Supplemental Material

Predisposing factors:

The autoimmune activity is controlled mostly by major histocompatibility complex (MHC) class I and class II molecules encompassing more than 200 intricately linked genes, the majority of which are involved in the immune response. A strong association between MHC-DR2 and anti-GBM disease was initially defined in 1978 by an HLA typing study which showed that 15 out of 17 patients with anti-GBM disease had HLA-DR2.[S1] It later became clear that the association was closely related to the DR15 subtype of DR2.[S2] Subsequently, based on studies using modern DNA sequencing techniques, DRB1*1501, DRB1*03 and DRB1*04 were shown to be associated with increased susceptibility to the disease.[S3]
Evidence for the significance of environmental factors in pathogenesis of anti-GBM disease comes from reports of clustering of cases as well as from reports of associations with environmental toxins such as organic solvents and hydrocarbons. Lung damage due to smoking has particularly been recognized as an important risk factor since pulmonary hemorrhage is rare in nonsmokers.[S4,S5]
The interaction between genetic and environmental factors can initiate or unmask the disease. HLA-DR15 susceptibility alleles may predispose individuals to anti-GBM disease by influencing reactivity of autoantigen specific T cells and altering the cytokine response.[S1] However, since these alleles are common in the healthy population, a second insult by an environmental factor may be required to precipitate tissue injury. In a subset of cases, the anti-GBM disease may be initiated by renal injury. There have been reports of anti-GBM disease following ureteric obstruction, urinary tract infection, lithotripsy and nephrectomy, suggesting that infectious or mechanical injury may be associated with release of autoantigens that may initiate disease in susceptible individuals.[S6,S7,S8]

The Antigen and the antibodies in anti-GBM disease:

The basement membrane contains type IV collagen and proteins including laminin, nidogen and heparan sulfate proteoglycans (most importantly, agrin) [Figure S1]. Type IV collagen forms the skeletal meshwork of basement membranes by the assembly of its six α chains (α1- α6).[S9,S10]
The collagen meshwork of glomerular and pulmonary basement membranes is composed mainly of α3, α4 and α5 chains. Each chain comprises of a long collagenous domain, a non-collagenous amino terminus 7S, and a non-

collagenous domain (NC1) at the carboxyl terminus [Figure S2, S3]. NC1 domains of the α3, α4 and α5 chains interact to form the α3.α4.α5(IV) triple helical extensively cross-linked molecule known as a protomer.[S11] These protomers dimerize at NC1 domain to form α3.α4.α5(IV) - α3.α4.α5(IV)NC1 hexamers which further interconnect with other hexamers to form the meshwork for the basement membrane. The target autoantigen in anti-GBM disease is the α3(IV)NC1.[S12,S13] The NC1 domain is composed of 232 amino acids where the autoantigen has been mapped to two epitopes designated EA and EB, at the amino acid residues 17-31 and 127-141, respectively[S14] [Figure S4]. The epitopes are partly buried during the assembly of α3.α4.α5(IV) - α3.α4.α5(IV)NC1 hexamers. Two types of these hexamers have been identified – the autoantibody reactive M-hexamers and the autoantibody impenetrable D-hexamers.[S15] The later are more abundant and have crosslinks between adjacent NC1 domains reinforced by sulfilimine bonds that help to maintain the crypticity of the Goodpasture epitopes and must be dissociated for autoantibody binding to occur. On the other hand, the less abundant M-hexamers lack the sulfilimine bonds allowing epitope unmasking and autoantibody binding, either spontaneously or induced by a concurrent inflammatory condition. The relative frequency and distribution of M and D types of hexamers may determine the likelihood of pulmonary hemorrhage in patients with anti-GBM disease.

The autoantibodies in patients with anti-GBM disease are most strongly reactive to α3(IV)NC1.[S16,S17] However, due to diversification of the immune response majority also show reactivity to α5(IV)NC1 and to a lesser extent α4(IV)NC1 domain. The antibodies may react with EA and EB epitope separately. Circulating anti-GBM antibodies may precede the onset of clinical disease by several months signifying the etiological role of additional factors. A small proportion of patients with anti-GBM disease who show characteristic linear glomerular deposition of antibody on immunofluorescence microscopy may not have demonstrable circulating antibodies with conventional assays. The presence of circulating anti-GBM antibodies is usually established by commercially available serum assay using a direct enzyme-linked immunoassay (ELISA).[S18] These assays use purified bovine a3(IV)NC1, recombinant antigen a3(IV)NC1 or a combination of both. Another alternative is indirect immunofluorescence microscopy that can detect the antibodies after patient's serum is incubated with sections of normal human kidney or commercially available sections from primate kidneys. Western blotting or biosensor techniques are more sensitive in picking up low-level antibodies that

might escape detection by indirect immunofluorescence.[S18] However use of both these techniques is limited to research laboratories.
The pathogenic antibodies are generally of the IgG class, in which IgG1 and IgG3 subclasses usually predominate. In rare cases, anti-GBM disease mediated by IgG4 antibody may be encountered.[S19,S20] It is noteworthy that healthy individuals may also have low levels of anti-GBM antibodies but they exclusively belong to IgG2 and IgG4 subclass and have low titer and avidity as compared to those in anti-GBM disease patients.[S21]

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Figures:

Figure S1:

Cartoon depicting the composition and important constituents of the glomerular and alveolar capillary basement membranes. The process of basement membrane formation starts with formation of a meshwork composed of collagen 4 (A). Additional proteins including laminin, nidogen and agrin (B) are added to the meshwork to form the normal basement (C).

Figure S2:

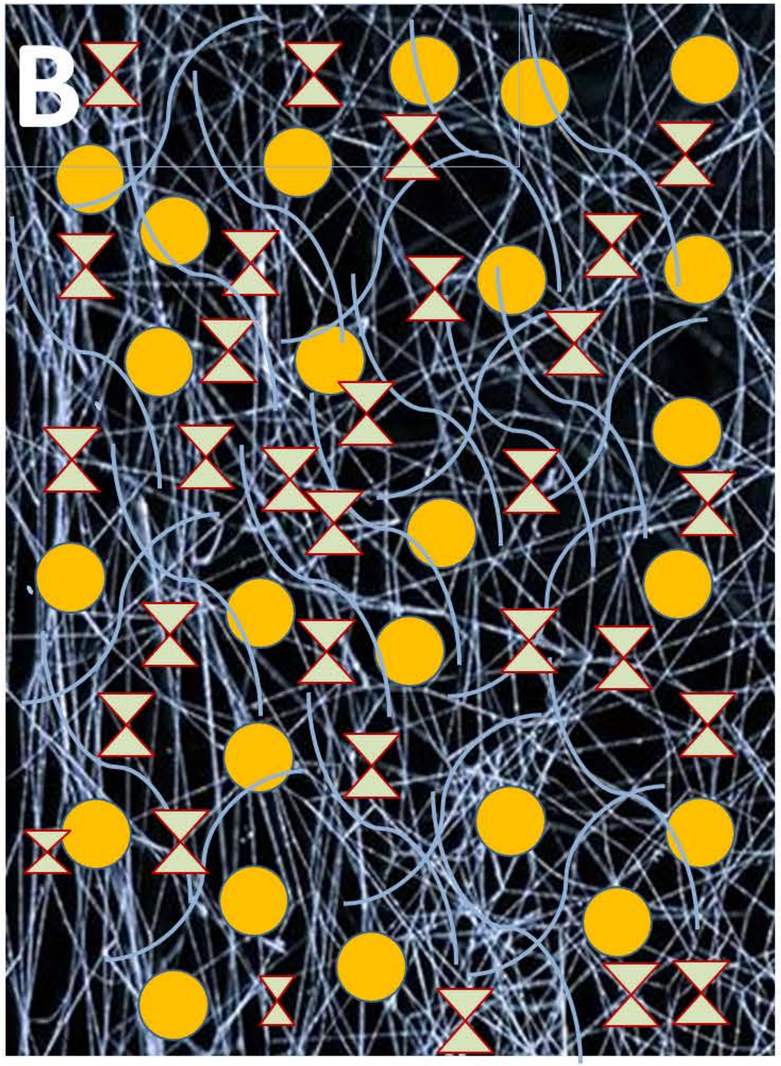
A diagram depicting a collagen 4 alpha chain composed of a long collagenous component with non-collagenous domain (7S) at the NH_2 terminal and another non collagenous zone (NC1) at the COOH terminal.

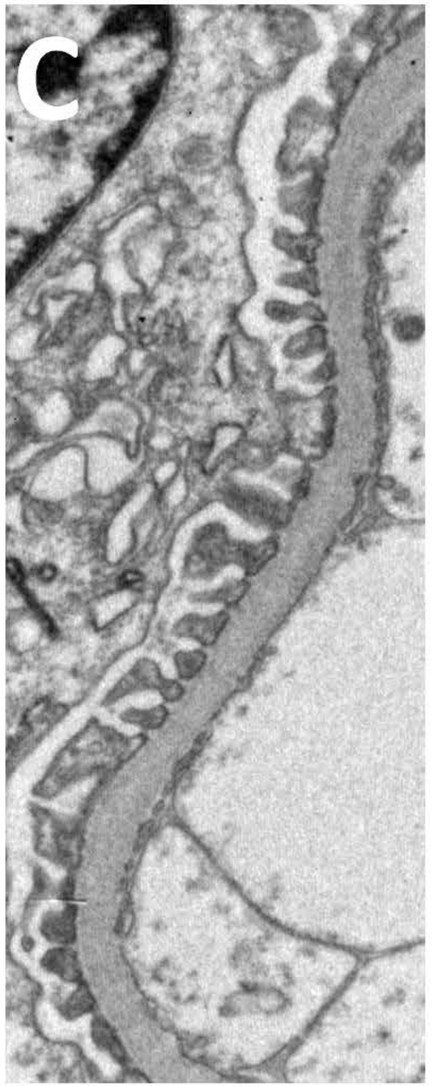
Figure S3:

Cartoon showing alpha 3, alpha 4 and alpha 5 chains of collagen 4. These chains join to form a triple helical extensively crosslinked molecule known as protromer. The protomers dimerize at NC1 domain to form alpha3,4,5NC1(IV) hexamers. The hexamers join to form the collagen 4 meshwork for the basement membranes.

Figure S4:

Cartoon showing typical structure of NC1 domain consisting of a polypeptide chain containing 232 amino acids. Two antigenic epitopes EA and EB are depicted in red.





Agrin Nidogen Laminin


7S
Collagenous
NC1
N
C

NC1

Alpha-3 (IV)

NH2

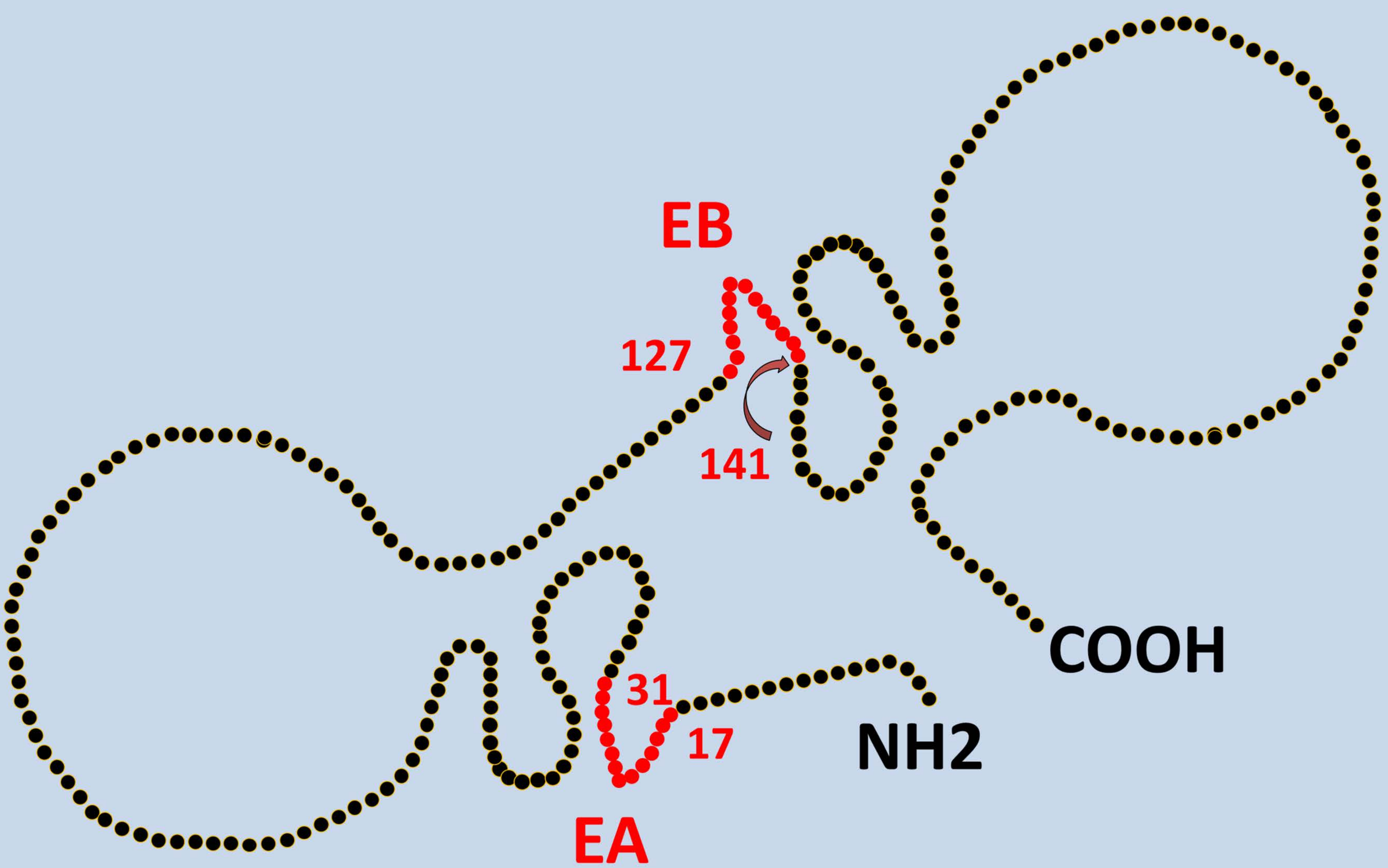
COOH

Alpha-4 (IV)

Alpha-5 (IV)

Protomer

Hexamer


EB
127
141
COOH
31
17
NH2
EA