Normal distribution of collagen IV in renal basement membranes in Epstein’s syndrome

Ichiro Naito, Shinsuke Nornura, Satoko Inoue, Megumi Kagawa, Shinichiro Kawai, Yoshimi Gunshin, Kensuke Joh, Chizuko Tsukidate, Yoshikazu Sado, Gengo Osawa

Abstract

Background—Epstein’s syndrome is defined as a subtype of Alport’s syndrome, and is distinguished from the other subtypes by accompanying macrothrombocytopenia. Mutations in collagen IV genes are known to be involved in the pathogenesis of typical Alport’s syndrome. However, the presence of an underlying genetic defect has not been demonstrated in Epstein’s syndrome.

Aim—To clarify the involvement of collagen IV in Epstein’s syndrome.

Methods—The distribution of the α(IV) chain was studied in renal specimens obtained from three patients with Epstein’s syndrome using chain specific monoclonal antibodies and an antigen retrieval procedure.

Results—The patients showed a normal distribution of α(IV) chains in α2(IV) and α2(IV) were expressed ubiquitously, whereas expression of α3(IV) through to α6(IV) chains was limited to the glomerular basement membrane, Bowman’s capsular basement membrane, and/or a portion of the tubular basement membrane.

Conclusions—These results suggest that genes other than those encoding α(IV) chains are responsible for the pathogenesis of Epstein’s syndrome.

(J Clin Pathol 1997;50:919–922)

Keywords: Alport’s syndrome; Epstein’s syndrome; macrothrombocytopenia; collagen IV; deafness

Alport’s syndrome is a progressive hereditary renal disease accompanying multiple abnormalities including sensorineural deafness, ocular abnormalities, macrothrombocytopenia, and diffuse leiomyomatosis.1 These abnormalities vary in expression and severity among families and patients.1 Epstein et al2 described two unrelated families affected with hereditary nephritis, deafness, and macrothrombocytopenia (Epstein’s syndrome). This is different from other subtypes of Alport’s syndrome by the presence of accompanying macrothrombocytopenia. Mutations in collagen IV genes are involved in the pathogenesis of X linked dominant1,3 and autosomal recessive Alport’s syndrome,4,5 and abnormalities in the α(IV) chain distribution have been demonstrated using immunohistochemical methods.8,9 Previously, we have studied all 51 exons of the COL4A5 gene in two cases of Epstein’s syndrome, but no mutations were found.7 This result does not belie the possibility that mutations in the COL4A5 gene might account for the pathogenesis of Epstein’s syndrome, because even in the X linked Alport’s syndrome, mutations in the COL4A5 gene have been demonstrated in fewer than 50% of patients.4,6 Epstein’s syndrome is known to be an autosomal dominant disorder,2 which suggests the involvement of COL4A3 or COL4A4, rather than COL4A5 in the pathogenesis of the disease.

Six different α(IV) chains have been identified genetically and immunohistochemically. We prepared monoclonal antibodies specific for the human α1(IV) through to α6(IV) chains and used them for the immunohistochemical analysis of fresh frozen sections of human tissues.10 Antigen retrieval has extended their application to paraffin embedded sections.11 A combination of monoclonal antibodies and antigen retrieval has enabled a retrospective study of the α(IV) chain in rare diseases. To clarify the involvement of collagen IV in the pathogenesis of Epstein’s syndrome, the distribution of all six α(IV) chains was determined in renal specimens obtained from Epstein’s syndrome patients using these immunohistochemical procedures.

Methods

Patients

Renal specimens were obtained from three patients from different families. The diagnosis of Epstein’s syndrome was based on the presence of a combination of nephritis, deafness, and macrothrombocytopenia. However, none of the patients presented ocular abnormalities.

Patient 1

At 3 years old patient 1 had been diagnosed with idiopathic thrombocytopenic purpura (ITP). Sensorineural hearing loss was recognised at 6 years of age. Giant platelets were found on her peripheral blood smear. The patient was treated for ITP but her platelet count remained in the range of 1–2 x 10^11/ml. Subsequently, the patient had proteinuria and microhaematuria. At the age of 16, she underwent a renal biopsy. Light microscopy revealed an almost normal glomerular shape and mild fibrosis in the interstitium, which contained foam cells. Electron microscopy revealed recirculation of the lamina densa in the glomerular basement membrane (fig 1).
All three patients satisfied three or four of the 10 criteria for the diagnosis of Alport’s syndrome proposed by Gregory et al. No history of hearing abnormality, renal abnormality, or macrothrombocytopenia existed in the families and relatives of these three patients. All 51 exons of the COL4A5 gene in patients 1 and 2 have been studied previously; however, no mutations have been demonstrated.

MONOCLONAL ANTIBODIES
The monoclonal antibodies used in the present study were H11 (anti-a1), H22 (anti-a2), H31 (anti-a3), H43 (anti-a4), H52 (anti-a5), and H63 (anti-a6). They were prepared against synthetic peptides of non-consensus regions of human a(IV) non-collagenous domains. Specificity was confirmed using western blotting and epitope mapping.

IMMUNOHISTOCHEMISTRY
Paraffin wax embedded renal sections were used for the analysis, although monoclonal antibodies are not very reactive with such sections; antigen retrieval was used to make the method more sensitive. The antigen retrieval procedure was as follows: the sections were heated in a small autoclave (KT2322, ALP Company Ltd, Tokyo, Japan) at 110–127°C for six minutes while they were immersed in 0.2 M HCl (pH 0.9). Subsequently, the monoclonal antibodies were applied to the sections. Bound monoclonal antibodies were detected using the LSAB kit (Dako, California, USA). To avoid technical errors, immunostaining was performed at least twice on sections heated at more than two different temperatures.

CONTROLS
Two renal specimens from patients without Alport’s syndrome or Epstein’s syndrome were stained according to the same method to act as positive controls. The recovery of antigenicity was poor in sections stored for extended periods. Renal specimens from patients 1, 2, and 3 were prepared between 1989 and 1994. Controls 1 and 2 were prepared in 1994 and 1989, respectively.

Results

CONTROLS
A combination of chain specific monoclonal antibodies and antigen retrieval revealed well demonstrated a1(IV) through to a6(IV) chains in the renal sections of controls 1 and 2. a1(IV) and a2(IV) chains were expressed ubiquitously in all basement membranes and mesangial matrices, whereas the other a(IV) chains showed a more limited distribution. a3 (IV) and a4(IV) chains were coexpressed in the glomerular basement membrane and in a portion of tubular basement membrane. They also appeared in Bowman’s capsular basement membrane at a low intensity. The a5(IV) chain was detected in the glomerular basement membrane, together with the a3(IV) and a4(IV) chains. a5(IV) and a6(IV) chains were detected in Bowman’s capsular basement membrane and a portion of the tubular basement membrane.
Collagen IV in Epstein’s syndrome

All three patients demonstrated significant positive staining for all α(IV) chains in the renal basement membranes. Figure 2 shows the results obtained from patient 1. Antigen retrieval demonstrated positive staining of α1(IV) to α6(IV) chains, with the same intensity and pattern of staining as seen in the controls. No significant abnormalities were found in the Epstein’s syndrome cases.

Discussion

Alport’s syndrome is classified into several subtypes according to its inheritance forms and accompanying symptoms. An X linked dominant form of Alport’s syndrome has been found to result from mutations in the COL4A5 gene, and an autosomal recessive form has been shown to result from mutations in the COL4A3 and COL4A4 genes. Immunohistochemical studies of α(IV) chains have revealed characteristic abnormalities in α(IV) chain distribution corresponding to the causative mutations in the collagen IV genes. Immunohistochemical abnormalities of the α(IV) chains in X linked dominant Alport’s syndrome have been well documented. In male patients, deletion mutations in COL4A5 result in the complete absence of α3(IV) to α6(IV) chains in renal basement membranes, whereas missense mutations (glycine substitution) result in a reduction in the antigenicity of these chains. In female patients, discontinuity of the antigenicity of α3(IV) through to α5(IV) chains has been described. In an autosomal recessive form of Alport’s syndrome, immunohistochemistry has revealed a complete absence of α3(IV) and α4(IV) chains associated with the partial absence of α5(IV) chains in the glomerular basement membrane. In the present study, Epstein’s patients demonstrated normal antigenicity in all α(IV) chains in the renal basement membranes, raising the possibility that genes other than those coding for collagen IV are involved in the pathogenesis of the disease.

Normal antigenicity of collagen IV has also been reported in X linked
recessive types\textsuperscript{10} of Alport’s syndrome patients, but the incidence of such findings is very low. In contrast, normal antigenicity was recognised in all three patients with Epstein’s syndrome. Thus, the appearance of normal antigenicity suggests that abnormalities of a glomerular basement membrane component other than collagen IV could be involved in the pathogenesis of the disease.

Laminin, nidogen, and proteoglycans are expressed in renal basement membranes.\textsuperscript{11} Laminin is a non-collagenous constituent of basement membranes that shows structural heterogeneity as a result of differences in the subunit chains. Investigation of the tissue localisation of these subunits has revealed that some are restricted to the glomerular basement membrane. Nidogen is a glycoprotein that is distributed abundantly in all basement membranes and has been found throughout the glomerular basement membrane. Heparan sulphate proteoglycan is present in the glomerular basement membrane, and has been found to play an important role in the selective permeability of the glomerular basement membrane.

Abnormalities in any of these glomerular basement membrane components can induce renal dysfunction, morphological abnormalities of glomerular basement membrane, and possibly macrothrombocytopenia. To identify the involvement of genes encoding these proteins, linkage studies must be performed using flanking marker genes.

In conclusion, to clarify the involvement of collagen IV in the pathogenesis of Epstein’s syndrome we investigated the distribution of the \(\alpha 1(IV)\) through to \(\alpha 6(IV)\) chains in renal sections from patients suffering from this syndrome. However, immunohistochemistry revealed no abnormalities associated with collagen IV molecules.