Amyloid in prostatic corpora amylacea

P A Cross, C J Bartley, J McClure

Abstract
Aim: To determine the presence and nature of amyloid in prostatic corpora amylacea using immunohistological studies.

Methods: Prostatic tissue from 18 transurethral and two open resection specimens was studied. Paraffin wax embedded tissue sections were stained with haematoxylin and eosin and the alkaline Congo red method with and without previous treatment with potassium permanganate. Sections were also stained with antibodies to amyloid A, \( \beta \), microglobulin, \( \lambda \) and \( \kappa \) light chains, prealbumin IgA, G, M, S100 protein, prostatic specific antigen, amyloid P component and CAM 5-2 (control and blocking studies were performed).

Results: The prostatic corpora amylacea universally showed the presence of amyloid. In all instances this contained \( \beta \), microglobulin.

Conclusion: Prostatic corpora amylacea represents a localised amyloidosis of \( \beta \), microglobulin origin that is unrelated to chronic renal failure and haemodialysis.

Methods
All surgical prostatic resection specimens for 1988 were retrieved. Those specimens which were normal or showed benign prostatic hyperplasia and contained corpora amylacea were studied further. There were 18 transurethral resections and two open prostatectomy specimens. Prostatic tissue from six necropsy cases and material from four cases of systemic amyloidosis of known type with prostatic disease were also used (table 1). Paraffin wax embedded tissue sections (cut at 4 \( \mu \)m) were stained with haematoxylin and eosin, or by the alkaline Congo red method with and without prior treatment with potassium permanganate (KMnO\(_4\)). Sections were also stained with the antibodies listed in table 2, using the avidin-biotin technique with and without digestion with trypsin. A blocking procedure using excess free human \( \beta \),M (Serotec) at a final concentration of 1 mg/ml was applied to tissue sections to assess the specificity of the reaction.

Results
Typical corpora amylacea lay within the prostatic glandular lumina in all the cases studied. Most of the corpora were Congo red positive, and gave apple-green birefringence with crossed polarising filters. The Congo red stain accentuated the lamellar appearance of the corpora, although the degree of staining was variable among corpora. The apple-green birefringence produced a Maltese cross effect (fig 1) in some corpora, the effect being more noticeable at the periphery. The size of the corpora did not seem to affect this reaction, except that the larger corpora were more likely to be either pigmented or calcified (these purely pigmented and calcified corpora were excluded from the immunohistochemical studies). Prior treatment with potassium perman-

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Amyloid cases</th>
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<tbody>
<tr>
<td><strong>Amyloid type</strong></td>
<td><strong>Clinical details</strong></td>
</tr>
<tr>
<td>1</td>
<td>A K</td>
</tr>
<tr>
<td>2</td>
<td>AA, K</td>
</tr>
<tr>
<td>3</td>
<td>A,M</td>
</tr>
<tr>
<td>4</td>
<td>A, ( \beta ),M</td>
</tr>
</tbody>
</table>
Table 2 Antibodies used

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Dilution</th>
<th>Species</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amyloid A</td>
<td>1 in 500</td>
<td></td>
<td>Calbiochem</td>
</tr>
<tr>
<td>f, M</td>
<td>1 in 500</td>
<td></td>
<td>Calbiochem</td>
</tr>
<tr>
<td>IgA, G, M</td>
<td>1 in 500</td>
<td></td>
<td>Dako</td>
</tr>
<tr>
<td>Prealbumin</td>
<td>1 in 500</td>
<td></td>
<td>Dako</td>
</tr>
<tr>
<td>SI100</td>
<td>1 in 500</td>
<td></td>
<td>Dako</td>
</tr>
<tr>
<td>Prostate SAg</td>
<td>1 in 500</td>
<td></td>
<td>Dako</td>
</tr>
<tr>
<td>Amyloid P component</td>
<td>1 in 250</td>
<td>P, Rabbit</td>
<td>Calbiochem</td>
</tr>
<tr>
<td>Cam 5.2</td>
<td>1 in 5</td>
<td>M, Mouse</td>
<td>Becton-Dickinson</td>
</tr>
</tbody>
</table>

F = Polyclonal, M = Monoclonal.

...ganate either much reduced or totally abolished the Congo red staining of the corpora.

Congo red positive dots were also noted within the prostatic glandular epithelium and were similar to those seen in the seminal vesicle. These were relatively uniform in size (about 1–2 μm) with some up to 6 μm, but variable in position within the cytoplasm of the epithelial cells. They did not produce apple-green birefringence, and the staining reaction was abolished by prior potassium permanganate treatment.

The corpora gave consistent immunohistochemical positivity for β2M (fig 2) with a patchy, inconsistent and weak positivity for the immunoglobulin heavy and light chains. The interstitial amyloid of the systemic amyloid cases gave results consistent with the vascular and stromal amyloid found in the other body tissues, independent of that of the corpora amylaceae. Prostatic epithelial cells (focally) and lymphoid cells were also positive for β2M (fig 3). The prostatic epithelial cells were strongly positive for both prostate specific antigen (PSA) and Cam 5.2; the corpora were not. The prostatic epithelial dots did not stain with any of the antibodies used.

Excess free β2M blocked the positive antibody staining of the corpora amylaceae.

Discussion

The Congo red positivity and apple-green birefringence of prostatic corpora amylaceae is not a novel finding, although it has been dismissed in the past. This combination of congophilia and apple-green birefringence is the most widely used criterion used to identify amyloid in tissue sections and is considered to be given by fibrillar protein depositions which have a β-pleated sheet configuration. The reduction of the congophilia by prior potassium permanganate treatment, initially thought to be an indicator of AA amyloid, is, however, also seen in β2M amyloidosis.

The finding of consistent and specific β2M positivity with prostatic corpora amylaceae has not been described before. β2M is an 11.8 kilodalton molecule that forms the light chain of the HLA class I antigen, and as such is found on all nucleated cells. It has strong homology with the other domains of both HLA class I and II antigens, as well as with that of the constant regions of the immunoglobulin heavy chains. The relatively recently described haemodialysis associated amyloid has been found to be due to the deposition of β2M. This presents clinically as either the carpal tunnel syndrome, a flexor tenosynovitis, or a destructive arthropathy. The deposition in chronic renal failure is due to failure of filtration across the renal glomerulus and certain types of dialysis membrane. Systemic, non-articular β2M deposition in these cases seems to be relatively minor and restricted to vessel walls. None of the 26 non-systemic amyloid cases studied had evidence of chronic renal failure. Battaglia was able to demonstrate Congo red positivity of prostate corpora, as well as Congo red positive...
dots in the prostatic epithelium, and "keratin" positivity (source and reactivity unspecified) of both of these immunohistochemically on frozen sections. He postulated that the corporal fibrils are formed by the shedding of the epithelial cells, the Congo red dots being groups of keratin fibrils. He therefore coined the term "apoptotic amyloid".

Prostatic epithelium contains four main types of cytokeratin (Moll numbers 5, 8, 18, 19) (Cam 5-2 datasheet; Becton-Dickinson, 1985), of which the monoclonal antibody Cam 5-2 should be able to recognise three (numbers 8, 18, 19). We were able to demonstrate strong, positive prostatic epithelial positivity of both Cam 5-2 and PSA, but were unable to show the corporal or epithelial dot cytokeratin positivity stated by Battaglia. This may reflect our use of paraffin wax (and not frozen) prostatic tissue, but digestion with trypsin of the paraffin wax material failed to show any corporal dot immunocytokeratin positivity. This lack of cytokeratin waxed tissue staining means either an alteration of the antigenic sites recognised by Cam 5-2 or absence of Cam 5-2 reactive cytokeratin in corpora amylacea. If corpora are derived (partly or wholly) from prostatic cells this latter possibility seems...
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unlikely, especially as there is strong electron microscopic evidence of a close similarity between prostatic epithelia and the corporal filament structure. The failure to demonstrate PSA in corpora suggests that no prostatic epithelial cell surface membrane material is present in corpora, assuming that the antigen is still present in an immunologically recognised form. The $\beta$-M in corpora can come either from nucleated cells (prostatic epithelial or any other) or from the absorption of free, filtered undergraded urinary $\beta$-M in cumulative small amounts onto a central nidos. The finding of spermatozoa within corpora shows that reflux into the prostate occurs. It is also known that $\beta$-M can undergo $\beta$-pleating in vitro to form an amyloid.

We conclude from our studies that the amyloid of prostatic corpora amylacea is derived from $\beta$-microglobulin. We suggest that the $\beta$-microglobulin arises from the urine by reflux and that corpora amylacea grow by apposition of the amyloid material on to a central nidus that is probably derived from the prostatic epithelium.

4 Arch Pathol 1965;80:487-94.