Anti-GBM antibodies in Goodpasture syndrome; anatomy of an epitope

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Introduction

Basement membranes are thin sheet-like extracellular structures that form an anatomical barrier wherever cells meet connective tissue. They provide a substrate for organs and cells and relay signals important for the differentiation and maintenance of the tissue. The importance of a functional basement membrane is illustrated in several pathological conditions where the glomerular basement membrane (GBM) is disrupted. An example is Goodpasture syndrome, where an autoimmune reaction to the GBM rapidly causes life threatening disease and loss of kidney function.

Structure of the GBM

Basement membranes are composed of several specific molecules such as type IV collagen, laminin, proteoglycans, entactin, and several other proteins found in small amounts in some restricted membranes. Both laminin and type IV collagen form large continuous matrices. Type IV collagen has self-aggregating properties and forms an irreversible and stable matrix in which the other basement membrane molecules are integrated. Each type IV collagen molecule is built up of three subunits, called \( \alpha(IV) \) chains. In the N-terminal end and in the large collagenous middle part, the three \( \alpha(IV) \) chains are intertwined into a triple helical structure, like most other collagens (Figure 1). In the C-terminal end each chain is folded into a separate globular structure, called the NCI domain [1]. Six genetically distinct \( \alpha(IV) \) chains are found, designated \( \alpha-1(IV) \) to \( \alpha-6(IV) \) (Table 1) [2].

Fig. 1. Schematic representations of 1a, the type IV collagen chicken wire network; and 1b, one type IV collagen molecule with its triple helical structure and a bound GP antibody in the c-terminal part of the NCI domain.

Specificity of anti-GBM antibodies

Goodpasture (GP) antibodies bind along the GBM and are seen as a linear deposition of IgG at direct immunofluorescence. This pattern is explained by the tissue distribution of the \( \alpha-3(IV) \) and \( \alpha-4(IV) \) chains...
containing network. This network is also found in the alveolar basement membrane in the lung, indicating the clinical condition to be a renopulmonary syndrome. When the GP antigen was first isolated, the antigen was found to be in a collagenase-resistant part of type IV collagen, the NC1 domain (Figure 1). If the hexameric NC1 domain is dissociated into monomers (the NC1 domain from one single z(IV) chain) and dimers, most of the reactivity is found to be directed against the z-3(IV) chain [6, 7]. Affinity chromatography has shown about 1% of GP patients’ total IgG to be anti-type IV collagen antibodies. This is an expected figure during normal immunization. The antibody IgG subclass pattern also indicates the occurrence of an autoimmunization process, which is characterized by a predominance of IgG1 and in some GP patients a large fraction of IgG4 as well [7]. Moreover about 90% of the anti-type IV collagen antibodies have been found to be z-3(IV) chain specific [8]. By using a monoclonal antibody to the z3(IV) chain in an enzyme-linked immunosorbent assay (ELISA), it was found that most of the GP antibody reactivity could be blocked. This indicates that there is one major epitope in most GP patients.

Epitopes of anti-GBM antibodies

The normal GP antibody recognizes a conformational epitope in the NC1 domain of the z-3(IV) chain. Although the epitope specificity of the autoantibodies involved in GP is very restricted, exceptions nonetheless exist. We found one patient to manifest antibodies to the z-1(IV) chain only, and none to the z-3(IV) chain. This patient did not suffer from progressive disease, but from a mild and stable form of the disease. The epitope for these z-1(IV) antibodies has been localized to the last four amino acids of the z-1(IV) chain, with the sequence MRRT [9]. This epitope was found to be linear, in contrast to the epitope found in conjunction with normal GP antibodies. Further evidence of variation in autoantibody specificity has been found in other patients with mild disease, though this remains to be studied in greater detail.

The GP epitope has been found to be a cryptotope, and only very little reactivity is found against the native hexameric structure (Figure 2). However, when the antigen is denatured and the hexamer dissociates into dimers and monomers, the reactivity is increased 10–15 fold. On the other hand, if the antigen is reduced and alkylated, almost all reactivity disappears, and less than 1/1000 of the binding to denatured antigen remains [9]. There is some clear evidence that the epitope specificity of the autoantibodies involved is a determinant of the disease progression rate, as shown by the patient with the z-1(IV) antibodies. If other fine differences in specificity could be identified as markers of disease and outcome in the future we will be able to develop new and more specific prognostic tools.

### Table 1. Type IV collagen chains and genes

<table>
<thead>
<tr>
<th>z(IV)-chain</th>
<th>Gene</th>
<th>Gene locus</th>
<th>Tissue distribution</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>z-1(IV)</td>
<td>COL1A1</td>
<td>13q34</td>
<td>All basement membranes</td>
<td>Co-localized with z-1(IV)</td>
</tr>
<tr>
<td>z-2(IV)</td>
<td>COL1A2</td>
<td>13q34</td>
<td>All basement membranes</td>
<td>Autoantigen in Goodpasture syndrome.</td>
</tr>
<tr>
<td>z-3(IV)</td>
<td>COL1A3</td>
<td>2q36</td>
<td>Ear, lens capsule, lung, kidney (GBM, small amount in distal TBM and Bowman’s capsule)</td>
<td>Mutations found in autosomal forms of Alport’s syndrome</td>
</tr>
<tr>
<td>z-4(IV)</td>
<td>COL1A4</td>
<td>2q36</td>
<td>Ear, lens capsule, lung, kidney (GBM, small amount in distal TBM and Bowman’s capsule)</td>
<td>Co-localized with z-3(IV)</td>
</tr>
<tr>
<td>z-5(IV)</td>
<td>COL1A5</td>
<td>Xq22</td>
<td>Skin, lens capsule, smooth muscle, kidney (GBM, distal TBM, Bowman’s capsule)</td>
<td>Mutations found in X-linked Alport’s syndrome</td>
</tr>
<tr>
<td>z-6(IV)</td>
<td>COL1A6</td>
<td>Xq22</td>
<td>Skin, lens capsule, ear, smooth muscle, kidney (Bowman’s capsule and distal TBM)</td>
<td>Deletion found in diffuse leiomyomatosis</td>
</tr>
</tbody>
</table>

TBM, tubular basement membrane.

### Anti-GBM antibodies in diagnosis and prognosis of the disease

Measurement of anti-GBM antibodies is of great value mainly in three clinical categories. (1) Patients with acute renal failure, where post- and pre-renal causes seem unlikely; (2) patients with increasing serum creatinine concentrations together with haematuria and casts; and (3) patients with pulmonary haemorrhage. In all three categories the finding of anti-GBM antibodies is of great value: it was found that most of the anti-GBM antibodies are linear, in contrast to the epitope found in conjunction with normal GP antibodies. Further evidence of variation in autoantibody specificity has been found in other patients with mild disease, though this remains to be studied in greater detail.
is that the two conditions constitute manifestations of one and the same underlying entity, is at present unclear. Having encountered families where one family member has GP and another has systemic vasculitis, we are inclined to favour the latter interpretation. As GP is a rapidly progressive disease, it is important to start therapy before tissue damage has advanced too far. The new ELISAs based on our current knowledge of the epitopes enables us to identify the patients early, and in the future it may well be possible to predict prognosis of the disease by studying the fine specificity of the antibodies.

Conclusions

Acknowledgements

References