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KAPPA:LAMBDA LIGHT CHAIN RATIO IN IgG ELUTED FROM RHEUMATOID ARTHRITIS SYNOVIUM

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SUMMARY

The light chain ratio in IgG eluted from rheumatoid arthritis synovial membrane was studied using quantitative immunodiffusion. The ratio obtained in synovial IgG was different from that of IgG in peripheral blood. In several instances a marked domination of one type light chain was noted in the eluted IgG, which also showed a limited electrophoretic dispersion on immunoelectrophoresis. This suggests that synovial IgG in rheumatoid arthritis is antibody, and not derived non-specifically from the immunoglobulin pool. Lambda type light chains dominated over kappa type in most eluates, suggesting preferential involvement of cells producing lambda type molecules.

INTRODUCTION

The basic immunoglobulin subunit consists of two heavy and two light chains held together of covalent disulphide bonds. There are two antigenic types of light chains, kappa (κ) and lambda (λ) (Mannik & Kunkel, 1963a; Fahey & Solomon, 1963). In IgG of the peripheral blood the two types of light chains occur in the approximate proportions 2:1 (κ:λ).

A wide assortment of antibodies manufactured in response to exogenous or endogenous antigens have been analysed for their content of κ and λ type light chains (Mannik & Kunkel, 1963b; Franklin & Fudenberg, 1964). The light chain ratio in these antibodies diverged markedly from the ratio in the γ-globulin pool of the individual. The 7S γ-globulin obtained from the surface of red blood cells in autoimmune haemolytic anaemia frequently contains only one detectable light chain type (Leddy & Bakemeier, 1965). Using fluorescent technique on kidney biopsy material from patients with various forms of glomerulonephritis Herdman *et al.* (1967) demonstrated a light chain ratio in IgG deposited on glomeruli different from that of IgG of the peripheral blood. These data seem to indicate that by studying the light chain ratio of IgG located in tissue it may be possible to determine if such IgG molecules represent antibody of more specific selection or a non-specific deposition of IgG from the immunoglobulin pool.

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Several investigators have shown that the synovial membrane in rheumatoid arthritis (RA) contains immunoglobulins, mainly IgG, as well as complement components (Kaplan & Vaughan, 1959; Mellors *et al.*, 1961; Rodman *et al.*, 1967), suggesting that an immune mechanism is operating in this disease. However, the presence of immunoglobulin in RA synovial tissue may also be the result of selective deposition due to trapping or to affinity of the tissue for γ-globulin. It may also be due to transudation or exudation, or may represent deposition of aggregated γ-globulin.

To investigate further the mechanism of deposition of IgG in the RA synovial membrane, these deposits were eluted and their light chain ratio determined. In several instances light chain ratio in immunoglobulins of the peripheral blood was also studied in the corresponding patient.

The results of the present study seem to indicate that IgG located in RA synovial tissue represents a special part of IgG.

MATERIALS AND METHODS

Synovial membrane from twenty-eight patients was obtained at knee joint synovectomy. The material was immediately frozen and kept at −40°C until processed. After thawing, approximately 10 g were homogenized with 50 ml phosphate-buffered saline (PBS), pH 7·5, in an ice-jacketed container, using an Ultra Turrax TP at high speed for 2–4 min. The homogenate was then spun at 3000 *g* in a refrigerated (+4°C) centrifuge for 30 min. The sediment thus obtained was washed repeatedly in PBS and spun until the supernatant fluid was clear. At this point the sediment, which consisted of particulate material essentially freed from unbound serum proteins, was mixed with 0·2 M glycine buffer, pH 3·2, and incubated at 37°C with constant stirring for 2 hr. Following incubation the mixture was spun at 3000 *g* for 30 min. The resulting supernatant fraction was immediately brought to pH 7·0 with 0·1 M NaOH and then dialysed against several changes of PBS (Lerner & Dixon, 1968). After dialysis, the eluted material was concentrated by negative pressure dialysis in collodion membranes and thus brought to a protein concentration of 2–4 mg/ml.

Eluates from knee joint synovium obtained at autopsy were also studied in four instances. They were all obtained from patients aged 30–50 years without joint disease, who had died from vascular catastrophe such as cerebral hemorrhage or myocardial infarction but who previously had been essentially well. To determine whether there was preferential fixation of IgG of κ or λ type in synovial tissue, γ-globulin (Fraction II) was added to part of the autopsy synovial tissue after homogenizing and washing twice. After incubating the mixture at 37°C for 2 hr, the homogenate was processed as usual.

The concentrated eluate was studied by immunoelectrophoresis (Scheidegger, 1955), agarose gel-electrophoresis (Laurell, 1965) and quantitative immunological technique using Oudin tubes (Oudin, 1952). Immunodiffusion in gel was used to screen eluates for presence of IgA and IgM. Immunoelectrophoresis of the eluates was used to obtain a general idea about the serum proteins present in the eluates, and as a screening procedure to select eluates for quantitation of IgG and light chains. The Oudin quantitations were done in duplicate, with the respective antiserum (anti-κ, anti-λ, anti-IgG, anti-IgA and anti-free light chain) incorporated in agar, and the concentrated eluate layered on top. The distance travelled by the precipitin band was compared with distances on a standard curve, prepared at the same time with the purified antigen in concentrations established by the Folin method

(Lowry *et al.*, 1951). This method shows a 5–10% variation on repeated or duplicate determinations (Lindström *et al.*, 1968).

Serum levels of κ and λ chains were determined using the same technique. All anti-κ and anti-λ antisera were adjusted to equal precipitin titre by quantitative precipitin curves (Heidelberger & Kendall, 1932) and $\kappa:\lambda$ ratios calculated as previously described (Lindström, Williams & Theologides, 1969).

Antisera

Human light chains of types κ and λ were obtained in the form of Bence Jones proteins from the urine of patients with multiple myeloma. Purification of the light chains was accomplished through zone electrophoresis on starch block (Kunkel, 1954) and gel filtration on Sephadex G-200. These purified Bence Jones protein gave a single line on immunoelectrophoresis with antisera to human IgG and human whole serum. They were used as antigen emulsified in Freund's complete adjuvant and injected at multiple sites subcutaneously in rabbits at weekly intervals. Intravenous injections of alum precipitated material were given after 4–5 weeks and the animal bled 10 days later. On immunoelectrophoresis the antisera gave a single line against whole human serum. In a few instances the antisera required some absorption with the opposite type light chain, to become monospecific.

Rabbit antisera specific for free human immunoglobulin light chains were prepared by the method of Tan & Epstein (1965). Rabbits were immunized with free light chains prepared from pooled normal IgG. The resulting antisera were absorbed with normal IgG to remove antibodies against antigens available on light chains bound to heavy chains, leaving antibodies against antigens exposed on free light chains only. The antiserum was rendered type specific for free κ and free λ light chains by absorption with a λ and a κ Bence Jones light chain preparation, respectively.

Goat anti-human whole serum and anti IgG, IgA, and IgM sera were obtained from commercial sources (Hyland Laboratories, Los Angeles, California).

RESULTS

Table 1 shows diagnostic subgroups in the twenty-eight patients studied as well as occurrence of immunoglobulins in the eluates, as detected by immunoelectrophoresis. In two of the three RA eluates which did not contain immunoglobulins, there was low activity of disease as judged by degree of synovitis ('burnt out' variety of RA) and in one of these two the orthopaedic surgeon noted a rather thin synovial membrane at surgery.

Immunoelectrophoresis showed the presence of albumin in all twenty-eight eluates. In most of the twenty-three RA eluates other serum-proteins such as transferrin, α_1-antitrypsin could be demonstrated. In the twenty eluates which contained IgG, the electrophoretic dispersion of this immunoglobulin was limited, as compared to serum IgG. Fig. 1 shows a representative immunoelectrophoretic pattern. Immune diffusion screening of eluates using commercial antisera against IgA and IgM showed trace amounts of IgA in several RA eluates; IgM could not be demonstrated.

Most of the RA eluates contained complement (β_{1C}), as shown by electrophoresis in gel containing antibodies to β_{1C}, a technique developed by Laurell (1966). Table 2 demonstrates that complement (β_{1C}) level correlated well ($P<0{\cdot}01$) with IgG content in the eluates. Fig. 2 shows a few eluates studied with this technique, as well as diluted normal human serum.

TABLE 1. Number of patients in diagnostic groups and occurrence of immunoglobulins (Ig) in eluates within each group.

Diagnosis	No. of patients	Occurrence of Ig in eluates
Rheumatoid arthritis	23	20/23
Osteoarthritis	4	0/4
Intermittent hydroarthrosis	1	0/1
Total	28	20/28

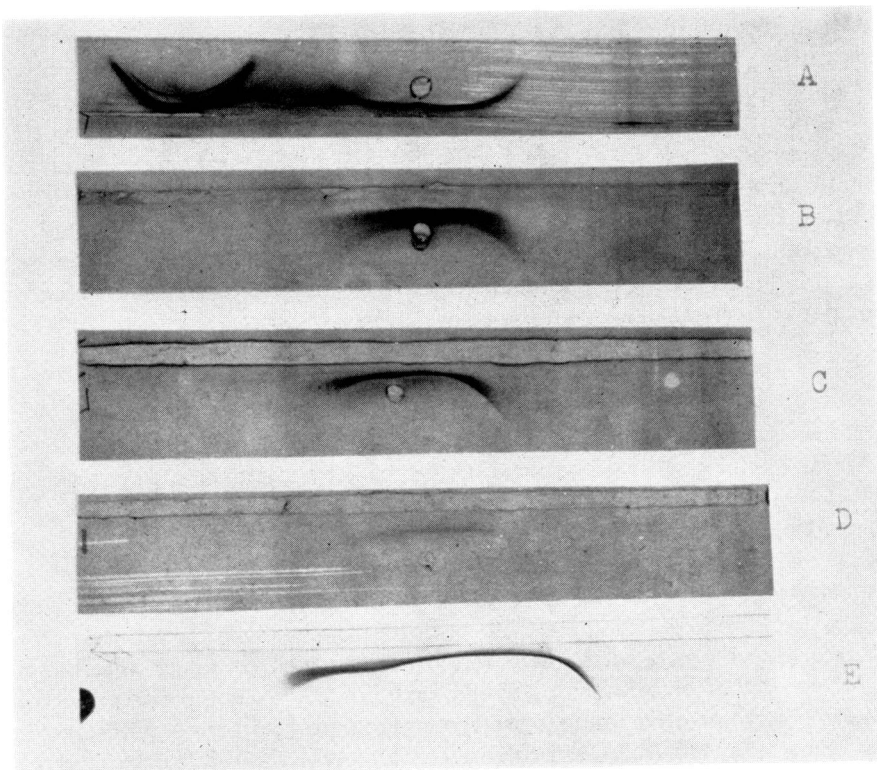

FIG. 1. Representative immunoelectrophoretic pattern. Shown is O.P. eluate (A–D) and O.P. serum (E). The following antisera were used: A, anti-normal human serum; B and E, anti-IgG; C, anti-λ; D, anti-κ. Note wider electrophoretic dispersion of serum IgG as compared to eluate IgG. Anode to the left.

Agar gel electrophoresis of eluates showed a fairly uniform pattern of albumin, other serum proteins and smear-like γ-globulin. There was no evidence of marked electrophoretic homogeneity in the γ-globulin region.

The twenty patients with rheumatoid arthritis whose eluates showed demonstrable amounts of IgG are listed in Table 3. The results of Oudin tube quantitation of IgG, κ chains and λ chains in the eluates are tabulated, as are the serum $\kappa:\lambda$ ratio and serum

TABLE 2. Quantitative data on IgG in synovial eluates, arranged in decreasing concentration. The β_1C values in eluates are expressed as percentage of the value of a pool of normal sera. ND = not determined

IgG concentration in eluates (mg/ml)	Complement (β_1C) in eluates
1·90	1·6
1·87	2·9
1·22	0·8
1·05	0·4
0·81	2·0
0·70	0·8
0·69	0·4
0·60	0·2
0·52	Trace
0·50	0·2
0·46	Trace
0·44	0
0·36	0·4
0·28	Trace
0·27	0·4
0·24	ND
0·23	0
0·21	0
Trace	0
Trace	0

rheumatoid factor titre. Eight eluates showed more material reacting with anti-λ than with anti-κ antisera, and in two of these there was only trace or very small amounts of κ-type light chain. Five eluates had more material reacting with anti-κ than with anti-λ; in this group three eluates lacked λ type light chains or showed only traces of this material. Seven eluates had approximately the same amount of κ and λ light chain. In only one case there was more material reacting with anti-light chain than with anti-IgG antisera (patient E.J.), in all other eluates it was less. In a few instances there was a marked excess of IgG-material (patients A.G., I.H., O.P. and E.L.). The serum $\kappa:\lambda$ ratios showed no correlation with ratios obtained in eluates. The average $\kappa:\lambda$ ratio of the serum from patients whose eluates

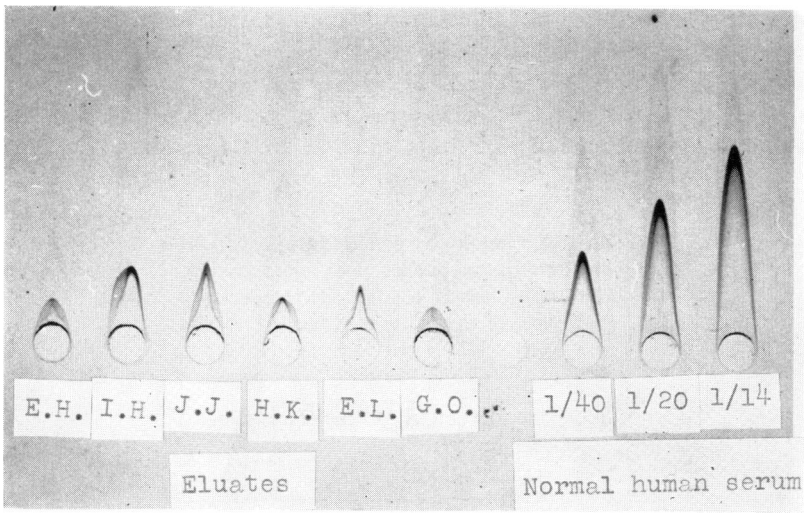

FIG. 2. Electrophoresis of selected eluates in agarose gel containing antibodies to complement (β_{1C}). The first six samples from the left are synovial eluates. The seventh well contains buffer and the last three different dilutions of normal human serum. Anode towards the top.

TABLE 3. Immunoglobulin quantitation on synovial eluates in mg/ml, serum $\kappa:\lambda$ ratio and Waaler–Rose titre

Patient (age and sex)	Serum Waaler–Rose titre	Serum $\kappa:\lambda$ ratio	Immunoglobulin quantitation in eluates (mg/ml)			Light chain type domination
			IgG	κ	λ	
A.G. (64 F)	ND	3·7	1·05	Trace	0·31	λ
L.S. (53 M)	128	2·9	0·52	0·09	0·24	λ
I.H. (52 F)	256	ND	1·90	0·25	0·84	λ
R.O. (26 F)	ND	4·1	0·46	0·06	0·20	λ
O.P. (36 M)	0	4·7	1·87	0·17	0·70	λ
E.L. (64 F)	ND	2·1	1·22	0·10	0·52	λ
D.G. (39 F)	256	4·7	0·70	0·14	0·28	λ
A.C. (66 F)	512	4·1	0·69	0·09	0·30	λ
S.J. (58 F)	256	1·7	0·44	0·25	0·11	κ
G.O. (49 F)	1024	ND	0·50	0·22	0	κ
D.R. (54 F)	256	2·7	0·24	0·15	0	κ
E.B. (43 F)	16	ND	0·28	0·20	Trace	κ
J.J. (64 F)	ND	ND	0·81	0·38	0·17	κ
H.K. (84 F)	512	1·7	0·27	0·13	0·13	=
M.L. (46 F)	0	1·8	Trace	0	0	=
M.L. (60 F)	64	ND	0·60	0·11	0·16	=
D.A. (61 F)	128	1·8	0·21	0·07	0·08	=
S.L. (58 F)	256	1·9	0·23	0·07	0·09	=
E.J. (57 F)	1024	3·1	Trace	0·05	0·08	=
E.H. (42 F)	ND	3·9	0·36	0·15	0·18	=

showed predominantly λ type light chains was 3·76. The average ratio for the group as a whole was 2·99. This ratio is higher than normal values previously reported, but is similar to ratios observed in sera of patients with lupus erythematosus (McKelvey & Fahey, 1965).

All eluates were screened for free light chains using immunodiffusion and antisera specific for free light chains of κ or λ type, respectively (Tan & Epstein, 1965). Free light chains of λ type could not be detected in this way. Two eluates (D.G. and I.H.) showed presence of κ type free light chains, and Oudin tube quantitation using the same antiserum, showed a concentration of 0·11 and 0·11 mg/ml, respectively. Thus, by subtraction it was found in these two eluates that the amount of κ type light chains bound to heavy chains was quite small, leaving λ type light chain to dominate strongly in the intact IgG molecules of these eluates (as it does in eluates A.G. and R.O.).

The eluates from synovial tissue obtained at autopsy (four cases) did not contain any immunoglobulins. Incubation of this synovial material with Fraction II resulted in some fixation of immunoglobulin which could be regained after acid elution. The κ:λ ratio in IgG eluted in this way was, however, that of normal IgG.

DISCUSSION

Immunological mechanisms are believed to play a role in the course of rheumatoid arthritis. Using immunofluorescent technique Kaplan & Vaughan (1959), Fish *et al.* (1966) and Rodman *et al.* (1967) have demonstrated deposits of immunoglobulins and β_{1C} and β_{1E} components of complement within rheumatoid arthritis synovium. Using fluorescein-labelled aggregated human γ-globulin, Mellors *et al.* (1961) have shown rheumatoid factor in plasma cells of rheumatoid synovial lesions, and its absence in non-rheumatoid diseased joints. The finding of low complement level in rheumatoid synovial fluid by Hedberg (1963) and Pekin & Zvaifler (1964) may reflect local utilization of complement in an antigen–antibody reaction. Neutrophils of rheumatoid synovial fluid contain cytoplasmic inclusions, consisting of IgG, IgM and complement (Rawson, Abelson & Hollander, 1965). All these data suggest that an immunological process is in operation in the rheumatoid arthritis joint, and it seems that the combination of rheumatoid factor and altered IgG may be an important factor in the pathogenesis of rheumatoid disease.

Studies by Edelman, Kunkel & Franklin (1958) have established that rheumatoid factor forms complexes with altered IgG. Such alteration is usually accomplished *in vitro* by heat aggregation which changes the shape of the molecule, exposing hidden reaction sites. Since this is not the likely mechanism by which γ-globulin is altered *in vivo*, other possibilities must be considered. To date, however, the quality of the synovial IgG has not been studied, and the alteration in the shape of its molecule, which makes it antigenic, is not well understood.

The working hypothesis in initiating this study was that the synovial IgG might be specific antibody, produced in response to an unknown antigen such as a micro-organism, virus or mycoplasma (Bartholomew, 1965; Hayflick & Chanok, 1965), and not γ-globulin from the general circulating pool. Also, the demonstration of a changed synovial IgG might shed some light on the molecular change in the local IgG, which makes it antigenic to IgM rheumatoid factor.

The data presented in this report indicate that the IgG eluted from the synovial membrane

in rheumatoid arthritis has a different κ and λ composition from that of the immunoglobulin pool of the peripheral blood. This suggests that IgG located in the synovial membrane is not derived non-specifically from the immunoglobulin pool, but rather that it is antibody. The demonstration of IgG bearing only one type of light chain and trace amounts or nothing at all of the other in several eluates strongly suggests that selective binding of a certain fraction of immunoglobulins has occurred.

The marked domination of one type light chain in eluted IgG noted in several instances, together with rather limited electrophoretic dispersion of IgG noted on immunoelectrophoresis, suggests that the synovial IgG is more homogeneous than IgG from the immunoglobulin pool. The same phenomenon has been reported by Leddy & Bakemeier (1965) concerning IgG eluted from the surface of red blood cells in autoimmune haemolytic anaemia, and by Feizi & Schumacher (1968) in cold agglutinins following infection with *Mycoplasma pneumoniae*. This finding may be due to a selective group of plasma cells involved in the synthesis of a specific antibody.

The positive correlation between amounts of IgG and complement (β_{1C}) observed in the eluates suggests some kind of binding between these proteins. It is interesting to note that Rodman *et al.* (1967) as well as Fish *et al.* (1966) observed that tissue localization of 7S γ-globulin in RA synovial membrane was closely approximated by concurrent staining for complement. Also Kinsella, Baum & Ziff (1969), using immunofluorescent technique, demonstrated IgG in association with the β_{1C} component of complement in most of the phagocytic lining cells from the synovial tissue of fifteen of seventeen patients with rheumatoid arthritis studied. The demonstration in this study of synovial IgG having antibody characteristics, together with its close association with complement suggests that they are taking part in an antigen–antibody reaction utilizing complement. In a reaction like that IgG might be considered to be specific antibody against some unknown antigen. It may be locally synthesized in the joint or it may represent part of circulating γ-globulin complexes of the intermediate type (9-17S) described by Kunkel *et al.* (1961), being deposited in the joint and binding complement.

It is noteworthy that there is an all over domination of λ type light chains in the IgG eluted from RA synovial membrane. In the seven eluates where the amount of κ and λ chains was about equal, a relative λ domination exists, since the normal ratio is 2:1 in favour of κ. The significance of this observation is not clear. It can be mentioned, however, that Epstein & Tan (1966) noted a preponderance of λ type free light chain in synovial fluid from patients with RA. These data suggest preferential involvement of cells producing λ type molecules.

When the eluates were screened for free light chain, only two (I.H. and D.G.) showed presence of free light chains, in both cases κ type, and in relatively small amounts. The majority of light chain encountered in the eluates must therefore have been linked to heavy chains, forming intact IgG molecules.

Subtracting the sum of light chains from the amount of IgG in individual eluate might give some estimate of immunoglobulin fragments not bound to light chains. Such calculations suggested the existence of isolated heavy chains or Fc-like material in several eluates (I.H., O.P., E.L. and A.G.). This is particularly interesting in the light of work by Owen *et al.* (1967) and Quismorio *et al.* (1968), who injected IgG fragments, which were closely related to Fc-pieces, into uninvolved knee joints of patients with RA, and were able to show that the fragments may be as much as fifty times as potent in inducing inflammation as whole IgG molecules from RA donors.

The special part of IgG demonstrated in RA synovium in this report may well be the 'altered' IgG capable of reacting with rheumatoid factor IgM or other types of anti-γ-globulin factors. This 'altered' IgG may be synthesized in response to a sustained stimulation by some as yet unknown antigen. The difference in light chain composition may represent only one aspect of the molecular change, which exposes hidden autoimmunogenic sites, thus making this variety of IgG antigenic to immunoglobulin producing cells.

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