Immunoglobulins A, G, and M in synovial fluid in rheumatoid arthritis

Reactive synovitis of local origin and in *post mortem* synovial fluid

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Hitherto immunoglobulins in the synovial media have been detected chiefly in the synovial fluid as phagosomes and in cellular and intercellular components of synovial tissue (Brandt, Cathcart, and Cohen, 1968; Lindström, 1970; Herman, Bradley, Ziff, and Smiley, 1971). Smiley, Sachs, and Ziff (1968) detected intensive synthesis of immunoglobulins in the synovial tissue *in vitro*; in patients with rheumatoid arthritis the findings were closely similar both qualitatively and quantitatively to those encountered in normal human spleen and lymph nodes.

Quantitative examination of the levels of immunoglobulins A, G, and M in the synovial fluid was carried out by Solnica, Kahn, Guino, Haïm, and de Sèze (1969), who examined 52 pathological synovial fluids, and by Veys, de Loore, and Mielants (1971), who investigated 144 such effusions. The study of Solnica and others (1969) has made it possible to compare the data with a control group consisting of four samples of *post mortem* synovial fluid. The object of the present study is to determine the levels of immunoglobulins A, G, and M in a series of pathological synovial fluids in rheumatoid arthritis and in reactive synovitis due to local causes, and to compare them with a series of samples of *post mortem* synovial fluid and with sera examined simultaneously in the patients under study.

Material

The series studied consisted of 160 samples of synovial fluid obtained by puncture-aspiration from the knee:

(1) The control group included 32 samples of *post mortem* synovial fluid obtained 2 to 20 hrs after death. According to clinical and necropsy diagnosis, none of the patients suffered from a disease accompanied by pathological changes in the joints, or from a disease accompanied by hypergammaglobulinaemia. The group consisted of

eighteen males and fourteen females; the mean age was 64 years (range 26 to 85).

(2) 72 samples of synovial fluid were obtained from patients with reactive synovitis: 32 post-traumatic effusions and 40 effusions in cases of osteoarthritis. The group consisted of 41 males and 31 females; the mean age was 52 years (range 19 to 87).

(3) In 56 cases, definite or classic rheumatoid arthritis was diagnosed according to the criteria of the American Rheumatism Association (1959) and their subsequent modification (Kellgren, Jeffrey, and Ball, 1961). There were 24 males and 30 females; the mean age was 54 (range 23 to 77). Rheumatoid factor was detected by the tube latex-fixation test in the titre $1 \geq 160$ and/or the haemagglutination test with sheep erythrocytes in the titre $1 \geq 64$ in forty patients in the serum and in 37 patients in the synovial fluid.

In Groups 2 and 3, the serum was examined simultaneously, and the serum: synovial fluid ratio was determined.

Methods

Samples of synovial fluid and serum were kept at -20°C . until tested. Quantitative determination of immunoglobulins A, G, and M was carried out by the method of simple radial immunodiffusion (Mancini, Carbonara, and Heremans (1965) as modified by Fahey and McKelvey (1965)) using commercial IDP sets (Institute of Sera and Vaccines, Prague) containing monospecific antihuman IgA (SwaHu/IgA), IgG (SwaHu/IgG), and IgM (SwaHu/IgM) swine antisera and the corresponding reference proteins. The standards used were verified at the Reference Centre for Immunoglobulins in Springfield; they are registered in the International Reference Centre for Immunoglobulins in Lausanne.

The data obtained were analysed by means of an ODRA 1204 computer. Both the log. *t*-test and the Wilcoxon rank test were used for their evaluation. The log. *t*-test was employed because the levels of immunoglobulins correspond to the logarithmic-normal gaussian distribution. The coefficient of variation (c.v.) was used for the evaluation of reproducibility. Each sign was examined

eleven times from the same sample of serum obtained by mixing 10 samples of serum from the same number of blood donors. C.v. was calculated according to the formula $\frac{s \times 100}{\bar{x}}$ (s = standard deviation, $\bar{x}$ = arithmetic mean).

The following c.v. values were obtained: 1.37 per cent. for IgG, 2.66 per cent for IgA, and 6.01 per cent. for IgM.

The activity of rheumatoid factor (RF) was tested by the tube latex-fixation test (Singer and Plotz, 1956) and the haemagglutination test with sheep erythrocytes (Ball, 1950). The titre $1 \geq 160$ in the latex-fixation test and the titre $1 \geq 64$ in the haemagglutination test with sheep erythrocytes were considered to be positive findings.

Results

The values obtained by examining the levels of immunoglobulins A, G, and M in the synovial fluid and in the serum of the groups studied are given in Tables I to III. The differences between the mean values of the levels of immunoglobulins of the main classes were highly significant ($P = 0.01-0.001$) in all three groups (Figure). The examination of the indices studied in the synovial fluid in reactive synovitis distributed into the subgroup of synovitis in osteoarthritis and the subgroup of post-traumatic

FIGURE Synovial fluid levels of IgA, IgG, and IgM in three groups of patients (the lines delimiting 95 per cent. range)

Table I Immunoglobulins A, G, and M in control group

Material tested		No. of cases	IgA $\bar{x}$ (95% range) (mg./100 ml.)	IgG $\bar{x}$ (95% range) (mg./100 ml.)	IgM $\bar{x}$ (95% range) (mg./100 ml.)
Post mortem synovial fluid		32	84.36 (61.68-115.38)	262.04 (147.15-466.63)	13.78 (8.79-21.61)

Table II Immunoglobulins A, G, and M in reactive synovitis of local origin

Material tested		No. of cases	IgA $\bar{x}$ (95% range) (mg./100 ml.)	IgG $\bar{x}$ (95% range) (mg./100 ml.)	IgM $\bar{x}$ (95% range) (mg./100 ml.)
Serum		72	459.97 (407.20-519.56)	1,384.74 (1,276.13-1,502.60)	94.68 (75.57-118.61)
Synovial fluid	Osteoarthritis	40	161.00 (128.91-201.08)	462.47 (285.21-749.89)	53.42 (43.45-65.68)
	Post-traumatic Effusions	32	182.12 (155.41-213.45)	687.82 (539.57-786.80)	44.39 (31.41-62.73)
	Total	72	170.74 (148.15-196.76)	557.41 (417.83-743.62)	48.53 (39.48-59.65)

Table III Immunoglobulins A, G, and M in rheumatoid arthritis

Material tested		No. of cases	IgA $\bar{x}$ (95% range) (mg./100 ml.)	IgG $\bar{x}$ (95% range) (mg./100 ml.)	IgM $\bar{x}$ (95% range) (mg./100 ml.)
Serum	RF-seropositive	56	531.56 (464.46-608.36)	1,679.96 (1,578.35-1,788.10)	124.16 (105.12-146.65)
	RF-seronegative	40	486.80 (416.55-568.90)	1,692.57 (1,569.45-1,825.25)	125.12 (102.76-152.33)
	Total	16	581.81 (436.68-776.96)	1,673.52 (1,469.77-1,905.53)	114.85 (93.07-141.74)
Synovial fluid	RF positive	37	274.60 (226.88-332.37)	937.96 (85.444-1,029.52)	77.99 (62.09-97.96)
	RF negative	19	217.51 (145.62-324.90)	746.47 (575.13-968.86)	53.10- (30.41-92.74)
	Total	56	251.21 (209.57-301.12)	886.02 (802.62-978.09)	75.50 (59.24-96.22)

joint effusions did not reveal any significant differences (Table II). The findings were similar when the data in the group of patients with rheumatoid arthritis was divided according to the presence or absence of rheumatoid factor in both synovial fluid and serum (Table III). The increase in immunoglobulins A, G, and M was highly significant ($P = 0.01-0.001$) as compared with the levels in the synovial fluid both in cases of synovitis in rheumatoid arthritis and in cases of reactive synovitis of local origin. The indices presented in Table IV were obtained by calculating the serum: synovial fluid ratio. Significant differences were found between the mean values of the indices for IgA ($P = 0.01-0.001$), and also for IgG ($P = 0.05-0.01$) and IgM ($P = 0.05-0.01$) in the evaluation of the findings in reactive synovitis of local origin and in rheumatoid arthritis. In the rheumatoid arthritis series, there were significant differences ($P = 0.05-0.01$) between the indices for IgA between the seropositive and seronegative cases, and between those with RF positive and RF negative synovial fluid.

Discussion

In the synovial fluid the control group may primarily consist of a series of *post mortem* samples obtained from dead subjects with normal findings in the joints. It is presumed that the *post mortem* findings are similar to finds *in vivo*. No comparison between the levels of immunoglobulins *in vivo* and *post mortem* from the same subjects could be found in the literature, assuming the *post mortem* changes in the synovial fluid to be similar to those in the serum, the data obtained from analyses of sera taken before and after death may be employed. According to Robinson and Kellenberger (1962), the electrophoretic pattern of components which are subject to change through *post mortem* haemolysis is labile. Within the first 24 hrs after death, gamma globulins containing mainly immunoglobulins were found to be most stable. Brazinski and Kellenberger (1970) demonstrated that, in the first 21 hrs after death, the levels of serum

immunoglobulins A, G, and M did not significantly differ from the values obtained *in vivo*. We therefore believe that in the synovial fluids also the levels of immunoglobulins of the main classes are relatively stable and reflect condition before death. The levels of immunoglobulins A, G, and M which were detected in the synovial fluid of the knee in a series of 32 cadavers without pathological changes in the joints may be regarded as the findings in the normal synovial fluid. A potential change due to an increased diffusion on increasing the gradient serum: synovial fluid was eliminated because hypergammaglobulinaemia had not been found in any of the cadavers before death. However, the physiological changes due to the age factor cannot be eliminated. Significant differences were established in the serum immunoglobulins in clinically healthy adult subjects of different ages (Buckley and Dorsey, 1970).

Increased local production and permeability of the synovial tissue together with decreased drainage of the synovial fluid by lymphatic pathways help to account for the significant increase in the levels of immunoglobulins A, G, and M (Fig. 1) in pathologically changed synovial fluid (Hannestad and Melbey, 1967). The importance of local production of IgG is suggested by a change in the kappa: lambda light chains ratio in favour of the lambda light chains found in immunoglobulin G eluted from the synovial tissue of patients with rheumatoid arthritis (Lindström, 1970). Herman and others (1971) found that the synovial tissue of patients with rheumatoid arthritis may produce five to nine times more IgG than the lymphocytes of peripheral blood. Smiley and others (1968) demonstrated *in vitro* that in 79 per cent. it was IgG, in 11 per cent. IgA, and in 10 per cent. IgM (only 10 per cent. of the new-formed IgM showed RF activity). The immune response of the synovial tissue was attributed by Smiley and others partly to its inflammatory reaction. In this connection, the share of local inflammation in the increase in immunoglobulins in the synovial fluid in cases of reactive synovitis due to local causes (*i.e.* in osteoarthritic and post-traumatic effusions) may

Table IV Serum: synovial fluid ratio for immunoglobulins, A, G, and M

Group		No.	IgA	IgG	IgM
			$\bar{x}$ (95% range)	$\bar{x}$ (95% range)	$\bar{x}$ (95% range)
Reactive synovitis of local origin		72	2.89* (2.56-3.23)	2.25† (1.99-2.50)	2.42‡ (2.08-2.44)
Rheumatoid arthritis	RF seropositive	40	2.07§ (1.72-2.41)	1.90 (1.67-2.13)	2.00 (1.47-2.52)
	RF seronegative	16	3.00§ (2.03-3.98)	2.34 (1.62-3.06)	2.24 (1.58-2.91)
	RF positive in synovial fluid	37	1.90¶ (1.56-2.32)	1.92 (1.75-2.10)	1.73 (1.37-2.09)
	RF negative in synovial fluid	17	2.56¶ (1.79-3.66)	2.29 (1.55-3.04)	2.43 (1.29-3.58)
	Total	56	2.34* (1.97-2.71)	2.00† (1.74-2.26)	2.01‡ (1.59-2.44)

* < 0.01, † < 0.05, ‡ < 0.05, § < 0.05, ¶ < 0.05.

also be considered. An immune response to the tissue changed by necrosis may be postulated. Aseptic necrosis in myocardial infarction represents a similar condition: Ebringer, Rosenbaum, Pincus, and Doyle (1971) observed a significant increase in IgG level in the serum 3 weeks after the onset of disease. Jasin and Ziff (1969) have demonstrated in experiments that immunoglobulins and specific antibody-synthesizing cells accumulate in chronic inflammatory foci produced not only by the local injection of antigen, but also by nonspecific inflammatory agents, and that the specificity of such antibody-producing cells reflects the overall immune status.

The reported results do not permit us to determine the share of individual factors in the significant changes in the levels of immunoglobulins A, G, and M in the synovial fluid in reactive synovitis due to local causes and in rheumatoid arthritis. However, they do not conflict with the conclusions of the above-mentioned studies. The absence of significant differences in IgM levels in the synovial fluid in rheumatoid arthritis depending on the presence of rheumatoid factor in the serum or in the synovial fluid is not surprising; the quantitative share of IgM-RF in the total amount of IgM is very small. In this connection, the significant differences (Table IV) between the mean serum : synovial fluid ratios for IgA in RF-positive and RF-negative rheumatoid arthritis represents, in our opinion, a valid observation. Solnica and others (1969) found significant differences between the IgA levels in the synovial fluid in cases

of RF seropositive and seronegative rheumatoid arthritis. In studies of changes in the serum immunoglobulin levels in rheumatoid arthritis, an increase in IgA is a very frequent finding (Barden, Mullinax, and Waller, 1967; Marcolongo, Carcassi, Frullini, Bianco, and Bravi, 1967).

Summary

The study of IgA, IgG, and IgM levels in a series of 160 samples of synovial fluid withdrawn from the knee of the same numbers of subjects showed significant differences in all three between the *post mortem* samples of synovial fluid taken from cadavers without pathological changes in the joints, and synovial fluid from joints showing inflammation of local origin (*i.e.* in cases of osteoarthritis and post-traumatic effusions) and from patients with rheumatoid arthritis. No significant differences were found between fluids from cases of RF positive and RF negative rheumatoid arthritis. In all three immunoglobulins, the serum levels were significantly higher than the synovial fluid levels both in cases of reactive synovitis of local origin and in cases of rheumatoid arthritis. In both these groups, however, there were significant differences between the mean serum : synovial fluid ratios. In the patients with rheumatoid arthritis, the same difference was observed for IgA between those who were RF positive and those who were RF negative.

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