain of IgG_3 when compared with the heavy chains of the other IgG-subclasses. In both the bound and unbound protein A fractions, no significant ^{14}C-labeled proteins were detected other than the IgG heavy and light chains.

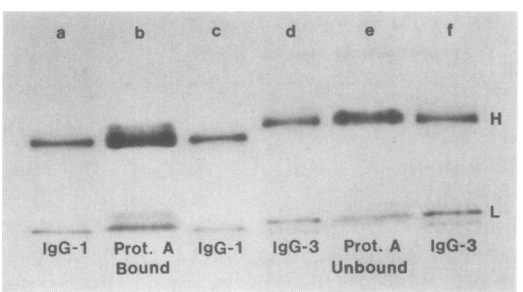

FIGURE 3 Radioactive fluorographic analysis of an SDS-PAGE separation of reduced, ^{14}C-labeled IgG isolated by the immunoadsorbent from culture, M.F., then fractionated on protein A. ^{125}I-labeled IgG_1 and $-_3$ served as markers for the heavy (H) and light (L) chains. (a and c) IgG_1 marker, 1,400 cpm, (b) synovial culture IgG bound to protein A, 300 cpm, (d and f) IgG_3 marker, 1,400 cpm, (e) synovial culture IgG not bound by protein A, 300 cpm.

IgG synthesis by control non-RA lymphoid tissues. Normal human synovial tissues contain very few lymphocytes and, thus, are not suitable for comparison with the RA synovial cultures. Therefore, the spleen from a patient with seronegative RA, lymph nodes from four patients without RA undergoing gallbladder surgery for cholecystitis and tonsils from five patients having tonsilectomy for recurrent tonsilitis were obtained. These were cultured in the same manner as the RA synovia, and the IgG isolated with the anti-IgG

TABLE III

IgG and IgG_3 Synthesis by Lymph Nodes and Tonsils of Non-RA Patients and the Spleen of a Patient with Seronegative RA

Culture	IgG percent soluble protein*	IgG_3 percent total IgG
Lymph nodes		
D.G.	32	35
V.G.	5	16
B.M.	30	21
J.S.	24	30
Tonsils		
O.H.	31	27
T.L.	26	23
L.O.	28	16
J.R.	24	21
B.S.	37	23
Mean±SE	26±2.8	23±1.9
Spleen		
D.S.	16	48

* Total soluble protein counts per minute determined by precipitation with 5% trichloroacetic acid before counting. Lymph node, tonsil, and spleen culture supernatants were passed over an anti-IgG immunoadsorbent, and the ^{14}C-labeled IgG was eluted with an acid buffer, neutralized and chromatographed on a protein A column. The IgG-fraction not bound (IgG_3) was then measured and expressed as a percentage of the total IgG applied to protein A.

immunoadsorbent also used to obtain synovial IgG. As seen in Table III, the nine nonrheumatoid lymphoid tissues synthesized an average of 23% of total IgG as IgG_3 (range, 16–35%), and the rheumatoid spleen culture produced 48% of its IgG as IgG_3 (Fig. 1).

Total IgG and IgG_3 in normal and rheumatoid sera. Since the IgG synthesized by rheumatoid synovia, non-RA lymph nodes, and tonsils, and the rheumatoid spleen all demonstrated a significant increase in the IgG_3 subclass over published normal serum IgG_3 levels (21) it was important to show that this enrichment for IgG_3 was not the result of an isolation artifact. Therefore, sera from normal subjects and rheumatoid patients were adsorbed on and eluted from an anti-IgG column. The purified serum IgG was then chromatographed on the protein A column. In both RA patients and controls, an average of 12% of the protein absorbance at 280 nm did not bind to the protein A column, and is presumed to be IgG_3 (Table IV). Since the accepted levels of IgG_3 in serum are 8 to 10% (21), the anti-IgG immunoadsorption and protein A fractionation procedures used for synovial cultures did not significantly enrich for IgG_3.

Confirmation of monoclonal restriction with isoelectric focusing. The increased level of IgG_3 in some culture supernatants above the level, which could be easily explained by the known synthetic rate for this subclass, prompted the analysis of the purified RA synovial IgG by isoelectric focusing (18). This method allows excellent resolution of minor differences in isoelectric point in the IgG region. In 5 of 15 IgG samples from synovial cultures thus far examined, significant monoclonal restriction in isoelectric point of the IgG has been found. An example of one of the RA synovial IgG samples showing such a restriction in isoelectric point is shown in Fig. 4.

TABLE IV
Comparison of IgG_3 Levels in Serum with That of Synovial Culture Supernatants from Patients with RA

Serum	Synovial IgG_3 (percent culture supernatant IgG)	Serum IgG_3 (percent total serum IgG)	Ratio
RA patients			
J.D.	9	12	0.8
M.F.	26	8	3.2
M.H.	43	6	7.2
F.L.	26 Mean = 29	15 Mean = 12	1.7
M.M.	57	17	3.4
M.P.	8	15	0.5
A.W.	32	9	3.6
Normal subjects			
M.G.		17	
W.H.		10 Mean = 12	
L.V.		9	

Serum IgG from seven RA patients whose synovium had also been cultured, and from three normal controls was isolated on the anti-IgG immunoadsorbent, the IgG acid eluted and measured using absorbance at 280 nm. The isolated serum IgG was then rechromatographed on a protein A column and the percentage of IgG_3 (unbound fraction) calculated. The synovial IgG_3 was calculated based on ^{14}C radioactivity as described in Table II.

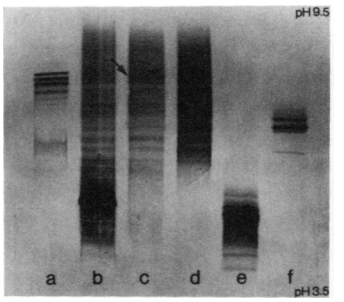

FIGURE 4 Slab gel isoelectric focusing of IgG isolated by adsorption on the anti-IgG column from RA culture supernatant, L.Wa. (a) Myeloma protein marker, (b) RA serum, (c) IgG from culture, L.Wa. Arrow points to monoclonal IgG band. (d) IgG of pooled Cohn Fraction II, (e) culture medium without synovium added, (f) erythrocyte lysate.

Absence of significant amount of rheumatoid factor in synovial IgG. The ^{14}C-labeled IgG from culture supernatants isolated on the immunoadsorbent was separated on Sephadex G-200 to establish the fraction that represented IgG-RF (Fig. 5). Any IgG-RF present in the culture supernatant, because of its high self-association constant of 10^{10} liters/mol (22), should exist

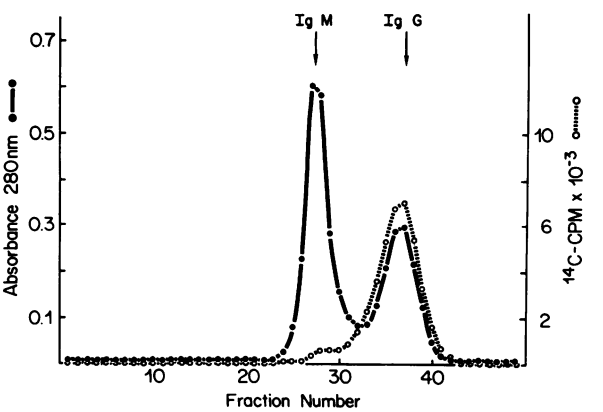

FIGURE 5 Sephadex G-200 chromatography of IgG from synovial culture supernatant. Total IgG from the supernatant was isolated on, and eluted from the anti-IgG immunoadsorbent, then chromatographed on Sephadex G-200. An IgG myeloma protein and an IgM Waldenstrom macroglobulin were added as size markers for absorbance at 280 nm.

as a dimer or a larger aggregate, and would be expected to migrate near the IgM protein marker. In contrast, non-RF IgG would comigrate with the IgG myeloma protein marker. When four culture supernatants (B. K., A. M., M. P., and L. J.) were fractionated on Sephadex G-200, an average of only 9% (range, 6 to 15%) of the ^{14}C-labeled IgG migrated near the IgM marker. Since this method does not differentiate between IgG-RF and other aggregated IgG, these data only set an upper limit for the IgG-RF in the RA synovial culture supernatants.

DISCUSSION

It is widely assumed that the antigen stimulating immunoglobulin synthesis by rheumatoid synovial lymphoid cells is IgG because of the IgM-RF and the IgG-RF in serum, and the staining of some synovial plasma cells by fluorescent, aggregated-IgG (1, 3, 23). Previous studies from this laboratory have analyzed the IgG produced by RA synovial fragments for other specific antibody activity. Repeated intramuscular and/or intraarticular immunizations with tetanus toxoid given before surgery in RA patients failed to induce production of specific antibody by synovium removed and studied in tissue culture up to 21 d after the in vivo immunization, although brisk synthesis of anti-tetanus antibody occurred in peripheral blood lymphocytes from the same patients when studied simultaneously (8). Thus, the day to day exogenous antigen contact of the RA patient does not influence the antibody specificity of the immunoglobulin produced by the synovial lymphoid infiltrate.

Because 80% of the immunoglobulin synthesized by RA synovia is IgG, and this IgG can be isolated in nearly pure form, synovial IgG was evaluated for restriction in subclass and light chain type. The larger size of the IgG-3 heavy chain when compared to the heavy chains of IgG_1, IgG_2, and IgG_4 is reflected in its slower migration in the SDS-PAGE shown in Figs. 2 and 3. The SDS-PAGE results also emphasize that very little protein other than IgG_3 is present in the synovial IgG fraction not binding to the protein A. The light chain data are so variable that they are difficult to interpret.

The use of DEAE-cellulose chromatography to obtain the IgG studied in the first nine synovial cultures introduced two potential artifacts: selection of the IgG_3 subclass over the other IgG subclasses due to differences in isoelectric point (24) and a loss ranging as high as 50% of the total IgG from the culture supernatants because of incomplete recovery from the DEAE-cellulose column (25). Uncertainty on the first point was resolved by using ^{125}I-labeled myeloma proteins of the IgG_1 and IgG_3 subclass and the demonstration that percentage recoveries from DEAE-cellulose of the two subclasses were the same. The greater total IgG recovery favored later use of the anti-IgG immmunoadsorbent, however. The large variation in the percentage of IgG represented by IgG_3 from one culture supernatant to another may reflect differences in an individual patient's disease activity at the time of surgery. The patients providing the cultured synovial tissues are not typical of most patients with RA. They had severe synovitis, which had been resistant to several forms of medical therapy. It is possible that some aspect of the medical therapy, which included nonsteroidal antiinflammatory agents, prednisone, gold compounds, and D-penicillamine in some patients, but not in others, may have altered the local immune response in the cultured synovia. Although no clearcut relationship could be found that was related to therapy, it is known that steroids, gold and D-penicillamine all have definite and variable effects on serum immunoglobulin levels and the titer of RF. A retrospective review of the clinical status of the first nine patients revealed that all had unusually active synovitis at the time of surgery. This might explain the reason for the larger amount of IgG_3-lambda isolated from the synovial cultures of eight of these nine patients when compared with many of the last 22 synovial cultures analyzed.

When levels of IgG_3 in the serum obtained at the time of surgery in seven of the patients were measured, (Table IV) IgG_3 averaged only 12% of the total IgG, and similar results were found in three normal control sera. The IgG_3, as percentage of the total IgG present in the synovial culture supernatant in four of these seven RA patients exceeded the percent in their serum IgG 3.2 to 7.4-fold. It is, therefore, likely that a net increase in synthesis of IgG_3 is responsible for the increase in the IgG_3 concentration in RA synovial cultures. The concentration of IgG_3 subclass in synovial culture supernatant is also significantly greater than the concentration of IgG_3 reported by others in normal serum (21), in rheumatoid serum (26), or in rheumatoid synovial fluid (26). Analysis of the recovered IgG fractions by SDS-PAGE and radiofluorography also demonstrated the increased amount of IgG_3 in most of the rheumatoid synovial cultures when compared with normal serum. The present observation that rheumatoid synovial tissues synthesize an increased amount of their IgG as IgG_3 supports the previous immunofluorescent studies of Munthe and Natvig (3). When they studied 34 RA synovial tissues with fluorescein-tagged antibodies against the IgG-subclasses, 25 to 50% of the plasma cells stained with the anti-IgG_3 antisera in 21 patients, whereas 50–75% of the plasma cells stained with anti-IgG_3 antisera in four additional patients.

Lymph node and tonsil tissues from nine non-RA patients synthesized up to 37% of their soluble protein as IgG with an average of 23% of this as the IgG_3 subclass. This amount would be compatible with the higher synthetic rate and greater catabolic rate for IgG_3, which is approximately twice that of the other IgG-subclasses (27, 28). The 48% IgG_3 of the total IgG produced by the cultured rheumatoid spleen is not surprising since a few patients with RA develop splenomegaly related to disease activity.

Another major objective of the study was to determine the percentage of synovial IgG that represented IgG-RF. In the four rheumatoid synovial cultures studied, <15% of the isolated ^{14}C-labeled IgG showed self-association when chromatographed on Sephadex G-200, and thus qualified as potential IgG-RF. Although this is not consistent with the conclusions of Munthe and Natvig (3), their data showed that 40–70% of synovial plasma cells did not stain with aggregated IgG.

The finding of the enrichment for the IgG_3 subclass and occasional monoclonal restriction in the IgG produced by RA synovial tissues in culture may be interpreted in at least two ways, with both applying to some synovia. Because the IgG_3 subclass has a shorter half-life than the other IgG subclasses (28), its increased level in culture supernatants may only reflect absence of the catabolic machinery that would be present in vivo. Tissues such as synovia with large numbers of macrophages may degrade newly synthesized IgG_3 more rapidly than lymphoid tissues such as tonsil or lymph node. This might explain the smaller amount of IgG_3 found in some synovial culture supernatants.

However, because of the presence of monoclonal restriction of the IgG in some synovial cultures, a second explanation, the stimulation of the synovial lymphocytic infiltrate by a homogeneous antigen that induces an immune response enriched with IgG_3 subclass must be considered. The fascinating observation of Lindstrom (29) who showed not only an increase in lambda light chains, but also a limited electrophoretic mobility of the IgG eluted from some rheumatoid synovial tissues also supports a restricted local immune response in rheumatoid synovitis. The present data showing up to 97% of the synovial IgG as IgG_3, the preliminary results showing monoclonal restriction of the IgG in some, but not all, of the synovial cultures (Fig. 4), and the observation in some rheumatoid synovia of up to 75% of plasma cells staining for IgG_3 (3) add support to the second explanation.

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