a higher proportion of the aldimine crosslinks than the centre of the plaque, and, furthermore, the proportion was considerably reduced in inactive scleroderma patients. The presence of these aldimine crosslinks establishes that new collagen is being laid down in the skin of scleroderma patients.

Cleavage of the crosslinks stabilizing the collagen fibre may relieve the symptoms by softening the skin. D-penicillamine is capable of cleaving the aldimine crosslinks of newly formed collagen but not the stable crosslinks of mature collagen. Clearly, D-penicillamine will only be effective on active scleroderma, and previous conflicting evidence on the value of this drug may be partly due to a failure to differentiate its effects on mature and immature collagen. In addition, *in vitro* tissue culture studies on scleroderma skin have shown a partial inhibition of collagen synthesis in the presence of D-penicillamine (Herbert, Jayson, Bailey, and Lindberg, 1974).

Two patients with active scleroderma were treated with D-penicillamine (1.5 g/day) and followed with sequential biopsies over 9 months. In both cases a significant decrease in the proportion of aldimine cross links was observed, together with a definite improvement in the clinical manifestations of the disease.

References

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Discussion on both papers

DR. W. C. DICK (*Glasgow*) May I congratulate this team on a valuable piece of work in a field which badly needs this kind of study. I am interested in the mechanism which turns a person from being normal to producing a more soluble collagen which has more crosslinks. Do you think this could be a depressor mechanism?

DR. HERBERT This is, of course, the most important question to answer. At present we are only showing the changes that have taken place, and not the initiating mechanism.

DR. ALNOFF (*Brussels*) You have chosen scleroderma as a model. Would you think that in situations, like in keloids, you would have something of the same sort?

DR. HERBERT I think it would be very well worth looking at.

DR. R. N. MAINI (*London*) One of the problems in dealing with patients with scleroderma is the clinical assessment of disease activity. Do you think your investigations might provide a basis of such an assessment?

DR. HERBERT Two patients were referred to us with exactly this problem and we were able to show the presence of new collagen, thus establishing that both patients had active disease. Whether this is always a justifiable conclusion, I don't know as yet.

DR. D. J. WARD (*Oswestry*) Would measurements of hydroxyproline help to assess disease activity and the effect of penicillamine?

DR. HERBERT Urinary hydroxyproline determinations would be valueless as they would give no indication of the precise biochemical processes taking place in the scleroderma skin.

DR. S. D. ROBERTS (*Belfast*) You said that there were in the 'active' cases areas of skin where the activity was clinically less obvious. Is it true then to say that in the patient with active scleroderma there is also inactive scleroderma; and that there are areas in patients with inactive scleroderma that may be active?

DR. JAYSON This differentiation of areas was only possible in certain patients who had well-defined plaques and it was in these that we took separate biopsies from the centre and the edge of the lesion, and it showed that in the centre of the lesion the collagen had apparently matured, whereas at the edge of the lesion there was still proliferation of new collagen being laid down. This was possible in only a small number of patients. The sequential biopsies were taken from adjacent sites. These were not close enough so that new scar tissue might be taken, but otherwise were closely comparable. Incidentally, we have had the problem in a patient who presented with an acute connective tissue disease with some debatable changes in the skin and there was some difference of opinion as to whether this was an acute scleroderma, but in fact the analyses did show a massive increase in the production of new collagen which confirmed the diagnosis.

Extracellular Release of Cathepsin D from Cells in Human Normal and Rheumatoid Synovial Membranes. By A. R. POOLE, R. M. HEMBRY, J. T. DINGLE, I. PINDER, E. F. J. RING, and J. COSH (*Strangeways Research Laboratory, Cambridge, and Royal Hospital for Rheumatic Diseases*)

The acid proteinase cathepsin D can degrade proteoglycan, a major constituent of cartilage. The erosion and loss of articular and patellar cartilage observed in rheumatoid arthritis may be related to the excessive extracellular activity of this and other proteases released from cells in rheumatoid synovia. Up to the present time there has been no available means of detecting the local extracellular release and presence of this and other enzymes. Techniques recently developed in the Strangeways Research Laboratory have made it possible to capture enzymes as they are released from cells.

This paper reports the first application of this method to an investigation of cathepsin D release from normal and rheumatoid synovia. So far tissues have been removed from knee joints of 3 patients undergoing meniscectomy (normal) and 11 patients undergoing synovectomy (rheumatoid), and immediately cultured for up to 24 hours with a specific antiserum to human cathepsin D. While cell viability was maintained, extracellular cathepsin D was trapped by antibody with the formation of insoluble immunoprecipitates: these were subsequently localized at or near cell surfaces with immunofluorescence microscopy.

There was relatively little evidence for cathepsin D release from cells in synovia of nonrheumatoid joints: only occasionally were intimal lining cells and histiocytes in the capsular tissue suspected of secreting cathepsin D and then only in small amounts relative to synovia from many rheumatoid patients.

An extensive release of this enzyme was noted in 5 out of 11 rheumatoid patients from cells in synovia adjacent to the edges of partially degraded articular and fibrocartilage. There was an equally significant release in 3 out of 5 patients of cathepsin D from many cells in pannus tissue remote from the synovium and overlaying partly degraded articular cartilage.

The extracellular release and presence of this enzyme was particularly striking in synovium bordering partially degraded patella cartilage (3 out of 4 patients). The most common morphologically identifiable and viable cell-type releasing cathepsin D was macrophage-like: all very active tissues contained many such cells, although nonsecreting cells of this type were frequently seen. Only rarely was the release of cathepsin D from chondrocytes detected, even in partially degraded matrix.

It is suggested that the pathological excessive degradation of proteoglycan in patella and articular cartilage, which was seen only in rheumatoid joints and is particularly marked adjacent to synovium and pannus tissue, may be in part caused by the extracellular activity of cathepsin D and other proteases, resulting from the excessive secretion of these enzymes from cells in synovial and pannus tissues.

Discussion

DR. J. BALL (*Manchester*) I would like to ask whether your antibody will penetrate hyaline cartilage and thus enable you to decide whether there is cathepsin release or not?

DR. POOLE We have done some very careful experimental work with radioactively labelled immunoglobulins to study their penetration into cartilage, and also immunofluorescent studies which have been published (Poole, Barratt, and Fell, 1973). Generally speaking, normal adult cartilage is largely impermeable to IgG antibody, but when the cartilage has been degraded and has lost a certain amount of proteoglycan it becomes significantly permeable to the antibody. Only in these situations is it possible to clearly detect any secretion of cathepsin D from chondrocytes. Our present studies, however, indicate that viable chondrocytes in degraded cartilage are not releasing detectable amounts of cathepsin D. The major contribution to the extracellular pool of cathepsin D appears to be from cells present in the synovium.

DR. M. I. V. JAYSON (*Bristol and Bath*) Did you relate the release of cathepsin D to the detailed histology of the synovium and in particular the amount of lymphocyte infiltration and the amount of surface cell hyperplasia?

DR. POOLE These studies are in progress. All I can say is that there seems to be no evidence to indicate that lymphocytes, polymorphs, or plasma cells are commonly secreting cathepsin D. We have not studied the possible relationship between the release of this enzyme and the actual immunological situation within the synovium, but this is something which we would very much like to look at further.

Reference

Poole, A. R., Barratt, M. E. J., and Fell, H. B. (1973) *Int. Arch. Allergy*, 44, 469

Histocompatibility Antigen (HL-A 27) and Its Relation to Disease. By D. A. BREWERTON, MAEVE CAFFREY, ANNE NICHOLLS, and D. C. O. JAMES (*Westminster Hospital, London*)

In a series of 75 patients with ankylosing spondylitis the histocompatibility antigen HL-A 27 (W 27) was found in 72 patients compared with 3 controls (Caffrey and James, 1973; Brewerton, Caffrey, Hart, James, Nicholls, and Sturrock, 1973a). The same antigen was identified in 32 out of 60 first-degree relatives. W 27 has also been reported in 35 out of 40 patients in an independent investigation (Schlosstein, Terasaki, Bluestone, and Pearson, 1973).

Reiter's disease and nonspecific urethritis have been studied, and HL-A 27 was found in 2 out of 33 controls, 3 out of 33 men with NSU and 25 out of 33 men with Reiter's disease (Brewerton, Caffrey, Nicholls, Walters, Oates, and James, 1973c). This series has now been extended and the first 50 patients with Reiter's disease assessed. HL-A 27 was present in 17 out of 32 patients with peripheral arthropathy alone and in all 18 patients with sacroiliitis or spondylitis.

Preliminary results are available on ulcerative colitis and psoriatic arthropathy. HL-A 27 has been present in 1 out of 21 patients with ulcerative colitis, and in 10 out of 13 patients with spondylitis associated with ulcerative colitis. Other workers have reported a modest association between psoriasis and HL-A 13 and W 17, but not HL-A 27 (Krain, Newcomer, and Terasaki, 1973). So far we have found HL-A 27 in 8 out of 23 patients with psoriatic arthropathy.

HL-A 27 has recently been reported in 20 out of 22 patients with Yersinia arthritis (Aho, Ahvonen, Lassus, Sievers, and Tiilikainen, 1973). This is the first example of an association between HL-A 27, arthropathy and an identified, infective agent.

A study is in progress of acute anterior uveitis as it presents in eye departments. A report of the first 50 patients has been published (Brewerton, Caffrey, Nicholls, Walters, and James, 1973b) and 79 have now been investigated. Associated diseases were present in 24—mostly ankylosing spondylitis, Reiter's diseases and associated disorders of the spine or sacroiliac joints. All of the patients with uveitis and rheumatic disease had HL-A 27. The antigen was not present in 3 men with active urethritis at the same time as their uveitis. 55 patients had no evidence of an associated disease and 17 had HL-A 27. Young women with HL-A 27 may present with acute anterior uveitis and no rheumatic symptoms in their 20s and early 30s, at the same age that men may present with ankylosing spondylitis.

The relationship of this finding to pathogenesis is unknown. It is possible that the inheritance of HL-A 27, or some closely related immune response, renders a small proportion of the population peculiarly susceptible to the effects of a variety of infective agents.

Discussion

DR. R. D. STURROCK (*Glasgow*) These results are very interesting. We confirm the findings of HL-A 27 in spondylitis, but using serum AJ we have found that in 50% of our patients who have HL-A 27 they also have associated AJ antigen. Now AJ was first suggested by Dr. Sandberg