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Synovial fluid in ankylosing spondylitis

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Ankylosing spondylitis is an inflammatory arthropathy which affects predominantly the sacroiliac joints and the spine. Other joints may be involved in up to 25 per cent or more cases (Kinsella, MacDonald, and Johnson, 1966), but in most of these it is the hips or shoulders which are affected. Spread of the disease to involve the knees and the more peripheral joints occurs much less frequently; therefore data on the synovial fluid are difficult to obtain.

The composition of the synovial fluid is of some interest since little is known about the aetiology of ankylosing spondylitis. Early histological changes may resemble those of rheumatoid disease (Cruickshank, 1951), but the clinical picture, age and sex of patient, prognosis, treatment, and marked tendency to bony ankylosis are all different in ankylosing spondylitis. Further, in rheumatoid disease, the presence of raised immunoglobulins and autoantibodies in the blood (Torrigiani and Roitt, 1967; Panush, Bianco, and Schur, 1971; Claman and Merrill, 1966), their presence in synovial membrane (Fish, Michael, Gewurz, and Good, 1966), the association with other autoimmune disorders, and the response to steroids all suggest that immune mechanisms play an important part in the pathogenesis. These are not features of ankylosing spondylitis, nor are the presence of nodules, lymphadenopathy, or splenomegaly. The stimulus provoking the immune response in rheumatoid disease is not yet known and there is a definite possibility that it is some kind of infective agent (Walton, 1968). The cause of ankylosing spondylitis is also unknown, though some cases are associated with urogenital infections or inflammatory bowel diseases (Mason, 1964).

Synovial fluid in rheumatoid disease contains high levels of IgG and IgM (Panush and others, 1971) and an increased cell count, mostly polymorphs, some of which have phagocytosed complexes of rheumatoid factor and IgG (Parker and Schmid, 1962; Williamson and Ling, 1965; Rawson, Abelson, and Hollander, 1965). Rheumatoid synovial fluid also contains high levels of 5-nucleotidase (Farr, Kendall, Shuttleworth, Meynell, and Hawkins, 1973) and lysosomal enzymes (Caygill and Pitkeathly, 1966) both of which reflect the disease activity in the joint. These changes in rheumatoid synovial fluid suggest an active inflammatory response with an immunological component.

We have examined the synovial fluid from a small number of patients with ankylosing spondylitis. We looked particularly at differential cell counts, 5-NT levels, and immunoglobulins in order to compare them with rheumatoid disease in terms of inflammatory activity, and to look for evidence for or against an immunological basis.

Material and methods

PATIENTS

Twelve specimens of synovial fluid were obtained from six patients with ankylosing spondylitis whose mean age was 34 years (Table I). These patients were clinically typical in all respects apart from peripheral joint involvement. The diagnosis was confirmed by classical appearances of bony ankylosis on spinal *x*-rays. None had nodules and all were Waaler–Rose negative. Only one patient showed erosive changes in the *x*-rays of peripheral joints.

Thirty specimens of synovial fluid were obtained from 26 patients with osteoarthrosis, of whom eighteen were female, and the mean age was 41 yrs (range 50 to 84). All had typical clinical features and definite *x*-ray changes.

48 specimens of synovial fluid were obtained from an unselected group of 37 rheumatoid patients of whom eighteen were female and the mean age was 49·5 yrs (range 26 to 70). According to the ARA classification, thirteen were classical, 21 definite, and three probable.

METHODS

We assessed all patients clinically, haematologically, biochemically, and performed serum Waaler–Rose, latex slide test, and immunoglobulin estimations.

We examined the synovial fluid, and determined total and differential white cell counts (Jenner-Giemsa stain), red cell counts, and estimation of IgG, IgM, IgA, and IgD levels (Mancini, Carbonara, and Heremans, 1965) and the Waaler-Rose titre (Greenbury, 1957). The 5-nucleotidase was measured by the method of Persijn, van der Slik, Kramer, and de Ruijter (1968) and modified by the inclusion of a blank to measure residual adenosine in adenosine monophosphate. In addition we estimated acid phosphatase (Gutman and Gutman, 1940) and glucose by autoanalysis, using neocuproin.

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Results

SYNOVIAL FLUID

Cytology

The results of the cell counts in the synovial fluid obtained from patients with the three disorders are given in Table II. This shows that compared with the unselected rheumatoid group the polymorphs were lower in the ankylosing spondylitis group, but the total and percentage lymphocytes were very much higher. The significances of the differences between ankylosing spondylitis and osteoarthrosis, ankylosing spondylitis and rheumatoid disease are also given.

Immunoglobulins and biochemistry

The immunoglobulins, 5-nucleotidase, acid phosphatase, and glucose results are given in Table III with the significance and *P* values as in Table II. This shows that the IgG and IgM values in synovial fluid of patients with ankylosing spondylitis are markedly raised, whilst the 5-nucleotidase and acid phosphatase are intermediate between the rheumatoid and osteoarthrosis groups.

The corresponding serum values for the immunoglobulins and 5-nucleotidase are given in Table IV. Serum IgG was significantly raised in the ankylosing spondylitis group compared with the rheumatoid group.

In order to compare patients with a similar degree of inflammatory activity, synovial IgG, IgM, IgA, and differential cell counts were estimated in a group of rheumatoid patients whose synovial 5-NT levels were matched with those of the twelve ankylosing spondylitic fluids. The results are shown in Table V. Although the mean lymphocyte count was higher in this selected group of rheumatoid patients as compared with the overall rheumatoid group they were still much lower than in ankylosing spondylitis. Furthermore, IgA was highly significantly lower in the selected group compared with ankylosing spondylitis.

Discussion

The most striking finding was the high concentration of all immunoglobulins and the large number of lymphocytes in the peripheral joint fluid of patients with ankylosing spondylitis. The high IgG, the IgM levels, and the lymphocyte counts were highly significant even when compared with those of the rheumatoid group, and the IgG was raised in the

Table I *Ankylosing spondylitis patients*

Case no.	I	II	III	IV	V	VI
Age (yrs)	25	35	35	31	54	25
Sex	M	M	F	F	F	M
Medications	Phenylbutazone Steroids	Indomethacin	Nil	Indomethacin Panadol	Phenylbutazone	Phenylbutazone Steroids
Radiotherapy	Nil	Yes	Nil	Nil	Nil	Nil
Iritis	Yes	Yes	Nil	Nil	Nil	Yes
Duration of ankylosing spondylitis (yrs)	10	15	17	6	10	10
Duration of peripheral joint symptoms (yrs)	11	14	16	8	11	11
Haemoglobin (g./100 ml.)	11·6	12·2	13·1	12·9	13·0	11·8
ESR (mm./1 hr Westergren)	20	96	35	75	21	22
Fibrinogen Total clottable (mg./100 ml.) Normal range 190–400	—	—	600	600	510	505
C-reactive protein (μg./ml.)	80	—	125	—	23	115
Albumin (g./100 ml.)	3·4	3·8	4·1	3·9	3·3	3·6
Globulin (g./100 ml.)	3·8	4·9	3·6	4·6	4·3	4·0
Total protein (g./100 ml.)	7·2	6·1	7·7	8·5	8·1	7·5

Table II *Synovial fluid white cell counts*

Diagnosis	*Osteoarthrosis Mean (SD) No. of cases*		*Ankylosing spondylitis Mean (SD) No. of cases*		*Rheumatoid arthritis Mean (SD) No. of cases*
Total white cell count (per cu. mm.)	300 (185) 27	HS $P < 0.01$	7137·5 (4339) 12	NS	9829 (7401) 40
Absolute polymorph count (per cu. mm.)	45·25 (31·26) 12	HS $P < 0.001$	3516·9 (3141) 12	HS $P < 0.005$	7490 (2000) 40
Percentage polymorphs	14·66 (15·87) 12	NS	46·0 (28·4) 12	NS	68·75 (23·26) 40
Absolute lymphocyte count (per cu. mm.)	198·5 (151·4) 11	HS $P < 0.001$	2054·3 (1276) 12	HS $P < 0.001$	866·3 (678·2) 36
Percentage lymphocytes	41·3 (16·1) 13	NS	37·6 (25·2) 12	HS $P < 0.001$	16·45 (13·0) 40
Absolute monocyte count (per cu. mm.)	132·7 (67·2) 11	HS $P < 0.001$	900·4 (480·3) 12	NS	824·4 (734·8) 34
Percentage monocytes	34·0 (12·8) 11	HS $P < 0.001$	14·0 (4·2) 12	NS	12·03 (10·6) 34
Absolute lymphocyte + monocyte counts (per cu. mm.)	331·3 (207·7) 11	HS $P < 0.001$	2955 (1425) 12	HS $P < 0.025$	1682 (1220) 36
Percentage lymphocytes + monocytes	79·5 (19·5) 11	HS $P < 0.01$	51·6 (27·5) 12	HS $P < 0.001$	26·1 (13·9) 34

serum. The possibility that contamination with blood contributed to these high levels was excluded by demonstrating low red cell counts on each specimen of fluid.

Immunoglobulin levels are known to be raised in rheumatoid disease (Torrigiani and Roitt, 1967; Vaughan, Barnett, Sobel, and Jacox, 1968; Veys and Claessens 1968). Panush and others (1971) showed that the IgG and IgM values were higher in the synovial fluid than those found in osteoarthrosis; the IgM was only raised in seropositive patients and in these the IgG was significantly higher. Less is known about the immunoglobulin status of patients with ankylosing spondylitis. Kriegel, Burger, Kapp, and Alexopulos (1969) studied the serum and found that the IgA was raised whilst the others were normal. Comparable studies on synovial fluids have not been performed though Wilkinson and Jones (1964) demonstrated higher α_2 and γ globulins compared with rheumatoid disease.

The second feature of the synovial fluid was the markedly increased number of lymphocytes and monocytes. Sigler, Bluhm, Duncan, and Ensign (1971) found a lower total white cell count with a preponderance of mononuclear cells in the fluid of peripheral joints when ankylosing spondylitis was compared with rheumatoid disease. In the latter, a high synovial lymphocyte count is found in the milder more chronic form of the disease (Ropes and Bauer, 1953), and we have found that the lymphocyte count is inversely proportional to the activity of the joint disease (Farr, Kendall, Shuttleworth, Meynell, and Hawkins, 1973). By comparison the total white cell count and polymorph count reflect activity.

In studies on the synovial membrane, 'lymphoid' follicles have been described in rheumatoid disease (Collins, 1949), and these are said to be a feature of autoimmune disease (Mackay and Burnet, 1963). Julkunen (1966) found synovial follicles in both ankylosing spondylitis and in rheumatoid disease.

Muirden and Mills (1971) noted that the small lymphocyte is the principal infiltrating cell in the synovial membrane and this cell is important in cell-mediated immunity. They also found that a heavy infiltrate of immunocytes tends to protect the cartilage and bone from damage. The observations of Turk (1970) on leprosy may be relevant. He showed that there was a deficiency of lymphocytes in the severe, progressive form of leprosy and a pronounced infiltration of lymphocytes in the lesions of the mild

Table III *Synovial fluid immunoglobulins and other results*

Diagnosis	*Osteoarthrosis* (*SD*) *No. of cases*		*Ankylosing spondylitis* (*SD*) *No. of cases*		*Rheumatoid arthritis* (*SD*) *No. of cases*
Immunoglobulins					
IgG (mg./100 ml.)	499·3 (±237) 21	HS P < 0·001	1995·2 (±1258) 10	HS P < 0·005	1127·62 (±415·7) 24
IgM (mg./100 ml.)	23 (±19) 21	HS P < 0·001	63·9 (±21·74) 10	HS P < 0·005	45·33 (±29·52) 24
IgA (mg./100 ml.)	104 (±71·5) 21	HS P < 0·001	342·6 (±255) 10	NS	336·78 (±202·6) 24
Others					
5-NT (i.u./l.)	11·97 (±6·0) 30	HS P < 0·01	17·83 (±7·7) 12	HS P < 0·005	35·28 (±21·01) 40
Acid phosphatase (whole) (u./100 ml.)	2·16 (±0·99) 18	HS P < 0·001	4·71 (±1·8) 8	NS	6·158 (±3·14 24
(supernatant)	1·38 4	NS	3·05 (±0·53) 8	NS	3·44 (±1·33) 26
Glucose (mg./100 ml.)	94·13 (±12·96) 16	NS	90·6 (±9·7) 8	NS	83·48 (±23·53) 27

Table IV *Serum immunoglobulin and 5-NT results*

Diagnosis	*Osteoarthrosis* (*SD*) *No. of cases*		*Ankylosing spondylitis* (*SD*) *No. of cases*		*Rheumatoid arthritis* (*SD*) *No. of cases*
IgG (mg./100 ml.)	1418 (±549) 16	HS P < 0·01	2453 (±1140) 6	S P < 0·025	1654 (±505·4) 20
IgM (mg./100 ml.)	95 (±52·8) 16	NS	121·8 (±30·85) 6	NS	93·9 (±39·5) 20
IgA (mg./100 ml.)	388·9 (±230) 16	NS	418 (±370·5) 6	NS	467·9 (±251·2) 20
IgD (mg./100 ml.)	5·75 (±7·7) 16		Nil		Nil
5-NT (i.u./l.)	8·53 (±2·45) 17	NS	10·67 (±5·27) 6	NS	11·39 (±5·29) 23

Table V *Synovial fluid values in ankylosing spondylitis compared with selected rheumatoid group (see text)*

Group	*Selected rheumatoid Mean (SD)*	*Ankylosing spondylitis Mean (SD)*
Percentage polymorphs	51·2 (32·4)	46·0 (28·4)
Absolute polymorph count (per cu. mm.)	4463·9 (4500)	3516·9 (3141)
Percentage lymphocytes	27·9 (24·4)	37·6 (25·2)
Absolute lymphocyte count (per cu. mm.)	938·6 (395·4)	2054·3 (1276)
Percentage monocytes	19·3 (10·9)	14·0 (4·2)
Absolute monocyte count (per cu. mm.)	935·0 (721·4)	900·4 (480·3)
Percentage lymphocytes + percentage monocytes	47·3 (30·6)	51·6 (27·5)
Absolute lymphocyte + monocyte counts (per cu. mm.)	1873·6 (893·2)	2955 (1425)
IgG (mg./100 ml.)	956·7 (325·2)	1995·2 (1258)
IgM (mg./100 ml.)	35·7 (23·1)	63·9 (21·74)
IgA (mg./100 ml.)	178·1 (146·2)	342·6 (255)

form. It is thought that cell-mediated immunity is necessary to eliminate the mycobacteria.

Our other findings relate to disease activity in the affected joints. In each instance in ankylosing spondylitis, total white cell count, polymorph count, synovial 5-NT, acid phosphatase, and glucose were intermediate between osteoarthrosis and rheumatoid disease.

Our observations suggest that, in ankylosing spondylitis, there is a modest inflammatory reaction in which immune mechanisms are playing a part and in which a vigorous lymphocyte response is exerting a protecting influence. These conclusions can only be tentative since we have only been able to study a small number of cases. Further work must be done to find out if those with peripheral joint disease form a distinct sub-group in which lymphocyte function and the reaction of the synovial membrane should be studied. The antigen or infective agent responsible remains to be discovered.

Summary

We have studied twelve specimens of synovial fluid obtained from the knees of six patients with ankylosing spondylitis. The results have been compared with those from a group with rheumatoid disease and a second group with osteoarthrosis. In ankylosing spondylitis, the lymphocyte count and immunoglobulin levels were significantly higher than in the other two groups, suggesting an immunological basis for this disease. The parameters of disease activity in the fluid, namely, synovial polymorph counts, 5-nucleotidase, and acid phosphatase in the spondylitis group, were intermediate between those of the rheumatoid and osteoarthrosis groups.

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References

CAYGILL, J. C., AND PITKEATHLY, D. A. (1966) *Ann. rheum. Dis.*, **25**, 137 (A study of β-acetylglucosaminase and acid phosphatase in pathological joint fluids)

CLAMAN, H. N., AND MERRILL, D. (1966) *J. Lab. clin. Med.*, **67**, 850 (Serum immunoglobulins in rheumatoid arthritis)

COLLINS, D. H. (1949) 'The Pathology of Articular and Spinal Diseases', pp. 175–183. Arnold, London

CRUICKSHANK, B. (1951) *Ann. rheum. Dis.*, **10**, 393 (Histopathology of diarthrodial joints in ankylosing spondylitis)

FARR, M., KENDALL, M. J., SHUTTLEWORTH, R., MEYNELL, M. J., AND HAWKINS, C. F. (1973) *Ibid.*, **32**, 326 (Source and significance of 5-nucleotidase in synovial fluid).

FISH, A. J., MICHAEL, A. F., GEWURZ, H., AND GOOD, R. A. (1966) *Arthr. and Rheum.*, **9**, 267 (Immunopathologic changes in rheumatoid arthritis synovium)

GREENBURY, C. L. (1957) Broadsheet No. 18, Association of Clinical Pathologists (The Rose-Waaler Test)

GUTMAN, E. B., AND GUTMAN, A. B. (1940) *J. Biol. Chem.*, **136**, 201 (Estimation of 'acid' phosphatase activity of blood serum)

JULKUNEN, H. (1966) *Acta rheum. scand.*, **12**, 188 (Synovial inflammatory cell reaction in chronic arthritis)

KINSELLA, T. D., MACDONALD, F. R., AND JOHNSON, L. G. (1966) *Canad. med. Ass. J.*, **95**, 1 (Ankylosing spondylitis: A late re-evaluation of 92 cases)
KRIEGEL, VON W., BURGER, R., KAPP, W., AND ALEXOPULOS, J. (1969) *Verh. dtsch. Ges. Rheumatol.*, **1**, 206 (Die Immunoglobuline bei ankylosierender Spondylitis)
MACKAY, I. R., AND BURNET, M. F. (1963) 'Autoimmune Diseases', pp. 148–149. Thomas, Springfield, Ill.
MANCINI, G., CARBONARA, A. O., AND HEREMANS, J. F. (1965) *Immunochemistry*, **2**, 235 (Immunochemical quantitation of antigens by single radial immunodiffusion)
MASON, R. M. (1964) *Proc. roy. Soc. Med.*, **57**, 533 (Spondylitis)
MUIRDEN, K. D., AND MILLS, K. W. (1971) *Brit. med. J.*, **4**, 219 (Do lymphocytes protect the rheumatoid joint?)
PANUSH, R. S., BIANCO, N. E , AND SCHUR, P. H. (1971) *Arthr. and Rheum.*, **14**, 737 (Serum and synovial fluid IgG, IgA and IgM antigammaglobulins in rheumatoid arthritis)
PARKER, R. L., AND SCHMID, F. R. (1962) *J. Immunol.*, **88**, 519 (Phagocytosis of particulate complexes of γ-globulin and rheumatoid factor)
PERSIJN, J. P., VAN DER SLIK, W., KRAMER, K., AND DE RUIJTER, A. (1968) *Z. klin. Chem. klin. Biochem.*, **6**, 441 (A new method for the determination of serum nucleotidase)
RAWSON, A. J., ABELSON, N. M., AND HOLLANDER, J. L. (1965) *Ann. intern. Med.*, **62**, 281 (Studies on the pathogenesis of rheumatoid joint inflammation. II. Intracytoplasmic particulate complexes in rheumatoid synovial fluids)
ROPES, M. W., AND BAUER, W. (1953) 'Synovial Fluid Changes in Joint Disease', p. 36. Harvard University Press, Cambridge, Mass.
SIGLER, J. W., BLUHM, G. B., DUNCAN, H., AND ENSIGN, D. C. (1971) *Clin. orthop.*, No. 74, p. 14 (Clinical features of ankylosing spondylitis)
TORRIGIANI, G., AND ROITT, I. M. (1967) *Ann. rheum. Dis.*, **26**, 334 (Antiglobulin factors in the sera from patients with rheumatoid arthritis and normal subjects)
TURK, J. L. (1970) *Brit. med. J.*, **3**, 363 (Contribution of modern immunological concepts to an understanding of diseases of the skin)
VAUGHAN, J. H., BARNETT, E. V., SOBEL, M. V., AND JACOX, R. F. (1968) *Arthr. and Rheum.*, **11**, 125 (Intracytoplasmic inclusions of immunoglobulins in rheumatoid arthritis and other diseases)
VEYS, E. M., AND CLAESSENS, H. E. (1968) *Ann. rheum. Dis.*, **27**, 431 (Serum levels of IgG, IgM and IgA in rheumatoid arthritis)
WALTON, K. W. (1968) *Int. Rev. exp. Path.*, **6**, 285. (Hypersensitivity and infection in the pathogenesis of the rheumatic diseases)
WILKINSON, M., AND JONES, B. S. (1964) *Ann. rheum. Dis.*, **23**, 22 (Electrophoretic studies of synovial fluid proteins)
WILLIAMSON, N., AND LING, N. R. (1965) *Ibid.*, **24**, 513 (Cellular reaction to complexes formed between rheumatoid factor and aggregated human gamma globulin)