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IgG subclass composition of rheumatoid arthritic sera and joint fluids

F. SHAKIB AND D. R. STANWORTH
From the Department of Experimental Pathology, University of Birmingham, Rheumatism Research Wing, Medical School, Birmingham B15 2TJ

Shakib, F., and Stanworth, D. R. (1976). *Annals of the Rheumatic Diseases*, **35**, 263–266. **IgG subclass composition of rheumatoid arthritic sera and joint fluids.** The IgG subclass composition of 24 matched pairs of synovial fluids and sera, from 24 patients with rheumatoid arthritis, was determined. The subclass composition of rheumatoid synovial fluid IgG was found to be qualitatively the same as that of the corresponding serum, differing only in certain quantitative aspects. A 7·8% increase in IgG1 level found in the sera of rheumatoid arthritis patients relative to that in the sera of normal individuals was accompanied by 6·8, 0·7, and 0·3% decreases in IgG2, IgG3, and IgG4, respectively.

Antigammaglobulins (i.e. rheumatoid factors) in sera from rheumatoid arthritis patients have been shown to be directed mainly against IgG, and thus the question of the nature of the autoimmunogenic stimulus of this major immunoglobulin class in rheumatoid arthritis is raised. For instance, although there is evidence that such antigammaglobulins react *in vitro* with at least three out of the four human IgG subclasses (Normansell and Stanworth, 1968; Natvig, Gaarder, and Turner, 1972) it was conceivable that an original IgG immunogen, presumably effective at localized sites such as the joints, was confined to a single subclass and might reflect on the nature of a preliminary antigen (extrinsic or intrinsic). Some recent evidence suggests that different types of antigens initiate antibody responses in different IgG subclasses (Yount and others, 1968; Anderson and Terry, 1968; Graham, Yount, and Roberts, 1973). Therefore, the IgG subclass compositions of 24 matched synovial fluid and serum specimens from rheumatoid arthritis patients were compared as a preliminary investigation to the proposed study of antibody IgG subclass profiles.

In earlier studies from this laboratory (Keogh, 1968) IgG was isolated from rheumatoid joint fluid and its susceptibility to cleavage by papain as an indirect indication of its subclass composition was determined. However, a recently developed quantitative immunodiffusion assay (Shakib and others, 1975) now provides a more satisfactory means of determining the relative amounts of IgG subclasses in the joint fluids of rheumatoid patients.

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Correspondence to F. Shakib

Materials and methods

RHEUMATOID SERA AND SYNOVIAL FLUIDS

Matched pairs of rheumatoid fluids and sera (taken at the same time) were obtained from patients with well-defined active rheumatoid arthritis. Samples were kept in the frozen state until required for testing.

HYALURONIDASE TREATMENT OF SYNOVIAL FLUIDS

To 1 ml of synovial fluid, 0·05 ml of hyaluronidase enzyme (Koch-Light Laboratories Ltd.) solution (made up by suspending 0·1 mg of the enzyme in 10 ml of 0·075 mol/l sodium phosphate buffer, pH 7·0 containing 0·075 mol/l sodium chloride) was added and the mixture then incubated at 37°C for 60 minutes.

QUANTITATION OF IgG SUBCLASSES BY RADIAL-IMMUNODIFFUSION

The method used in the measurement of IgG subclasses has been described elsewhere (Shakib and others, 1975).

Results

In the radial immunodiffusion method of Mancini, Carbonara, and Heremans (1965) it is assumed that all unknown serum samples and standard solutions will diffuse equally well in the agar-antiserum medium. However, would the rather high viscosity of synovial fluid tend to retard the movement of its protein components, e.g. IgG, through the agar-antiserum layer? Five synovial fluid samples were treated with hyaluronidase for 60 minutes at 37°C. IgG subclass levels of the five samples were estimated before and after treatment with hyaluronidase. In order to

eliminate the 'between plate' variation of the method and to minimize 'within plate' error (due to variation in the thickness of the agar-antiserum layer), both treated and untreated samples were assayed on the same plate adjacent to one another. The difference between each pair of results, i.e. before and after hyaluronidase treatment, was found to be within the 'within plate' variation of the radial immunodiffusion method reported previously (Shakib and others, 1975). Results are shown in Table I.

The quantitative study of IgG subclasses was then carried out on 24 matched pairs of human (5 males, 19 females) synovial fluids and sera taken from patients with well established rheumatoid arthritis. To eliminate the 'between plate' variation of the assay method, matched pairs of fluid and sera were estimated on the same plate. Results for individual rheumatoid patients are shown in Table II and the data summarized in Table III.

Discussion

All four IgG subclasses were found in rheumatoid synovial fluids in the same proportion as that of the corresponding sera. In most cases the concentration of a particular subclass in the synovial fluid was less than that in the corresponding serum; the mean serum to synovial fluid ratio was found to be 1·2 for total IgG and each of its subclasses except IgG3, which showed a ratio of 1·4 (possibly due to the susceptibility of this subclass to denaturation in the physical

Table I *Effect of hyaluronidase treatment on measurement of IgG subclasses in synovial fluid*

Sample no.	*IgG1 (g/l)*		*IgG2 (g/l)*		*IgG3 (g/l)*		*IgG4 (g/l)*	
	A	*B*	*A*	*B*	*A*	*B*	*A*	*B*
1	8·5	8·4	1·2	1·22	0·57	0·57	0·039	0·041
2	4·0	4·2	0·94	0·93	0·32	0·31	0·029	0·028
3	16·8	17·0	0·83	0·80	0·49	0·49	0·019	0·019
4	6·0	6·0	1·4	1·45	1·12	1·12	0·031	0·03
5	9·0	8·9	2·2	2·22	1·1	1·11	0·030	0·03

A = before treatment; *B* = after treatment.

Table II *Results of the quantitative study of total IgG and its heavy chain subclasses in 24 matched pairs of rheumatoid synovial fluids and sera*

Sample no.	*Sex*	*Rose-Waaler reactivity*	*Total IgG (g/l)*		*IgG1 (g/l)*		*IgG2 (g/l)*		*IgG3 (g/l)*		*IgG4 (g/l)*	
			S	*F*	*S*	*F*	*S*	*F*	*S*	*F*	*S*	*F*
1	F	−	18·60	10·20	12·70	8·50	2·35	1·20	1·45	0·57	0·086	0·039
2	F	−	15·80	11·20	12·00	7·10	1·25	2·58	0·98	0·61	0·027	0·018
3	F	+	11·20	7·80	8·50	6·00	1·75	0·92	1·38	0·82	0·026	0·019
4	M	+	11·00	6·20	8·20	5·20	2·05	0·92	0·92	0·40	0·074	0·032
5	F	−	8·60	5·80	6·50	4·00	0·94	0·94	0·75	0·32	0·028	0·029
6	M	?	7·80	9·80	6·10	9·00	0·84	0·94	0·55	0·62	0·062	0·076
7	F	−	14·00	13·00	11·00	10·00	2·08	2·08	0·58	0·56	0·053	0·05
8	F	−	11·10	8·30	9·00	9·05	0·90	0·71	0·24	0·225	0·074	0·056
9	F	+	29·00	17·00	22·90	13·90	3·05	1·75	1·40	0·84	0·048	0·039
10	F	+	20·00	20·00	17·00	16·80	1·10	0·83	0·74	0·49	0·021	0·019
11	F	?	9·40	4·80	9·00	3·60	0·96	0·92	0·30	0·12	0·019	0·029
12	F	?	8·00	5·60	6·00	4·00	0·82	0·68	1·33	0·90	0·024	0·068
13	F	+	9·40	10·00	7·50	7·70	1·38	1·12	0·70	0·90	0·042	0·029
14	F	+	7·90	9·00	5·00	6·00	1·28	1·40	0·80	1·12	0·033	0·031
15	F	+	15·80	9·40	10·00	5·00	3·45	3·05	2·35	1·18	0·024	0·023
16	F	−	14·00	21·00	10·60	16·00	1·85	1·75	0·94	1·35	0·11	0·19
17	F	−	11·50	12·80	10·00	9·00	1·48	0·94	0·32	0·30	0·027	0·022
18	F	+	14·70	13·20	11·80	10·50	1·50	1·30	0·92	1·00	0·039	0·019
19	F	+	14·80	14·20	12·00	11·00	1·30	1·40	0·96	1·30	0·16	0·19
20	M	+	13·00	7·60	9·00	6·00	1·30	0·73	1·32	0·66	0·14	0·068
21	F	−	11·60	11·50	8·12	10·80	1·30	1·00	1·15	0·76	0·055	0·052
22	M	−	16·80	12·70	14·20	9·00	3·00	2·20	1·40	1·10	0·052	0·03
23	F	+	8·90	9·20	7·05	7·50	0·85	0·90	0·55	0·64	0·09	0·086
24	M	+	11·50	10·30	8·50	7·45	2·05	1·30	1·52	1·26	0·023	0·022

S = serum; *F* = synovial fluid.

Table III *Comparison between distribution of IgG subclasses in 111 normal sera and in 24 matched pairs of rheumatoid synovial fluids and sera*

IgG	*Normal serum, Shakib* et al. *(1975)*			*Rheumatoid serum*			*Rheumatoid synovial fluid*		
	Mean (g/l)	*% Total*	*Range (g/l)*	*Mean (g/l)*	*% total*	*Range (g/l)*	*Mean (g/l)*	*% total*	*Range (g/l)*
IgG1	8·01	71·5	3·70–12·30	10·11	79·3	5·00–22·90	8·46	80	3·60–16·80
IgG2	2·17	19·4	0·46–4·30	1·61	12·6	0·82–3·45	1·31	12·4	0·68–3·05
IgG3	0·94	8·4	0·15–2·45	0·98	7·7	0·24–2·35	0·75	7·1	0·12–1·35
IgG4	0·08	0·7	0–0·185	0·055	0·4	0·019–0·16	0·051	0·5	0·018–0·19
IgG	11·75		7·70–18·50	13·10		7·80–29·00	10·85		4·80–21·00

environment of the synovial fluid during storage). This was, however, demonstrable only by statistical analysis, since synovial fluid levels of subclasses of IgG higher than that of the corresponding serum were found in at least 50% of patients. This pattern, which could be due to a local synthesis of IgG in the joint space, did not always occur in all subclasses at the same time and was not a function of the presence or absence of rheumatoid factor as indicated by the Rose-Waaler test.

Regression analyses have shown poor correlations between the levels of total IgG and its subclasses in rheumatoid synovial fluids and the levels in the corresponding sera; total IgG, IgG1, IgG2, IgG3, and IgG4 showing correlation coefficients of 0·64, 0·64, 0·69, 0·59, and 0·77 respectively. A 7·8% increase in serum IgG1 level found in rheumatoid arthritis patients was accompanied by 6·8, 0·7, and 0·3% decreases in serum IgG2, IgG3 (no change in absolute amount), and IgG4, respectively. These changes in the level of IgG1, IgG2, and IgG4 were shown by the t-test to be significant ($P < 0·02$–$0·001$) when compared to levels of the corresponding subclasses in 111 normal sera. The possibility that this disproportional distribution is due to an actual sex difference (80% of patients studied were females, and rheumatoid arthritis has a higher incidence in females than in males) in the serum level of IgG subclasses was unlikely, since previous work (Shakib and others, 1975) did not show any sex difference in the normal population.

The fact that all rheumatoid arthritis patients studied here were receiving steroid therapy could explain the low level of serum IgG2, but not the unchanged IgG3 and the high IgG1 levels found. Follow-up studies of a group of renal transplant patients receiving steroid therapy have shown that all IgG subclasses are susceptible to such treatment (unpublished observation). The patterns of IgG subclass distribution observed here, i.e. high IgG1 with low IgG2, was also noticed in a normal individual in response to antitetanus immunization (unpublished observation). This would perhaps be consistent with the suggestion of infection as a possible aetiological factor in the genesis of rheumatoid arthritis as proposed sometime ago by Hollander and others (1962).

The finding that all IgG subclasses are represented in rheumatoid synovial fluid, and in a proportion similar to that of the corresponding serum, does not necessarily invalidate the suggestion that a single subclass or a population of related subclasses might be the stimulus for rheumatoid factor production (R. Keogh and D. R. Stanworth, in preparation).

It is conceivable that antibodies representative of certain IgG subclasses may pass selectively into the joint spaces during the initial phase of rheumatoid arthritis, in response to the presence of an infectious agent (Hollander and others, 1962). Subsequent denaturation or unfolding of these (e.g. as a result of combination with specific antigen) may bring about the exposure of new γ chain antigenic determinants which provide the stimulus for anti-IgG (i.e. rheumatoid factor) formation (as discussed previously by Henney and Stanworth, 1966). Furthermore, although the resultant rheumatoid factors are predominantly of the IgM class, IgG rheumatoid factors are now known to be present in the serum and joint fluids of rheumatoid arthritis patients; and these, like IgM rheumatoid factors, could be involved in the formation of deleterious antibody antigen complexes which contribute to the induction of localized inflammatory reactions.

In order to obtain further information about such possible roles of immunoglobulin of the IgG class in the joints of rheumatoid patients, either as secondary immunogen or as antiantibody, it will be important to try to show antibody activity within the IgG subclasses detectable in synovial fluids as described here. Such an investigation is now in progress.

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