

Metabolic alterations in human synovial lining cells in pigmented villonodular synovitis

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SUMMARY Synovial tissue from 3 patients with villonodular synovitis has been examined by quantitative cytochemistry. Considerable changes in the metabolism of the synoviocytes have been observed. These included a massive rise in the activity of glucose 6-phosphate dehydrogenase to levels even greater than those found in rheumatoid arthritis, and a significant rise in the activity of 2 glycolytic enzymes, glyceraldehyde 3-phosphate and lactate dehydrogenase. Lysosomal naphthylamidase showed raised activity with no latency. The sulphhydryl content in the synoviocytes was raised, as was the amount of available phospholipid. This pattern of metabolic alterations is similar to that found in human rheumatoid synoviocytes.

Even in a tissue composed of many cell types, such as rheumatoid synovial tissue, it is possible to measure the biochemical activity of each cell type specifically by means of quantitative cytochemistry. By such procedures apparently characteristic alterations in the metabolism of rheumatoid synoviocytes have been recorded (Chayen *et al.*, 1971; Butcher *et al.*, 1973; Henderson *et al.*, 1978a, b, 1979). During these studies on the metabolic activity of the synovial lining cells biopsies were obtained from 3 patients with unequivocal villonodular synovitis. Since this is a relatively rare condition (Jaffe *et al.*, 1941; Byers *et al.*, 1968), the aetiology of which is not established, some aspects of the metabolic activity of the synovial lining cells in these specimens have been investigated.

Clinical details

Case 1 (specimen 1978). Male, aged 61, with 3-year history of intermittent swelling and pain in the right knee. Pigmented villonodular synovitis, diagnosed by arthroscopy, confirmed at operation.

Case 2 (specimen 1979). Female, aged 66, with 2-year history of recurrent pain and swelling of the right knee. Repeated aspirations yielded grossly blood-stained fluid. At arthroscopy pigmented

villonodular synovitis was diagnosed, and this was confirmed histologically on biopsy. An attempt was made to treat the condition by the instillation of 5 mC of ⁹⁰yttrium, but after some amelioration of symptoms for 6 weeks there was a recurrence of swelling and pain of the right knee.

Case 3 (specimen 2055). Female, aged 31. Partial synovectomy of the left knee in 1966, after which the swelling recurred. Synovectomy in 1977, at which the specimen was taken. Through a medial parapatellar incision blood-stained fluid was found. There was gross involvement of the whole of the synovial membrane with pigmented villonodular proliferation. Both femoral and tibial joint surfaces were discoloured and eroded, as was the patella. Both menisci were degenerate.

Materials and methods

Pieces of synovial tissue, approximately 4 mm³, were chilled by precipitate immersion in n-hexane (BDH 'free from aromatic hydrocarbons' grade, boiling range 67–70°C). After not longer than 1 minute the specimens were removed from the hexane and stored at –70°C in corked dry glass tubes. Specimens were sectioned at 10 μm in a Bright's cryostat maintained at a cabinet temperature of –25 to –30°C with the knife cooled to –70°C by having solid carbon dioxide packed around its haft. The sections were flash-dried from the knife on to slides taken from the ambient

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temperature of the laboratory (Chayen *et al.*, 1973). To examine the histology some sections were stained with toluidine blue and others with haematoxylin and eosin.

DETECTION OF STRUCTURAL REACTIVE GROUPS

Perls's Prussian blue reaction: Sections were treated with a 1% solution of potassium ferrocyanide (Pearse, 1968); ferric ions (Fe^{3+}) in the tissue react with the ferrocyanide to produce the intensely coloured, insoluble ferric ferrocyanide (Prussian blue).

Chèvremont-Frederic reaction: The principle of this method is that sulphhydryl groups are such strong reducing agents that they can reduce ferricyanide $[\text{Fe}(\text{CN})_6]^{3-}$ to ferrocyanide $[\text{Fe}(\text{CN})_6]^{4-}$. The latter reacts with ferric ions to produce Prussian blue (as described above). This method gives a measure of the sulphur-containing amino acids which are in the reduced (-SH) state. When serial sections are treated with dithionite (a strong reducing agent) all the oxidised -SH groups, that is, the disulphide bonds (-S-S-), are reduced to the -SH state and so become reactive with ferricyanide. Thus the total amount of sulphur-containing amino acids in the section can be quantified (Chayen *et al.*, 1973).

Acid haematein reaction: This method is used for the demonstration of unsaturated phospholipids (Chayen, 1968). The method was that described by Chayen *et al.* (1973).

ENZYME ASSAYS

The activity of dehydrogenases was measured by the reduction of purified neotetrazolium chloride to yield the insoluble red formazan when the enzyme, in the cells, oxidised its specific substrate in the presence of optimal concentrations of the preferred coenzyme (NADP^+ or NAD^+) and the intermediate hydrogen-acceptor phenazine methosulphate. The sections were stabilised by the addition of 20% polyvinyl alcohol (BO5/140, from Wacker Chemicals Ltd.). Optimal concentrations of reactants for all these procedures, applied to synovial tissue, have been given in the relevant reference. Glucose 6-phosphate dehydrogenase, the first and rate-limiting step of the hexose monophosphate shunt, was assayed by the method of Butcher *et al.* (1973), lactate dehydrogenase (EC 1.1.1.27) by that of Henderson *et al.* (1978b), and glyceraldehyde 3-phosphate dehydrogenase (EC 1.1.1.12) by the procedure of Henderson (1976). The activity of the last two enzymes gives a measure of glycolysis.

Lysosomal naphthylamidase activity and its latency, which is a measure of the state of the lysosomal membranes, were assayed as described by

Bitensky and Chayen (1977). Results are expressed in terms of percentage bound activity, which is calculated from the expression: $(\text{total activity} - \text{free activity}) \times 100 / \text{total activity}$. The free activity is that found on incubation of sections for a given period in the substrate medium. Total activity is that found when the permeability barrier (to the entry of substrate) of the lysosomal membrane is removed. This is achieved by preincubating sections in an acidic buffer, as described by Bitensky and Chayen (1977).

MEASUREMENT

For each reaction 15–20 fields, each containing 2–3 synovial lining cells, were measured in each of duplicate or triplicate sections by means of a Vickers M85 scanning and integrating microdensitometer ($\times 40$ objective; scanning spot diameter $0.5 \mu\text{m}$ for measuring the deposits of formazan; $\times 100$ oil immersion objective; scanning spot of diameter $0.25 \mu\text{m}$ for measuring the reaction product of naphthylamidase). The integrated relative absorption per unit field, recorded by the microdensitometer, was converted to units of absolute integrated extinction per unit field by suitable calibration.

Results

HISTOLOGY

Patient 1: The synovial lining showed considerable hyperplasia with nodules composed of compressed synovial villi. In the underlying loose stroma there were collections of chronic inflammatory cells, which in places had formed into lymphoid follicles. Haemosiderin pigment, which gave a positive Perls reaction, was obvious both in the lining cells and in pigment-filled macrophages.

Patient 2: The specimen showed fairly dense cellular connective tissue interspersed by cleft-like vascular channels with a hyperplastic synovial lining covering the surface. Haemosiderin pigment was abundant. Chronic inflammatory cells were not evident.

Patient 3: There was marked heterogeneity in the histological appearance of this specimen. In places there was marked nodular villous hyperplasia, while in others a more solid connective-tissue-vascular mass was present. A striking feature was giant-cell transformation of the lining cells both in the lining layer and deep to it. In both the villous and more solid areas the sublining cell tissue contained dense infiltrates of foamy macrophages which stained with the fat-dye, oil red O. Haemosiderin pigment was abundant in the lining cells, giant cells, and macrophages. There was no evidence of chronic inflammatory cell infiltrate.

BIOCHEMICAL ACTIVITIES

Glucose 6-phosphate dehydrogenase activity was remarkably high (Table 1), being equal to, or far exceeding, the greatest activity found in rheumatoid synoviocytes.

Table 1 Glucose 6-phosphate dehydrogenase activity in synovial lining cells

Tissue	Enzyme activity (Integrated extinction $\times 10^3/10$ min)
Villonodular synovitis:	
Specimen No. 1978	1338
1979	1295
2055	606
Nonrheumatoid (mean \pm SD)	119 \pm 93 (n = 14)*
	106 \pm 22 (n = 8)†
Rheumatoid (mean \pm SD)	496 \pm 209 (n = 18)*
	432 \pm 12 (n = 7)†

*Results of Henderson (1977). †Results of Butcher *et al.* (1973).

The total lysosomal naphthylamidase activity, measured after removal of the influence of the lysosomal membrane, was also markedly raised in the synoviocytes in these specimens (Table 2). Almost all of this activity was manifest activity: specimens 1979 and 2055 had no measurable latent activity, and the lysosomes in the synoviocytes in specimen 1978 showed 5% latent activity.

The activities of two glycolytic enzymes, glyceraldehyde 3-phosphate and lactate dehydrogenase, were markedly raised over those found in relatively normal synovial lining cells (Table 3).

In the 1 specimen examined (No. 1979) all the sulphur-containing amino acids were in the reduced (-SH) state in that reduction with dithionite did not increase the reaction for sulphhydryl groups. The acid haematein reaction for freely available phospholipids gave fairly strong responses in all three specimens.

Table 2 Total naphthylamidase activity in synovial lining cells

Tissue	Enzyme activity (Int. ext. \times 1000/15 m)	Rel. abs./unit field/30 m†
Villonodular synovitis		
Specimen No: 1978	533	
1979	698	
2055	1040	
Non-rheumatoid (mean \pm SD)	232 \pm 10 (n = 4)*	140 \pm 63 (n = 9)
Non-rheumatoid after recent trauma (mean \pm SD)		312 \pm 93 (n = 8)
Rheumatoid (mean \pm SD)	358 \pm 75 (n = 4)*	223 \pm 93 (n = 18)

*Results of Henderson (1977). †Results of Chayen *et al.* (1971). Although it is not possible to relate these values of 'relative absorption' to 'integrated extinction', the former values are given as an indication of the degree to which recent trauma and the rheumatoid state affect this activity.

Table 3 The activities of lactate dehydrogenase (LDH) and glyceraldehyde 3-phosphate dehydrogenase (GAPD)

Tissue	Enzyme activity (int. ext. \times 1000/10 m)	
	GAPD	LDH
Villonodular synovitis		
Specimen No. 1978	54	—
1979	—	464
2055	333	686
†Nonrheumatoid	9 \pm 8 (n = 6)*	156 \pm 77 (n = 6)*
†Rheumatoid	110 \pm 84 (n = 7)*	654 \pm 328 (n = 14)*

†Results expressed as mean \pm SD. *Results of Henderson *et al.* (1978c). — means not measured.

Discussion

Presumably because this disease is uncommon, no biochemical studies have been reported on the metabolism of synoviocytes in pigmented villonodular synovitis. The results in 3 cases investigated in the present study indicate that major metabolic alterations occur in these cells in this condition. Glucose 6-phosphate dehydrogenase activity was considerably raised (Table 1), even above the values found in synoviocytes of rheumatoid synovial tissue (Butcher *et al.*, 1973; Henderson, 1977). Lysosomal latency (bound naphthylamidase activity) was virtually nonexistent, very much as was found by Chayen and Bitensky (1971) with synoviocytes of rheumatoid synovial tissue (bound activity of 1%, range: 0–5%, n = 13 as against 44% for non-rheumatoid synoviocytes, range: 31–57%, n = 8). Total lysosomal naphthylamidase activity was markedly increased even over that found in synovial cells lining rheumatoid synovial tissue (Table 2). This increased content of a lysosomal enzyme is in accord with these cells engulfing nondigestible matter (Dingle, 1975). The activities of both glycolytic enzymes studied were of the levels found in rheumatoid synoviocytes, which Henderson *et al.* (1978b) showed to be higher than in synovial lining cells from joints free from rheumatic disease. Similarly the -SH:-S-S ratio (almost 100% sulphhydryl) and the availability of cellular phospholipids (the acid haematein reaction) were very much as have been found in rheumatoid but not non-rheumatoid synovial lining cells (Butcher *et al.*, 1973; Henderson *et al.*, 1978a). Thus the metabolic behaviour of the synovial lining cells in these 3 specimens of villonodular synovitis was similar to, or exaggerated above, that found previously as typical of the synoviocytes of rheumatoid tissue.

It has been argued, for example, by Chayen and Bitensky (1971), that the biochemical changes in the synoviocytes, previously considered characteristic for rheumatoid tissue, were related to the erosion of cartilage and joint destruction. There is some

evidence (McMaster, 1960; Schajowicz and Blumenfeld, 1968) that villonodular synovitis, if left untreated for a sufficient time, can also produce such changes. Certainly in case 3 (No. 2055) both femoral and tibial joint surfaces as well as the surface of the patella were found at operation to be discoloured and eroded, and both menisci were degenerate. Moreover, there is a degree of similarity between villonodular synovitis and haemophilic arthropathy, in which the destruction of the joint is common (Crevelde *et al.*, 1971; Mainardi *et al.*, 1978). Consequently the biochemical findings in these cases of villonodular synovitis are not inconsistent with the view that such changes may be associated with the degradation of joints.

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