Further characterisation of a spontaneously occurring antibasement membrane antibody

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SUMMARY A spontaneously occurring antibasement membrane antibody has been further characterised according to antigenic specificity, immunoglobulin class, and tissue localisation using immunofluorescent and immunoelectron microscopic techniques. The autoantibody reacted with the basement membrane of kidney tubules, Bowman's capsule, and the epithelial portion of ileum but not with the basement membrane of skin, cornea, glomerulus, or oesophagus. It also reacted with bile canaliculi, sarcolemmal sheath, and salivary duct. On electron microscopy the antibody was distributed along the basement membrane of Bowman's capsule and renal tubules. Some reactivity against collagen was observed. Antibody activity was found in both IgG and IgM fractions. In immunodeficiency disorders, the autoantibody was found only in patients with selective IgA deficiency.

A spontaneously occurring autoantibody reacting with selected membranes and distinct from other basement membranes was described by us in 1971 (Ammann and Hong, 1971a). This antibody was found in patients with coeliac disease and IgA deficiency. Seah *et al.* (1971) had previously described an autoantibody with similar tissue localisation, which they termed reticulin antibody and which they found primarily in patients with coeliac disease and dermatitis herpetiformis. Subsequent studies by Alp and Wright (1971) and von Essen *et al.* (1972) confirmed both the tissue localisation of the autoantibody and the occurrence in coeliac disease. Rizzetto and Doniach (1973) reported on the occurrence of reticulin antibodies in an extensive evaluation of 3628 sera from patients with a wide variety of disorders. They defined the tissue localisation of the autoantibody, the specificity for certain disease states, and the immunoglobulin class distribution. They further suggested that five types of autoantibody could be demonstrated and termed these: reticulin R₁, reticulin R₂, Kupffer cell, sinusoidal adherent cell cytoplasmic, and reticulin sinusoidal Rs.

In this report we describe detailed studies on the tissue localisation and distribution of antibody

reactive with basement membrane using immunofluorescent and immunoelectron microscopic techniques. This autoantibody appears to be identical with that described by Seah *et al.* (1971) and the reticulin R₁ antibody described by Rizzetto and Doniach (1973).

Material and methods

Blood samples were obtained from patients, immediately separated, and stored frozen at -20°C . These samples were used to perform indirect immunofluorescence at 1-10 serum dilutions on various tissues obtained from rat and human sources, as previously described (Ammann and Hong, 1971b).

Electron microscopy was performed as follows: Normal rat kidney was cut and placed in ice-cold 1% glutaraldehyde in 0.1 M cacodylate buffer for 45 minutes. Blocks were washed in buffer and 35 μ slices were cut on a tissue chopper. This was followed by incubation with either the patient's purified IgG or normal human serum for 45 minutes at room temperature and the sections were washed three times with 0.1 M cacodylate buffer. These, plus a third control preparation not incubated with serum, were all incubated in ferritin-labelled goat anti-human IgG for 30 minutes, washed three times with 0.1 M cacodylate buffer, fixed in

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buffered 1% glutaraldehyde, post-fixed in 1% osmium, and prepared for electron microscopy.

Collagen was prepared from the skin of newborn infants and was supplied to us by Rothbard and Watson (1972). This preparation was used for absorption of antibody in a concentration of 1 mg/ml with varying amounts of serum.

The autoantibody was separated into IgG and IgM fractions, using sodium sulphate precipitation and Sephadex G200 column chromatography. The patient from whom this autoantibody was obtained lacked serum IgA.

Results

Separation of the serum into IgG and IgM fractions and subsequent indirect immunofluorescence with anti-IgG and anti-IgM antibody indicated that autoantibody activity was present in both immunoglobulin fractions. No autoantibody activity was present in IgA as the patient lacked serum IgA.

After absorption of the autoantibody with highly purified collagen, no appreciable change in the distribution or intensity of autoantibody reactivity could be demonstrated by immunofluorescent techniques. Table 1 summarises the distribution of reactivity of the autoantibody against various tissues. Figure 1 demonstrates a typical pattern on rat renal tubules.

Table 1 *Tissue reactivity of antibasement membrane activity*

Structure	Anti-IgG fluorescent antibody reactivity
Bowman's capsule	+
Glomerular basement membrane	—
Kidney tubule basement membrane	+
Liver portal tracts	+
Sarcolemmal sheath	+
Epithelial cell basement membrane (ileum)	+
Oesophagus basement membrane	—
Salivary duct (striated duct) basement membrane of serous cells	+
Cornea basement membrane	—

Electron microscopy using ferritin-labelled anti-human gamma chain indicated that the autoantibody reacted in the same distribution on renal basement membranes as was found using the immunofluorescent method. The ferritin-labelled anti-human IgG was seen along many tubular basement membranes and in some endothelial cells of capillaries in tubular areas (Fig. 2). There was no ferritin anti-human IgG along glomerular basement membrane of glomerular capillary endothelium. In addition, it was present in the rat plasma in both tubule and glomerular capillary lumens, indicating that the reagent penetrated to these areas and cross-reacted with rat IgG. In certain areas activity against collagen fibres was demonstrated (Fig. 3).

Fig. 1 *Fluorescent photomicrograph of isolated IgG on rat kidney section. Positive staining of basement membrane of Bowman's capsule and tubules is seen. × 40*

Fig. 2 Electron micrographs of ferritin molecules within the basement membrane (B) of this tubular cell can be seen (arrow). There is also some ferritin in the endothelial cell (E) lining the capillary lumen (L). The scale is one micron.

Table 2 summarises the distribution of antibasement membrane antibody in various clinical immunodeficiency disorders. It was not found in 40 normal adults and children.

Discussion

Spontaneously occurring antibasement membrane antibody has been described with varying histological distributions. In Goodpasture's syndrome, an autoantibody is found by immunofluorescence which reacts with the basement membrane of the renal glomerulus and lung (Lerner *et al.*, 1967; McPhaul and Dixon, 1969). Autoantibody reacting with the basement membrane of skin and oesophagus has been found in pemphigoid (Chorzelski *et al.*, 1966; Beutner *et al.*, 1967). The autoantibody described in this report reacts with the basement membrane of Bowman's capsule and kidney tubules (Fig. 1) but not with the glomerulus or basement membrane of oesophagus or cornea. Reactivity was also observed with basement membrane of the epithelial cells of

villi taken from the ileum, sarcolemmal sheath of muscle, and the portal tracts of the liver. In some sections reactivity with collagen fibres could be seen (Fig. 3). From these studies it cannot be determined if the autoantibodies are reacting with shared antigenic components or if two distinct autoantibodies are present. Attempts to absorb the autoantibody with collagen did not reduce antibasement membrane activity.

The importance of these studies is in the observation that both basement membrane and collagen reactivity were observed on electron microscopic evaluation whereas dual reactivity could not be distinguished by immunofluorescent studies. It is likely, therefore, that the antibasement membrane antibody described by us (Ammann and Hong, 1971b), the antireticulin antibody described by Seah *et al.* (1971), and the R₁ antibody described by Rizzetto and Doniach (1973) have similar, if not identical, antigenic components, namely, basement membrane and collagen.

The studies of Seah *et al.* (1971) and the detailed

Fig. 3 Electron micrograph of a small cluster of collagen fibres (C), which have a scatter of ferritin molecules (arrows) on and near their surface. There is also some ferritin in the basement membrane (B) of this tubule cell. The scale is one micron.

Table 2 Antibasement membrane antibodies in immunodeficiency disorders

Disorder	No. tested	No. positive
Coeliac disease with selective IgA deficiency	3	3
Selective IgA deficiency without coeliac disease	40	1
Hypogammaglobulinaemia	12	0
Thymic hypoplasia	6	0
Chronic granulomatous disease	10	0
Severe combined immunodeficiency	5	0
Normals (2 to 40 years)	40	0

investigations of Rizzetto and Doniach (1973) are in agreement as to the tissue localisation of antibody reactivity. A nodular fluorescence is observed surrounding the portal tracts of the liver with discrete wave-like bands extending into the sinusoidal walls, a honeycomb appearance on smooth or striated muscle, staining of Bowman's capsule, and reactivity in the peritubular area of the kidney. We have extended the number of tissues examined and find reactivity along the basement membrane of epithelial

cells of ileum and basement membrane of salivary duct (striated duct) serous cells (Table 1). No reactivity was observed against basement membranes of cornea, glomerulus or oesophagus, indicating that these basement membranes are distinct antigenically from those of Bowman's capsule, epithelial cells of gastrointestinal tract and kidney tubule, and the portal tract of the liver. It is of interest that the distinct distribution of antibasement membrane antibody detected in Goodpasture's syndrome, pemphigoid, and in coeliac disease corresponds to distinct differences in biochemical composition, as described by Kefalides (1969).

Several experimental studies suggest that antibodies to collagen and basement membrane have similar patterns of distribution by immunofluorescent techniques. Nagasawa and Shibata (1969) used human glomerular basement membrane as antigen and studied the tissue distribution of antibody in rat tissue. Antibody activity was demonstrated against the basement membrane of Bowman's capsule, renal tubular glomerulus, sarcolemmal muscle sheath, and

liver sinusoids. However, using histochemical techniques, Velican and Velican (1970) demonstrated structural heterogeneity between glomerular, capsular, and tubular basement membranes. Using antibody to human infant skin collagen, Rothbard and Watson (1972) demonstrated reactivity by immunofluorescent methods in a pattern of distribution similar to that obtained using antibasement membrane antibody. These studies emphasise the difficulty in distinguishing antibasement membrane activity from anti-collagen activity by immunofluorescent methods alone.

Rizzetto and Doniach (1973) stressed that connective tissue elements are a mixture of amorphous proteins and mucopolysaccharides in which there are fibrous proteins related to collagen. They suggested that electron microscopic studies would be necessary to see if the antigens detected by spontaneously occurring autoantibody are fibrillary or belong to amorphous proteins and mucopolysaccharides. Our studies clearly demonstrate both basement membrane and collagen reactivity. The term 'reticulin' antibody (Seah *et al.*, 1971) or 'R₁' antibody (Rizzetto and Doniach, 1973) would, therefore, appear to be appropriate general terms when this autoantibody is detected by immunofluorescent methods. The antibody activity may be further defined as against basement membrane and/or collagen by electron microscopic techniques.

In clinical disorders, the autoantibody has been found to be primarily associated with coeliac disease. Although we originally described the autoantibody in patients with selective IgA deficiency coeliac disease (Ammann and Hong, 1971b), Seah *et al.* (1971) and Alp and Wright (1971) found the autoantibody to be present in patients with coeliac disease, the majority of whom had normal IgA values. In a study of 3628 sera from patients with a variety of diseases, Rizzetto and Doniach (1973) found antibody to reticulin to be most frequently present in dermatitis herpetiformis, coeliac disease, chronic heroin addicts, Sjögren's syndrome, myasthenia gravis, vitiligo, alopecia, and rheumatoid arthritis. However, they did not distinguish between the various subtypes of antibody to reticulin present in the various diseases. Spontaneously occurring antitubular basement membrane antibody has been described after renal transplantation (Wilson *et al.*, 1974) and in methicillin-induced interstitial nephritis (Baldwin *et al.*, 1975).

We studied the sera of 76 patients with immunodeficiency to determine the relationship between antibody against reticulin and specific immunodeficiency disorders. Although the number of patients in some categories was small, only one patient who did not have coeliac disease was found

to be positive. This patient had selective IgA deficiency and oedema of unknown aetiology. Thus, the autoantibody appears to be confined to immunodeficient patients with selective IgA deficiency.

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