Subclasses IgA₁ and IgA₂ in serum and synovial fluid in rheumatoid arthritis and reactive synovitis of local origin

Z. HRNČÍŘ AND M. TICHÝ

From the Second Medical Department, Charles University Faculty of Medicine, Hradec Králové, Czechoslovakia

SUMMARY Subclasses IgA₁ and IgA₂ in serum were examined in 40 patients (28 cases of rheumatoid arthritis and 12 cases of reactive synovitis of local origin) and also in synovial fluid of the knee joint in 17 of these patients. The levels of IgA₁ and IgA₂ in serum were statistically significantly higher than in synovial fluid in both groups of patients ($P=0.0237-0.0018$), but significant correlations between serum and synovial fluid for IgA₁ ($r=0.8855$, $P=0.0010$) and for IgA₂ ($r=0.7630$, $P=0.0124$) were found only in cases of rheumatoid arthritis. A percentage evaluation revealed a significant disproportion ($P=0.0028$) in favour of IgA₁ in synovial fluid during rheumatoid arthritis. The analysis of proportions of IgA subclasses and rheumatoid factor has shown no significant relationships.

In cases of rheumatoid arthritis with positive rheumatoid factors (RF) in serum or in synovial fluid, higher values of total IgA in serum (Dreher, 1977) or in serum and in synovial fluid (Solnica *et al.*, 1969; Hrnčíř *et al.*, 1972) than in RF-negative patients have been repeatedly observed. A significant positive correlation and a direct linear regression were also observed between the levels of total IgA in synovial fluid and RF in serum (Komatsubara *et al.*, 1976) and in synovial fluid (Hrnčíř *et al.*, 1977). On the other hand, rheumatoid arthritis is one of the diseases with frequent occurrence of selective deficiency in total IgA in serum (Natvig *et al.*, 1971). In the cited references we found no reports on IgA dysimmunoglobulinaemia examined at the level of IgA₁ and IgA₂ subclasses. This is the subject of the present study.

Material

The series under examination consisted of 40 patients. Twenty-eight patients (7 men and 21 women, age range 26-74 years, average age 47) who had definite or classical rheumatoid arthritis (Ropes *et al.*, 1959) and their subsequent modification (Kellgren *et al.*, 1961). Serum was examined in all patients

while in 9 patients, synovial fluid of the knee joint was also examined. Twelve patients (6 men and 6 women, aged between 19 and 75, average age 48 years) suffered from reactive synovitis of the knee joint of local origin, ie, during osteoarthritis of following injury. Serum was examined in all of them while synovial fluid was also examined in 8. Pooled serum obtained from 15 blood donors (7 men and 8 women, aged between 25 and 58 years, average age 48) from the East Bohemian region was also examined.

Methods

Blood and synovial fluid were collected simultaneously. The samples of serum and synovial fluid were kept at -20°C until examined. Before examination, the synovial fluid was condensed using Minicon B-15 (Amicon, USA) to 70 g/l of total protein. Two hours before the actual examination the synovial fluid was incubated at $+37^{\circ}\text{C}$ with testicular bull hyaluronidase, concentration 1 ml/m (Hyaza, Sevac, Prague).

The IgA₁ and IgA₂ subclasses were determined by the method of simple radial immunodiffusion (Mancini *et al.*, 1965) using monospecific IgA₁ and IgA₂ antisera (RaHu/IgA₁ and RaHu/IgA₂, Nordisk Immunological Laboratories). The results were

evaluated as percentages and also calculated in mg/100 ml. The starting point for evaluating the percentages was the total of means of IgA₁ and IgA₂ precipitation rings. Values in mg/100 ml were calculated from the percentages of IgA₁ and IgA₂ and from the total IgA (mg/100 ml) in the sample under investigation. The level of total IgA was determined by simple radial immunodiffusion (Mancini *et al.*, 1965) using a commercial ISP set (Sevac, Prague) containing a monospecific antihuman swine anti-serum (SwaRu/IgA) IgA and a standard registered in the International Reference Centre for Immunoglobulins in Lausanne.

Rheumatoid factor was examined by the tube latex fixation test (Singer and Plotz, 1956) and by the haemagglutination test with sheep red cells (Ball, 1950). Mathematical analysis was carried out by means of ODR 1204 and Hewlett-Packard computers using the non-paired *t*-test for proportional numbers, the non-paired log *t*-test for bilateral alternatives, and the method of correlation and regression analyses.

Results

Data obtained by evaluation of subclasses IgA₁ and IgA₂ according to percentage distribution are given in Table 1. Statistical analysis revealed a significantly higher percentage of IgA₁ than IgA₂ ($P=0.0028$) in synovial fluid during rheumatoid arthritis. No other statistically significant differences ($P>0.05$)

were observed in the percentages of IgA₁ and IgA₂ subclasses in the diseases under investigation.

Levels of IgA₁, IgA₂, and total IgA expressed in mg/100 ml are presented in Table 2. Levels of all 3 parameters were always significantly higher in serum than in synovial fluid ($P=0.0237-0.0018$). On the other hand, the comparison of the levels of IgA₁, IgA₂, and total IgA in the same sample showed no statistically significant differences ($P>0.05$). Higher levels of IgA₁, IgA₂, or of total IgA in synovial fluid than in serum have not been found in any of the patients examined. In the pooled serum sample from blood donors, the value of total IgA was 307 mg/100 ml of which 61.8% was subclass IgA₁ (190 mg/100 ml) and 38.1% was subclass IgA₂ (117 mg/100 ml).

On evaluating the relationships between the levels of total IgA, and IgA₁ and IgA₂ subclasses in serum and synovial fluid of patients with rheumatoid arthritis, significant positive correlations and direct linear regressions were determined. The values for IgA₁ and IgA₂ subclasses are given in Figs. 1 and 2; for total IgA, the correlation coefficient (*r*) was 0.08571 ($P=0.0022$). In reactive synovitis of local origin, on the other hand, no significant relationships between the levels in serum and synovial fluid were found for any of the given 3 parameters for IgA₁, $r=0.2062$ ($P>0.05$), for IgA₂, $r=-0.0493$ ($P>0.05$), and for total IgA, $r=0.1330$ ($P>0.05$).

Using the methods of correlations and regression analyses, no significant relationships were found

Table 1 Blood serum (BS) and synovial fluid (SF) IgA₁ and IgA₂ subclasses (percentage distribution)

IgA subclass	Diagnosis	Sample tested	No.	$\bar{x}$	95% Confidence limits	
IgA ₁	Rheumatoid arthritis	BS	28	50.3	54.1	46.5
		SF	9	53.5*	56.9	50.1
	Reactive synovitis	BS	12	50.9	55.5	46.3
		SF	8	51.8	56.0	47.7
IgA ₂	Rheumatoid arthritis	BS	28	49.6	53.5	45.8
		SF	9	46.3*	49.6	43.1
	Reactive synovitis	BS	12	49.0	53.6	44.4
		SF	8	48.7	53.2	44.2

* $P=0.0028$.

Table 2 Blood serum (BS) and synovial (SF) IgA₁ and IgA₂ subclasses and total total IgA in mg/100 ml

	Diagnosis	Sample tested	No.	$\bar{x}$	95% Confidence limits	
IgA ₁	Rheumatoid arthritis	BS	28	182	214	150
		SF	9	111	147	76
	Reactive synovitis	BS	12	180	232	128
		SF	8	89	104	75
IgA ₂	Rheumatoid arthritis	BS	28	168	192	144
		SF	9	92	123	61
	Reactive synovitis	BS	12	154	188	120
		SF	8	88	117	59
Total IgA	Rheumatoid arthritis	BS	28	335	405	304
		SF	9	204	270	137
	Reactive synovitis	BS	12	305	359	251
		SF	8	177	220	135

between the levels of IgA₁ and IgA₂ subclasses and total IgA on the one hand and on the other hand, the log functions of reciprocal values of absolute RF titres in the latex fixation test and the haemagglutination test with sheep red cells.

Fig. 1 Regression analysis for mutual relations between pairs of examined samples of serum and synovial fluid in rheumatoid arthritis. Direct linear dependence with significant deviation of the regression line from zero ($P < 0.05$) was found for IgA₁ subclass. $n=9$, $\log y = 0.7099 \log x + 1.1107$, $r=0.8855$, $P=0.0010$.

Fig. 2 Regression analysis for mutual relations between pairs of examined samples of serum and synovial fluid in rheumatoid arthritis. Direct linear dependence with significant deviation of the regression line from zero ($P < 0.05$) was found for IgA₂ subclass. $n=9$, $\log y = 0.6540 \log x + 1.1160$, $r=0.7630$, $P=0.0124$.

Discussion

The examination of IgA₁ and IgA₂ subclasses has so far been carried out in IgA typing monoclonal IgA gammopathies (Overdorfer *et al.*, 1973). However, IgG₁-IgG₄ subclasses were also examined in polyclonal dysimmunoglobulinaemias including rheumatoid arthritis (Morrel *et al.*, 1975; Shakib and

Stanworth, 1976). When examining IgG₁-IgG₄ subclasses in serum and synovial fluid of patients with rheumatoid arthritis, Shakib and Stanworth (1976) found the same qualitative composition in the two media. However, on quantitative analysis the levels of IgG₁-IgG₄ subclasses in synovial fluid of some of the patients were found to be higher than in the corresponding sera. This indicates local formation in rheumatoid synovial fluid in some patients.

Local formation of immunoglobulin in rheumatoid synovial fluid *in vitro* has been demonstrated already in 1968 (Smiley *et al.*, 1968); IgG was found in 79% IgA in only 11%, and the rest was IgM. The amount of locally produced total IgA is so small that it cannot significantly influence the values of quantitative indices used for the study of relationships between the findings in serum and in synovial fluid; this can be demonstrated by most of the results obtained from the examination of total IgA and of IgA₁ and IgA₂ subclasses in the present study (Table 2, Figs. 1 and 2). The finding of a significant disproportionality in the percentage distribution of subclasses IgA₁ and IgA₂ in synovial fluid of patients with rheumatoid synovitis is therefore highly interesting (Table 1); it might indicate preferential formation of IgA₁ subclass in rheumatoid synovial fluid. The relationships between the levels of total IgA and RF observed in other series (Komatsu *et al.*, 1976; Hrnčíř *et al.*, 1977) have not been confirmed in the present study for the subclasses IgA₁ and IgA₂ with respect to RF.

References

- Ball, J. (1950). Serum factor in rheumatoid arthritis agglutinating sensitized sheep red cells. *Lancet*, **2**, 520-524.
- Dreher, R. (1977). Serum-Immunoglobulin-Konstellationen bei der rheumatoiden Arthritis. *Zeitschrift für Rheumatologie*, **36**, 3-21.
- Hrnčíř, Z., Salavec, M., Tichý, M., and Vavrina, J. (1977). Humoral immunity of synovial fluid. *Sborník Vědeckých prací Lékařské fakulty Karlovy University V Hradci Králové*, **20**, 1-102.
- Hrnčíř, Z., Tichý, M., Salavec, M., and Vavrina, J. (1972). Immunoglobulins A, G and M in synovial fluid in rheumatoid arthritis. *Annals of the Rheumatic Diseases*, **31**, 325-329.
- Kellgren, J. H., Jeffrey, M. R., and Ball, J. (Editors.) (1963). *The Epidemiology of Chronic Rheumatism*. Proceedings of the Symposium in Rome, 1961, vol 1, p. 324. Blackwell, Oxford.
- Mancini, G., Carbonara, A. C., and Heremans, J. F. (1965). Immunochemical quantitation of antigens by single radial immunodiffusion. *Immunochemistry*, **2**, 235-240.
- Morell, A., Skvaril, F., Barandum, S. (1975). *IgG-Subklassen der menschlichen Immunoglobuline*, 1st ed., p. 103. Karger, Basel.
- Natvig, J. B., Harboe, M., Fansa, O., and Tveit, A. (1971). Family studies in individuals with absence of gamma-A globulin. *Clinical and experimental Immunology*, **229-23**.

- Oberdorfer, A., Schnauffer Karin, Lange, H. J., and Neiss, A. (1973). Zur Verteilung von Paraproteinämien nach Geschlecht und Alter der Patienten, Paraprotein-Klassen, -Subklassen und-Leichtkettentypen). *Z. Klin. Chem. Klin. Biochem.*, **11**, 51-64.
- Ropes, M. W., Bennett, G. A., Cobb, S., Jacox, R., and Jessor, R. A. (1959). Diagnostic criteria for rheumatoid arthritis. *Annals of the Rheumatic Diseases*, **18**, 49-53.
- Shakib, F., and Stanworth, D. R. (1976). IgG subclass composition of rheumatoid arthritic sera and joint fluids. *Annals of the Rheumatic Diseases*, **35**, 263-266.
- Singer, J. M., and Plotz, C. M. (1956). The latex fixation test. *American Journal of Medicine*, **21**, 888-892.
- Smiley, J. D., Sachs, C., and Ziff, M. (1968). *In vitro* synthesis of immunoglobulins by rheumatoid synovial membrane. *Journal of Clinical Investigation*, **47**, 624-632.
- Solnica, J., Kahn, M. F., Guino, J., Haim, T., and Sèze, S. de (1969). Études immunologiques sur les liquides synoviaux. II. Taux des immunoglobulines IgA, IgG, et IgM chez les sujets normaux et au cours de différentes affections rhumatismales. *Revue du Rhumatisme*, **36**, 191-198.
- Komatsubara, Y., Hiramatsu, S., Hongo, I., Mieda, A., Soda, T., and Botan, Y. (1976). Multivariate analysis of serum proteins in rheumatoid arthritis. *Scandinavia. Journal of Rheumatology*, **5**, 97-102.