P component in the synovium in rheumatoid and osteoarthritis

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SUMMARY P component is present in amyloid deposits, normal serum, and normal tissues in relation to elastic fibres. Its pathological role in inflammatory synovitis was investigated. Its distribution was determined immunohistologically in 33 synovia: 15 rheumatoid; seven osteoarthritic; seven traumatic controls; and four infected biopsy specimens. P component was present in two circumscribed distributions: extracellular fibrils in dense fibroelastic tissue of the more fibrotic synovia; and in the arterial wall, where it was confined to a single elastic lamina in some cases and in others showed reduplication and fragmentation. These were not related to amyloid material. It shows no disease specificity, but P component categorises the nature of the pathological reaction and is typically in biopsy specimens showing the development of chronic fibrosis. There was close codistribution of P component with elastic tissue, though this was not absolute. P component had a different distribution from C reactive protein (in synovial lining cell layer), and fibronectin, which was absent from fibrotic areas. Understanding the pathological interactions of P component may help elucidate why some synovial reactions remain inflammatory and others progress to chronic fibrosis.

Structural glycoproteins are a heterogeneous group of extracellular connective tissue matrix proteins.¹ They include fibronectin, laminin, P component, and related glycoproteins. The distribution of fibronectin and laminin has been described in the synovium in rheumatoid arthritis and related disorders.^{2–7} P component has not been previously examined in normal or diseased synovium. It is a serum protein with marked homology to C reactive protein.^{8–9} P component is not an acute phase reactant in humans, though it is in mice.¹⁰ Tissue P component was originally described as an invariable component of amyloid deposits.¹¹ Its distribution is now known to be more extensive, with a significant structural role in glomerular basement membranes,¹² and more widely in tissues in relation to elastic fibres.¹³

To investigate whether P component has a pathogenic role we examined its distribution in the inflamed synovia from patients with rheumatoid arthritis and also studied osteoarthritic and non-inflammatory synovial biopsy specimens. For com-

parative purposes we evaluated its relation to fibronectin, laminin, and C reactive protein.

Materials and methods

Thirty three operative synovial biopsy specimens were obtained from 15 patients with rheumatoid arthritis, seven with osteoarthritis, four with tuberculosis of the synovium, and seven with torn menisci and other non-inflammatory traumatic conditions. The distributions of P component, laminin, fibronectin, and C reactive protein were studied by indirect immunohistochemistry on 6 µm sections of fresh frozen tissue, on ethanol fixed, paraffin embedded tissue by the Saint Marie method,¹⁴ and formal fixed, paraffin embedded tissue after enzyme digestion.¹⁵ Both immunofluorescence and immunoperoxidase techniques were employed. Tissue sections were also stained by haematoxylin and eosin and by Weigert's stain for elastic tissue. Further paraffin sections were stained by alcoholic Congo red and amyloid deposits sought by both polarised and fluorescent light.

Monospecific antisera employed were as follows: goat antirabbit P component (Atlantic Antibodies 053–03); goat antihuman fibronectin (Sigma F-

1509); rabbit anti-C reactive protein (Dako A073); rabbit anti-laminin (BRL 6265SA). Appropriately labelled second antisera for indirect immunofluorescence and immunoperoxidase techniques were obtained from Dako. Control sections were incubated with non-immune goat and rabbit sera in place of the primary antisera. All control sections were negative. Sections were counterstained with haematoxylin.

Results

All synovial biopsy specimens contained some immunoreactive P component, though in certain instances only small amounts were present. Immunohistological staining for P component showed a similar pattern with formal fixed paraffin embedded tissues, cold ethanol fixed tissue, or fresh frozen material. Two patterns of immunoreactivity for P component were discerned: firstly, fibrillar immunoreactivity in dense fibroelastic tissue beneath the synovial lining cells (Fig. 1) and secondly, vascular related immunoreactivity. There was perivascular positivity around most vessels larger than capillaries. Some arteries showed increased immunoreactive P component in the vessel walls (Fig. 2). In others it was confined to a single internal lamina. Vascular basement membranes were negative for P component.

There was a close relation between the distribution of P component and that of elastic fibres. Vessels with abundant immunoreactive P compo-

nent in their wall corresponded with those showing conspicuous reduplication of their internal elastic laminae on conventional histological staining (Fig. 2). Fibrillar P component was in a similar distribution to that of stromal elastic fibres. The codistribution was not complete, however, and there was frequently more immunoreactivity for P component than elastic fibre staining. At no time was it possible to demonstrate any staining for amyloid.

Comparisons of immunoreactive P component, C reactive protein, fibronectin, and laminin showed no evidence to suggest they were codistributed. C reactive protein was confined to the synovial lining cell layer, which showed only weak positivity. Fibronectin was far more widely distributed with reactivity within the synovial lining cell layer, articular fibres in the underlying subsynovial connective tissue, and in close relationship to small synovial blood vessels. Laminin was present in all vascular basement membranes but not in internal elastic laminae. P component contrasted with these in being more closely associated with fibrosis, whereas the others were more prominent in areas of synovial proliferation.

There was no clear relation between diagnostic category and the amount of immunoreactive P component. Its distribution, however, showed a distinct pattern in relation to the pathological changes in the synovium. Control biopsy specimens showed only very small amounts of P component. In the inflamed synovia, principally in some cases of rheumatoid arthritis and the patients with tubercu-

Fig. 1 Immunoreactive P component in a fibrillar distribution among dense fibroelastic tissues.

Fig. 2 Consecutive sections of a small artery showing similar distribution of positive staining. (a) Immunoreactive *P* component; (b) Weigert's elastic stain.

Fig. 3 *P* component in the walls of small blood vessels showing evidence of reduplication.

lous synovitis, P component was conspicuously absent from areas showing inflammatory cell infiltration. In contrast, biopsy specimens with early fibrotic changes showed more prominent P component (Fig. 3). It was especially prominent in the blood vessel wall at these sites, where it frequently suggested evidence of reduplication of the elastic laminae and related structures.

Discussion

P component is a minor structural glycoprotein of normal and diseased synovia. It is closely related to elastic fibres in the synovium, in keeping with previous observations of other normal and pathologically involved tissues.¹⁶ Its distribution is distinct from that of C reactive protein, laminin, and fibronectin. These differences are noteworthy because there is marked structural homology between P component and C reactive protein,^{8,9} and in vitro P component dimers specifically bind fibronectin in a calcium dependent manner.¹⁷ Laminin is confined to blood vessel basement membranes.⁷ Fibronectin has a wider distribution in the synovial lining cell layer, in relation to fibrillar extracellular matrix proteins, and around blood vessels.²⁻⁴ C reactive protein is located in the synovial lining cell layer.¹⁸ This coincides with its relatively low synovial fluid level,¹⁹ suggesting deposition in the lining cells.

Tissue amyloid deposits always contain P component.¹¹ Indeed it is only recently that P component has been recognised as a component of normal tissue. None of the patients we studied was considered to have systemic reactive amyloidosis nor was there evidence of amyloid deposits in the synovium. Goffin *et al*²⁰ and Ladefoged²¹ have both reported material with some histological features of amyloid deposits in the synovium; though the evidence that they are true amyloidotic material is not strong. The distribution of P component is also different from that of the non-collagenous reticulin component which is present in many tissues,²² including the inflamed synovium.²³ This tissue component is extracted by distilled water purification, as are amyloid fibrils. At the ultrastructural level rheumatoid and osteoarthritic synovia contain large amounts of fibrillar protein material, though its exact nature is uncertain (C J Morris and C Hollywell, unpublished observations). Although by conventional standards synovial P component is not related to amyloid deposits, the exact nature and distribution of extracellular fibrillar proteins in arthritis is a complex area of which relatively little is known.

P component is related to early fibrotic change

and is a weak marker of this process in the synovium. It is not associated with inflammatory cell infiltrates. The absence of any specific relation with a single diagnostic category is similar to findings with other connective tissue proteins, and is typical of many synovial histological changes. Nevertheless, the suggestion that P component may characterise early fibrosis is theoretically interesting, though at this stage it is of limited diagnostic value.

Vascular changes are common in synovial biopsy specimens, and vasculitis is a frequent extra-articular feature of rheumatoid arthritis. Inflamed small blood vessels in the synovium contain relatively little P component, but larger arteries have far more P component in their walls, frequently suggestive of reduplicative changes in the arterial wall. Completely or partially occluded vessels often contain large amounts of P component. The presence of vessel wall P component may be particularly significant in the pathogenesis of rheumatoid vasculitis, and further studies are needed in this area.

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