THE CASE OF THE TWO DISPARATE DISEASES: A MEDICAL MYSTERY

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THE MYSTERY

The propositus is a young white male who at the age of 16 was evaluated for gross hematuria. Full urologic evaluation failed to find the etiology of the hematuria. It was known at the time that the mother had some variety of "nephritis" which also was characterized by hematuria but had fairly stable, renal function. When a marked hearing deficit was appreciated, a diagnosis of hereditary hematuria with deafness was entertained (Alport's syndrome). The patient had two brothers who were screened at that time. Both brothers were shown to have hematuria, one with sensorineural hearing deficits and one with an elevated serum creatinine. One additional brother seems to have hematuria but no renal insufficiency. The propositus was placed on chronic hemodialysis at the age of 20 and underwent a 2/4 match cadaver renal transplant at the age of 24. The transplant functioned immediately with urine flow rates up to as much as 3 liter/day with a steady decline in serum creatinine. On the sixth hospital day the patient developed graft tenderness, fever, and a rising serum creatinine with hematuria which was partially responsive to bolus steroids. A biopsy performed on the 14th hospital day revealed evidence for acute rejection and a second course of anti-rejection therapy was given. On the 21st hospital day, the patient developed hematuria and rapidly declining renal function. He underwent transplant nephrectomy which discovered interstitial infiltrates and crescentic glomerulonephritis with areas of frank infarction. The tissue was thought to be consonant with acute rejection. He returned to hemodialysis where he did reasonably well until the age of 29 when a second cadaveric allograft was attempted. The second attempt had similar results with initial function followed by decline in renal function unresponsive to anti-rejection treatment characterized by rapid progression to oliguria and by hematuria. Again morphology of the failed graft revealed round cell infiltrates and a crescentic glomerulonephritis. He returned to hemodialysis.

Interestingly, both affected brothers subsequently required hemodialysis. One sib required dialysis at the age of 14, the year after discovery of the disease in the propositus. He received a cadaver renal transplant at the age of 16 which had urine flow but little clearance in recovery room. Over the first six hospital days the creatinine slowly declined from 10.1 to 5.1 mg/dl. The slow decline in serum creatinine was felt to be related to an underlying, asymptomatic rejection and 4 grams of bolus steroids were administered without altering the clinical status of the patient. On the 12th hospital day, the patient developed acute hematuria and became suddenly anuric. He was returned to dialysis and underwent a nephrectomy one month thereafter. Again the pathologic interpretation of the nephrectomy specimen was read as acute rejection with round cell infiltrates in the interstitium, but again with an interesting crescentic glomerulonephritis which was not remarked upon at that time but discovered upon reexamination at a later time in the face of the transplant experience of the third sib.

The third sib, who had only microscopic hematuria at the age of 18 when his older brother, the propositus, was found to have renal failure declined rapidly into uremia requiring hemodialysis at the age of 22. A cadaver transplant was found for the third brother in the same year as his two other sibs, which functioned immediately and dropped the serum creatinine to 2.5 mg/dl by the ninth hospital day. By the 15th hospital day the serum creatinine began to rise and the patient exhibited gross hematuria. Steady decline in renal

function precipitated return to hemodialysis two months after the placement of the allograft. A transplant nephrectomy was performed and again was characterized by round cell infiltrate and crescentic glomerulonephritis.

In brief, three young brothers with hereditary hematuria and deafness received a total of four transplants, all of which failed in a manner unresponsive to conventional anti-rejection medicines. The pathologic constant was an interstitial infiltrate and a crescentic glomerulonephritis, the latter of which is an uncommon finding in uncomplicated cellular rejection. Because of the similarities between the morphology of the transplant nephrectomy specimens and the rapidly progressive renal failure clinical course of Goodpasture's disease, a serum collected for virology studies of the propositus during the admission for the second allograft was screened for the presence of anti-glomerular basement membrane antibodies which were found present in high titer.

A mystery is posed. Is the appearance of two disparate diseases, Alport's syndrome and anti-GBM nephritis in a transplant a mere curious concatenation of events or are the diseases inexplicably tied in a causal manner? A renal transplant is susceptible to all of the diseases that can assault the normal kidney. Is it reasonable to believe that Goodpasture's disease is an incidental finding in these patients with Alport's? Does the history of four graft losses in three brothers with a similar unusual morphology suggestive of Goodpasture's disease argue that these two entities must be tied together?

It is the purpose of these grand rounds to bring the requisite scientific and clinical clues together to solve this very real mystery posed at the bedside. To solve this mystery, I will be sorting out various clues that might be gleaned from an explication of each of the disease entities that have been presented, Alport's syndrome and anti-GBM nephritis. As each clue falls in place, we may be closer to the solution of the mystery of the "Case of the Two Disparate Diseases".

ALPORT'S SYNDROME: Clue #1

Dickinson in his book, "Diseases of the Kidney and Urinary Derangements", published in 1875 first described genetically transmitted renal disease in the English literature in a family which initially presented with albuminuria and microscopic hematuria. Later, Guthrie described familial, recurrent, idiopathic hematuria in a large family with some affected members exhibiting hematuria even at birth in the Lancet in 1902. When renal failure ensued in this same family, Hurst was able to add to the concept of familial hematuria a lethal outcome in his paper in the Guy's Hospital Review in 1923. A.C. Alport reexamined this index family in 1927 drawing particular attention to the concomitant occurrence of sensorineural deafness in some members afflicted with nephritis and hematuria and to the importance of sex in the penetrance of the clinical phenotype. Subsequently the eponym, the Alport's syndrome, has been used to describe the progressive and hereditary renal disease which begins in early childhood and is characterized by recurrent or even persistent hematuria, progressive proteinuria and renal failure associated with a neurosensory hearing loss with a more rapid and more severe progression to renal failure found in afflicted males.

Classical Alport's syndrome is one of a series of hereditary glomerulopathies which may lead to renal failure (Table I) most which reflect a familial disorder related to protein excretion.

Table I. Heredofamilial Diseases with Prominent Glomerular Involvement

Classic hereditary chronic nephritis with deafness (Alport's syndrome)
Variant hereditary chronic nephritis (with or without deafness) with hyperprolinemia with ichthyosis with macrothrombocytopenia with Charcot-Marie-Tooth disease with ocular abnormalities
Familial "benign" hematuria with thin glomerular basement membrane
Angiokeratoma corporis diffusum universale (Fabry's disease)
Osteo-onychodysplasia (nail-patella syndrome)
Partial lipodystrophy and membranoproliferative glomerulonephritis
α_1-Antitrypsin deficiency with glomerulonephritis with hepatic fibrosis and subepidermal immunoprotein deposits
Urticaria-deafness-amyloidosis (Muckle-Wells syndrome)

Only a few of the disorders can be truly considered part of the differential diagnosis of familial hematuria. The most interesting differential diagnosis to make in the face of hematuria in the absence of renal insufficiency is that of thin glomerular basement membrane disease, a condition thought to be completely benign which was first described in San Antonio (Figures 1 and 2).

Figure 1

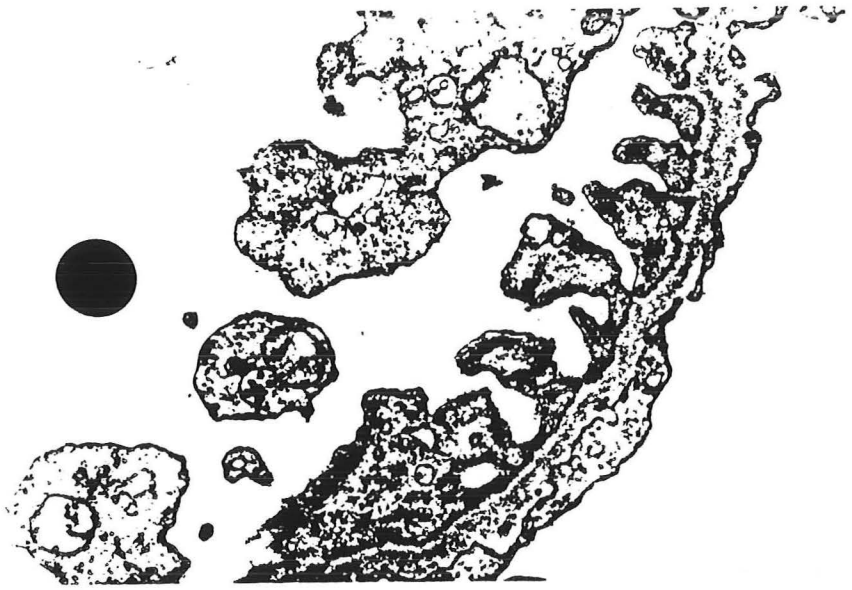


Figure 2

In contrast, all the variant clinical presentations of the classic chronic nephritis which when coupled to deafness one calls Alport's syndrome represent truly no different pathogenic entity as will become clear at the end of the solution of our mystery.

Classic Alport's syndrome, then, is a triad of inherited hematuria, progressive azotemia, and inherited deafness as described by A. Cecil Alport at St. Mary's Hospital in Paddington, London. Alport's singular contribution to the literature was the recognition of the fatal outcome for the majority of cases and for the recognition of the deafness. Alport had available to him for study a later generation of the initial series. As was described by Hurst, nephritis, hematuria, minimal hyperalbuminuria, and renal failure were found in this later generation. Alport writes,

> "...on January 4, 1924 I was asked to see a boy belonging to the third generation of this family...The boy's great grandmother... lived to the age of 90 years old and was extremely deaf. Her daughter had a severe attack of hematuria...but less severe attacks after the births of her younger children... She is very deaf, but her general health is excellent. Her two elder brothers both suffered from hematuria and died in childhood. One sister is deaf and another slightly so, but they do not suffer from the disease of their kidneys. All of her children with the exception of one male child developed hematuria...and catching cold or influenza brought on attacks. It will be seen from this analysis that nearly all the children of the three generations of one family suffered from hematuria or nephritis associated with deafness. The deafness which has not been stressed in the literature on the subject is one of the most distressing features of this extraordinary disease."

Most of the clinical features that we in modern time associate with Alport's syndrome were described in that paragraph by A. Cecil Alport. The most common feature that brought the initial family to medical attention is microscopic or gross hematuria, which often leads to a great deal of urologic evaluation in young children today in families not yet identified. The hematuria may be present as early as birth in male members of the family. Gross hematuria can be precipitated by exercise, eating of certain foods, or upper respiratory infections. Minimal proteinuria is common, so common that congenital proteinuria was felt by some to be a separate illness. However, frank nephrotic syndrome is an uncommon feature of Alport's syndrome, although it has been described. There has been some confusion concerning whether patients have hypertension associated with Alport's syndrome. Initial reports felt that hypertension was reasonably common, but hypertensive subjects all had near end stage or end stage azotemia. When carefully examined, afflicted children in the absence of frank azotemia are not hypertensive. The hypertension found in the azotemic patients is felt to be on the basis of volume expansion and not a result of a nephritic diathesis. As the deafness is an important differential point to distinguish Alport's syndrome from other hereditary hematurias and as the deafness might provide an important clue to the solution of the mystery in our index patient, it is reasonable to examine more closely the nature of this unique deafness (Table II).

Table II

DEAFNESS

1. Of the sensori-neural variety
2. Gamut from total deafness to detectable only by formal audiometry
3. 30-50% of patients with a hereditary hematuria syndrome exhibit deafness
4. A variant of hereditary hematuria is devoid of hearing problems
5. Presence of or severity of deafness unrelated to severity of renal disease

The deafness can run the gamut from total deafness to that detectable only by formal audiometry. It clearly is of a sensorineural variety. This audiogram is from a male who died with nephritis at the age of 24 (Figure 3). It shows the reduction in hearing acuity in both left and right ears as frequency is increased. The defect can be exhibited at even modest frequencies but it is most pronounced at the high decibel levels. Thus, this variety of deafness has been incorrectly called a high frequency hearing loss. Although it is true that the high frequencies are most affected, one can observe hearing deficits across

the frequency range. The deafness may either be complete when first discovered or be a progressive hearing loss. Detection can be early, even in childhood, like hematuria. If studied early enough the defect can be present in early infancy.

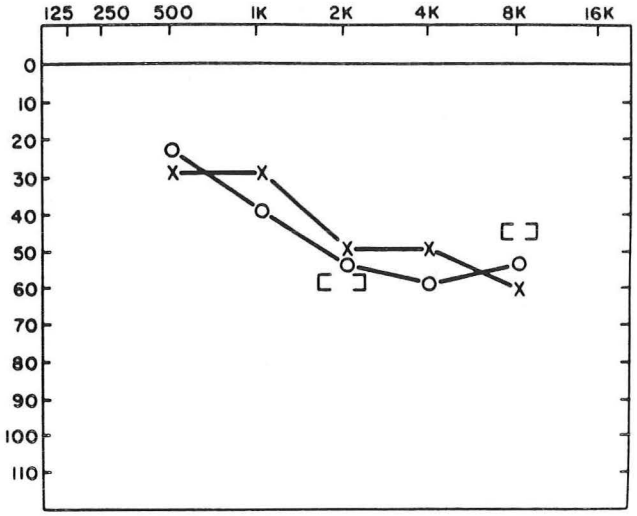


Figure 3

The tracing that is shown in Figure 3 is called a type-II, Bekesy tracing with a normal tone decay which are features of a sensorineural hearing deficit. If one tests responses with thermal stimuli, one can detect increasing vestibular neural recruitment again suggesting a sensory defect of the vestibular-cochlear apparatus. Although not invariant in patients with hereditary hematuria, as many as 50% of the patients with this disease will exhibit some degree of deafness, even if detectable only by formal audiometry. Interestingly the degree or severity of the deafness is completely unrelated to the severity of renal disease. The presence or absence of total deafness does not predict which of the patients will have a dire outcome from their nephritis.

Histology of the cochlear apparatus in affected patients with Alport's disease include such defects as loss of cochlear neurons, atrophy of the spiral ligaments, loss of hair cells, partial degeneration of the basalis vascularis, and an unusual basophilic staining material in the substrial zone of the spiral ligament (Figure 4).

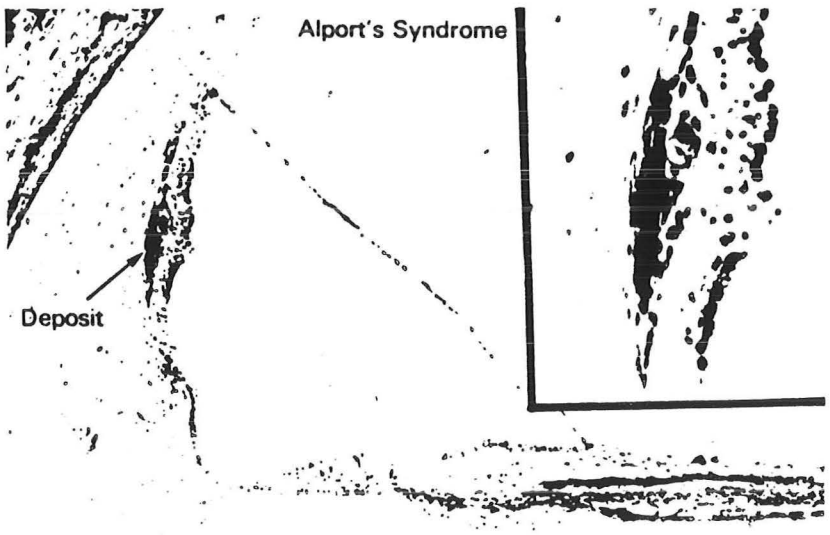


Figure 4

By electron microscopy, there appears to be fracturing in the basement membrane of the same strial ligament. Presently the basophilic staining or deposit material is thought to be secondary to a defect causally related to the fracturing of this basement membrane. Fractured basement membranes will be a theme of these rounds and clearly constitute one important clue to the solution of our mystery.

Structural defects in patients with hereditary hemorrhagic nephritis are not confined to the vestibulocochlear system, it has been learned in the last 30 years. Although Jaworski in the German ophthalmology literature first characterized an unusual lens abnormality, anterior lenticonus, in several patients with hemorrhagic nephritis, lens and capsular abnormalities were not clearly recognized as a possible related finding to renal disease until Sohar's review in 1956. Arnott and colleagues in a classic paper in 1966 clearly demonstrated that lens and capsular abnormalities were associated with that disease that A. Cecil Alport had described. It is now clear that a wide variety of ocular manifestations may be part of the clinical entity we call Alport's syndrome. These abnormalities include the classical anterior lenticonus, spherophakia and rarely posterior lenticonus (Figures 5 and 6).

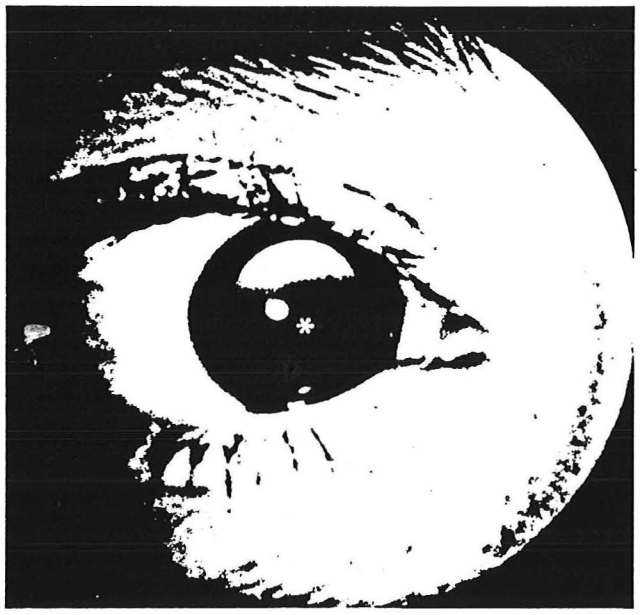


Figure 5

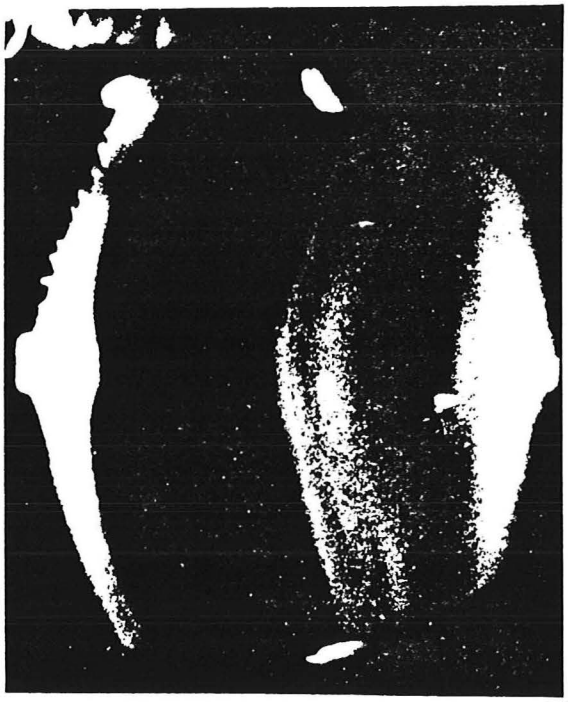


Figure 6

Thompson and colleagues in Birmingham, England attempted to examine whether ocular abnormalities were more common in patients with mild renal disease or serious renal disease. For study, they had 42 individuals belonging to 18 separate families, the majority of which had biopsy proven nephritis of the Alport's variety with hematuria and mild proteinuria but no renal failure and 19 patients from 11 different families, the majority of which also had positive biopsies with severe renal disease. Ten patients in these two groups had ocular changes associated with their Alport's disease. In the Table one can see that the majority of the patients were male, that there was a wide range of retinal findings, and that half of the patients had severe renal disease and the other half less severe (Table III).

Table III *Ocular signs in 10 patients with Alport's syndrome*

No.	*Sex*	*Age*	*Deaf*	*Renal Status*	*Ocular signs*
1	M	6	–	Haematuria	Posterior polymorphous dystrophy
2	M	14	+	Mild proteinuria	Posterior polymorphous dystrophy
3	M	15	+	Mild proteinuria	Perimacular flecks
4	M	18	+	CRF—dialysis	Arcus juvenilis anterior lenticonus
5	M	20	+	CRF—dialysis	Perimacular flecks
6	M	21	+	CRF—transplant	Posterior subcapsular lens opacities
7	M	30	+	CRF—dialysis	Arcus juvenilis
8	F	31	–	Haematuria	Macular flecks
9	F	33	+	CRF—transplant	Anterior lenticonus posterior subcapsular lens opacities
10	F	36	–	Haematuria	Flecks above upper temporal vessels (R)

CRF = Chronic renal failure.

From this study it has been stated that the posterior polymorphous dystrophy is the most common and the most specific retinal lesion associated with Alport's disease characterized histologically by a thickening of Descemet's membrane (a basement membrane) with fracturing within that membrane. The anterior lenticonus is associated with thinning of the capsule the result of a defect in the lens epithelial basement membrane. In the Thompson study other abnormalities of the retina were found to be present in patients with Alport's disease, such as "retinitis pigmentosa" and "retinal flecks" (Figures 7 and 8).

Figure 7

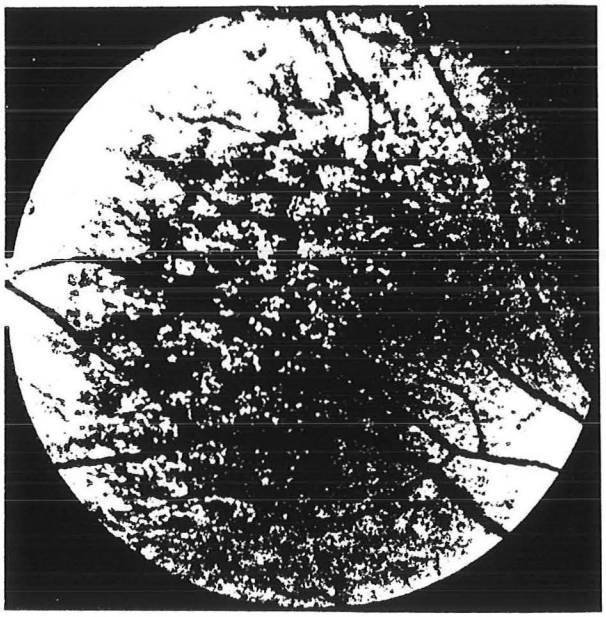
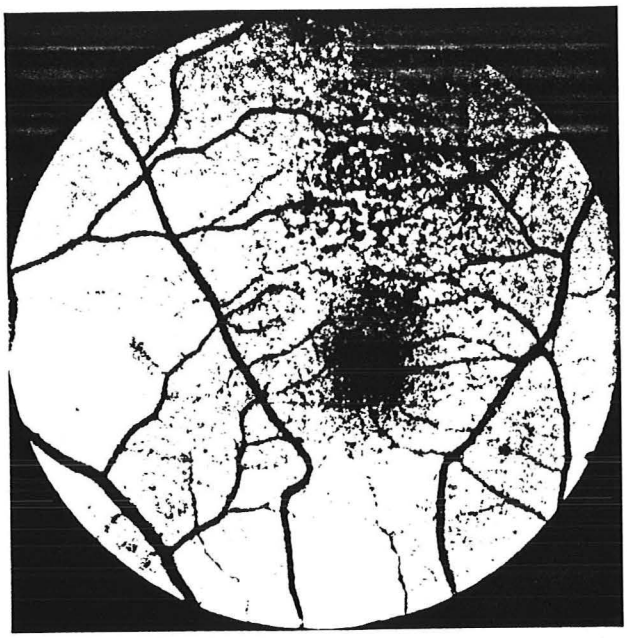


Figure 8



It was the feeling of the Thompson group that these other retinal abnormalities described, in contrast to the structural defects, are associated with the progression of renal failure and either are harbingers of impending renal insufficiency or manifestations of that problem.

The classic work relating ocular manifestations of Alport's to potential defects in the basement membranes in the globe is that of J. A. Govan from the Department of Ophthalmology at Guy's Hospital. Govan recognized the concomitant appearance of ultrastructural abnormalities in the basement membrane of the kidney, the eye, and the cochlea. The fractured basement membrane of the vas spirale, and of the basement membranes in the posterior portion of the globe and of the capsular lens he felt was part of a common basement membrane defect inherited by patients with Alport's disease. These speculations bring us closer to the solution of our mystery and provide an additional clue to the concomitant occurrence of Alport's disease and Goodpasture's disease in transplanted subjects.

To further develop the theme of a common basement membrane defect in a syndrome that involves, in the least, the kidney, the eye, and the ear, I would like to examine the pathology of the kidney in patients with Alport's syndrome. The history of pathology in this entity is laden with misconception and misunderstanding. We owe much to G.D. Perkoff who made the study of Alport's syndrome an important element of his life's work. Unfortunately, he was so overwhelmed by the morphologic changes in the renal interstitium that he believed Alport's syndrome to be an interstitial nephritis. Indeed, the disease has often been miscalled "hereditary interstitial pyelonephritis" or "hereditary interstitial nephritis". Gross appearance of the kidneys removed at autopsy or in patients deceased with this disorder supported the initial confusion. The subcapsular surface appears finely granular, and although course scarring seen in chronic pyelonephritis is lacking, the pitting on the surface suggested to some an interstitial nephritis pattern. In advanced cases, tubular atrophy and

dropout become important with the interstitium being replaced by fibrosis and a nonspecific chronic inflammatory infiltrate (Figure 9a).

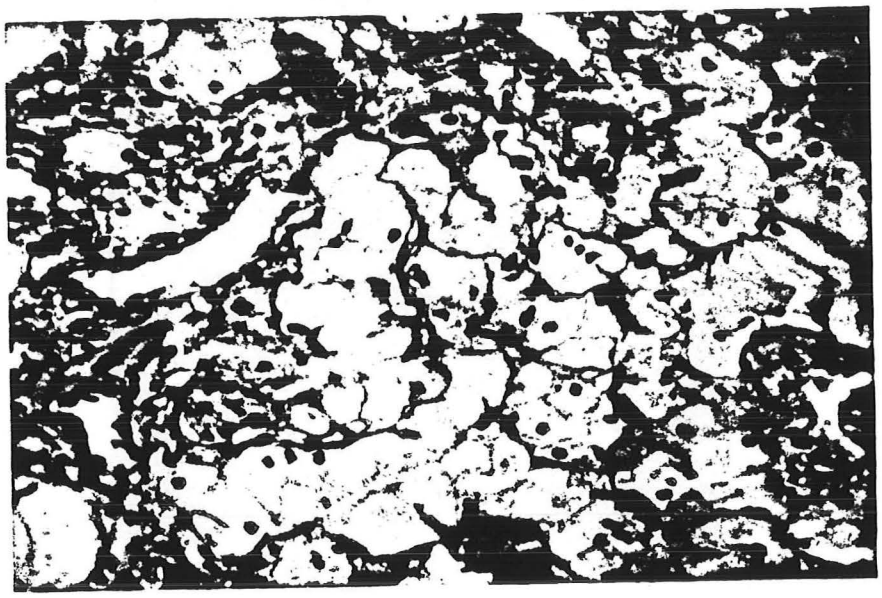


Figure 9a

Interstitial abnormalities are so prominent that the important glomerular lesions were overlooked for many years. Prominent foam cells are present in the interstitium often occurring in large clusters in the reanl cortex (Figure 9b).

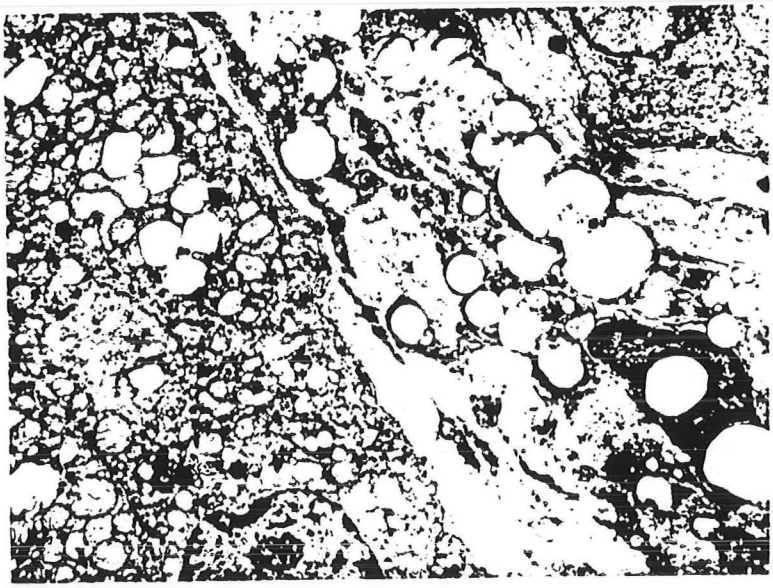


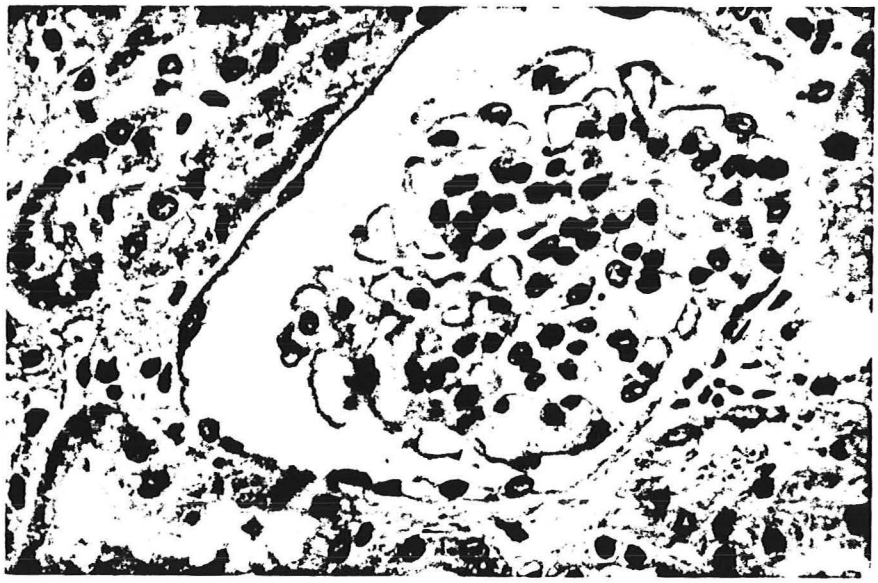
Figure 9b

The foam cells were once thought to be pathognomonic of this disease. It is now clear that foam cells are found widely in any proteinuric disease although cortical foam cells remain suggestive of Alport's. Even after glomerular defects were appreciated, the interstitial abnormalities were so overwhelming that some in the earlier literature continued to call this entity a mixed pathologic picture comprised of both glomerulonephritis and interstitial nephritis as did

Krickstein in 1966.

In contrast to this, the glomerulus appears to be the focus of important pathologic changes in this disease. Almost any pattern may be seen by light microscopy with an increase in mesangial substance, mesangial hypercellularity, localized areas of necrosis all described as common features by Heptinstall (Figure 10).

Figure 10



Frank focal proliferative glomerulonephritis with crescents has also been described. Segmental global glomerulosclerosis, focal thickening of the basement membrane, and messangial changes are all part of a nonspecific light microscopic picture that can be seen as originally described by Antonovych in 1969 and emphasized by G.S. Spear. As it became clear that Alport's syndrome was primarily a disease of the glomerulus, an initial assumption was advanced that Alport's was another variety of immunologically mediated renal disease. This notion was rapidly dispelled by Spear and colleagues as they found the glomerulus devoid of immune complex reactants as adjudged by immunofluorescence.

Spear and Slusher in 1972 pointed directly to the integrity of the basement membrane as the locus of the defect in the Alport's syndrome. They, and now a host of others, demonstrated a characteristic, if not pathognomonic finding, universal splitting of the basement membrane into a series of electron lucent areas which look like interweaving walls or lammeli. In some regions the basement membrane might be thickened, in other areas thinned (Figures 11 and 12). There is an absence of electron dense deposits confirming the negative immunofluorescent studies. Although Gary Hill argued in 1974 that basement membrane splitting might not be unique to Alport's syndrome as he was able to find examples of such splitting on a focal basis in a number of different diseases, he failed to find the universal splitting characteristic of Alport's in any other entity. I prefer to read Hill's paper as actually supportive of the pathognomonic nature of universal splitting of the basement membrane in Alport's syndrome. There are both practical and theoretical consequences for

concentration on the basement membrane as the pathogenic locus in Alport's syndrome. Practically, although one can see focal splitting of the basement membrane in other diseases, and although one can perhaps find a case of essential hematuria without such splitting, the finding on electron microscopy of universal, diffuse splitting of the basement membrane should raise in the clinician's mind a diagnosis of Alport's syndrome.

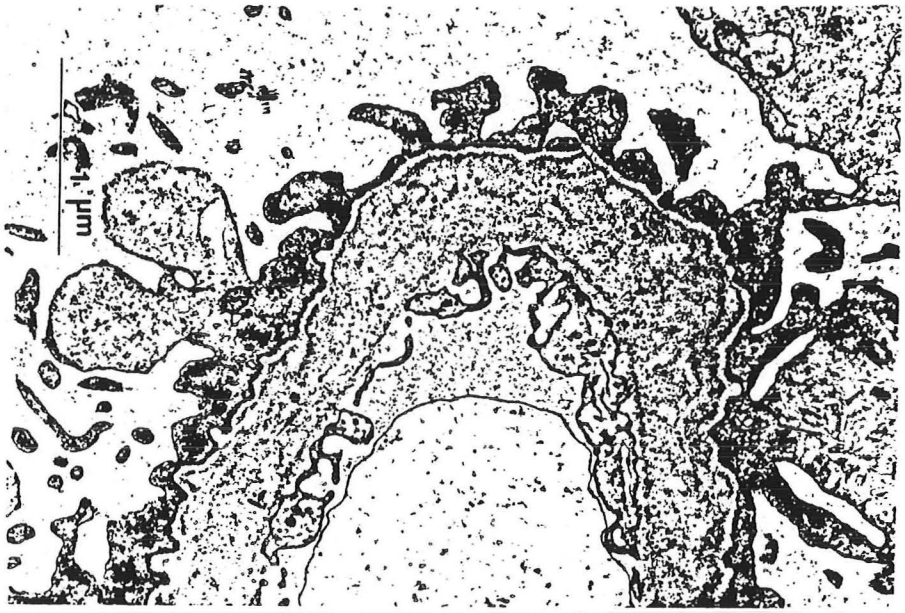


Figure 11

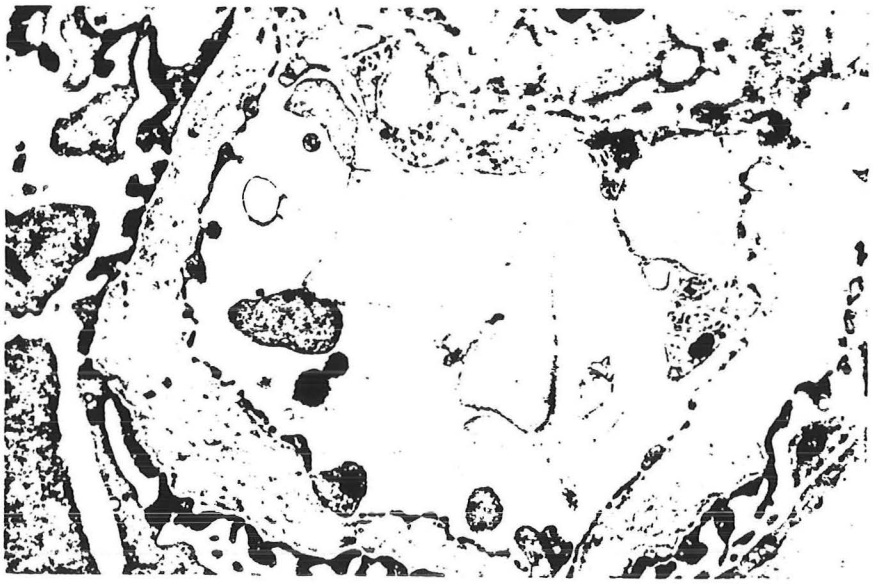


Figure 12

Theoretical structural defects in the basement membranes of the kidney, the lens capsule, in the inner ear all point to a potentially common anatomic and/or biochemical abnormality inherited in patients with Alport's syndrome. The nature of this abnormality might actually help to solve the mystery of the "Case of the Two Disparate Diseases".

Nosologically, some have sought to classify Alport's syndrome into unique entities based on genetics and on appearance of renal failure in childhood or adulthood. In my view, such nosological gymnastics are less interesting. This Ven diagram shows the complexity out of which the "splitters" can make of Alport's syndrome in which five separate types are defined (Figure 13).

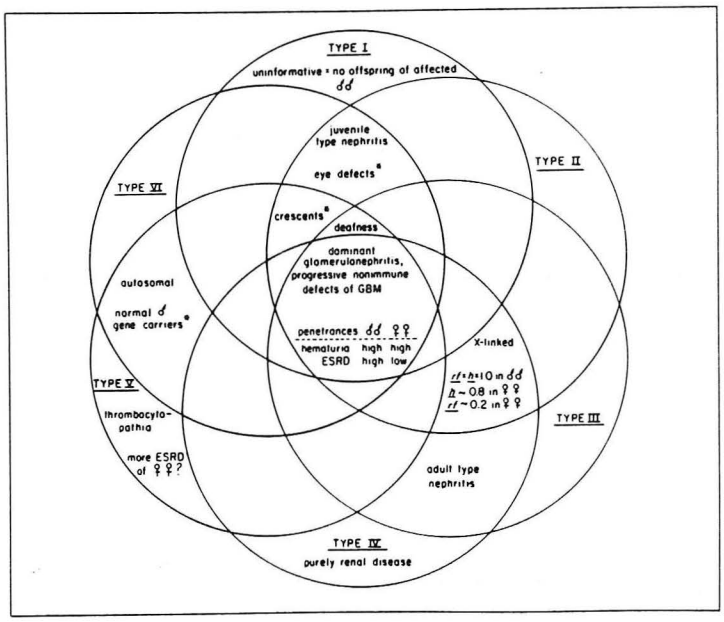


Figure 13

The overlapping segments of the circle become so confusing as to be of little use clinically. The disease, interestingly, is relatively common and constitutes approximately 3% of the patients with chronic renal failure in children and about 14% of the patients receiving a kidney transplant in 1975. I dare say this figure has fallen with the advent of transplantation in diabetic subjects, but clearly the disease is not an extraordinarily rare one. Part of the nosologic discrimination is based on the heterogeneity of genetic inheritance of Alport's disease. The initial British family from which the Alport's report was generated suggested a Mendelian dominant inheritance with incomplete modes of penetrance such that males were more affected. Other kindreds follow autosomal recessive patterns of inheritance. Feingold in Paris examined 41 separate kindreds supporting evidence for autosomal dominant, X-linked dominant and autosomal recessive forms of transmission in different families. The most interesting observation with respect to inheritance deals with an abnormality discovered in the long arm of the X-chromosome by Atkin and colleagues in 1988. The Alport's syndrome locus is now located to a site on the long arm of the X-chromosome distal to the p 19-2 marker. With the chromosomal site determined it is hoped that the precise base pair defect can be described in the very near future for some of the types of Alport's syndrome. Restriction fragment length polymorphisms have been created and are presently being analyzed.

An explication of the clinical syndrome of Alport's syndrome, then, provides several important clues to the solution of our mystery. A structurally or chemically defective basement membrane present in the kidney, the eye and the ear has drawn our attention. It is now time to move to a new path of inquiry

in search for new clues to the solution of our mystery.

CLUE #2: GOODPASTURE'S DISEASE

The association of lung hemorrhage, proliferative glomerulonephritis, and rapidly progressive renal insufficiency was recognized by Earnest Goodpasture in 1919 in a young man who developed this constellation of findings subsequent to an influenza viral infection. Although the index case probably did not have Goodpasture's Disease as we understand it today, Stanton and Tange coined the eponym Goodpasture's syndrome to describe cases of pulmonary hemorrhage accompanying necrotizing glomerulonephritis. As will become clear, an identical clinical syndrome can be created in laboratory models which develop or are administered antibodies directed against the glomerular basement membrane. A confusion has grown in the medical literature out of the difference between the clinical presentation recognized by Stanton and Tange and the pathogenetic mechanism which accounts for some of the cases. Goodpasture's syndrome as used by Stanton and Tange is reflective of a clinical triad alone; to wit, pulmonary hemorrhage, hematuria with proliferative and necrotizing glomerulonephritis, and rapidly progressive renal failure. Many different disorders may present with this triad as shown in Table IV.

Table IV

GOODPASTURE'S SYNDROME

DIFFERENTIAL DIAGNOSIS

1. Anti GBM ab nephritis
2. SLE
3. Other vasculitides especially Wegner's
4. Nephritis with heart failure

Anti-GBM antibody nephritis can certainly cause all three elements of Goodpasture syndrome, but probably is the rarest etiologic form of the syndrome. Lung hemorrhage, necrotizing GN, and renal failure can be seen in a small number of cases with systemic lupus erythrematosis. The triad may also be found with other systemic vasculitides especially Wegner's granulomatosis. Indeed the index case of Goodpasture probably was a case of vasculitis and not anti-GBM antibody nephritis. Interestingly, in numeric terms, the most common association of hematuria, renal failure and nephritis is garden variety nephritis associated

with volume overload and heart failure. It is appropriate then, to separate Goodpasture's syndrome from Goodpasture's disease. The syndrome is correctly applied to patients with the clinical triad discussed regardless of etiology. Goodpasture's disease ought to be reserved for those patients who exhibit elements of the triad associated with proven anti-glomerular basement membrane antibody production. To further confuse the nosologic picture, rapidly progressive glomeulonephritis, the variety initially associated with Goodpasture's syndrome has a rather large differential diagnosis (Table V).

Table V

RAPIDLY PROGRESSIVE GN

1. Anti GBM nephritis
2. Idiopathic Crescentic GN
3. Cryoglobulinemia
4. Post infectious GN
5. SLE
6. Other vasculitis
7. SBE
8. Hemolytic uremic syndrome
9. Hennoch-schonlein purpura
10. Post partum renal failure
11. Malignant hypertension

Although anti-GBM nephritis or Goodpasture's disease is listed first, it is one of the rarest varieties of rapidly progressive GN. Even idiopathic crescentic glomerulonephritis, a morphologic picture by light microscopy similar to Goodpasture's disease without evidence of anti-GBM antibody formation is probably more common.

Although patients from age 40-80 have been observed to have Goodpasture's disease, the majority of cases occur in young adult males in the third and fourth decades of life (Figure 14).

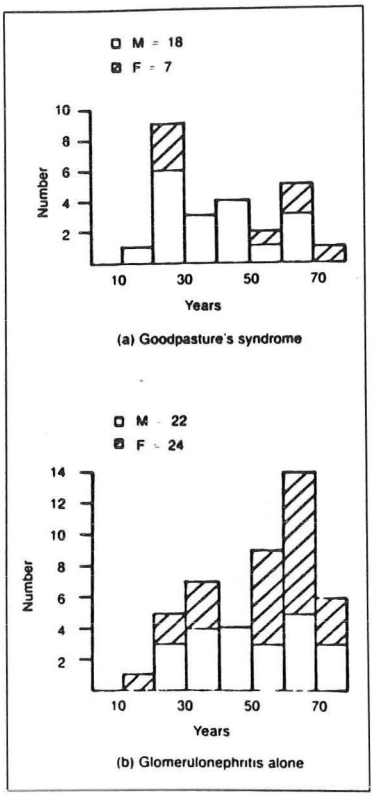


Figure 14

Since Goodpasture's disease reflects the presence of anti-GBM antibody, not all cases have all three elements of the triad which defines Goodpasture's syndrome. Pulmonary findings are common in most subject and may precede or be found simultaneous with renal findings in up to 70% of reported cases. Lung hemmorhage runs the gamut from intra-alveolar bleeding detected only by the careful physician on the basis of lab abnormalities and/or a chest x-ray to frank exsanguinating hemoptysis. If lung findings precede renal disease, the latent period averages no more than three months. In a smaller group of patients, the onset of Goodpasture's disease may be heralded by renal signs and symptoms followed by pulmonary systems in a mean of three months later. In the major review of Wilson and Dixon in 1973, 28 of 32 patients with anti-GBM antibodies were in frank renal failure from 1-14 months after onset of disease with a mean of 3 1/2 months. Fully, one half of the 32 patients died despite intervention by dialysis or transplant, eight from respiratory therapy in their series. Three quarters of the patients presented with hemoptysis, almost half with dyspnea, and 40% with gross hematuria (Table VI). The episodes of gross hematuria and/or hemoptysis seem to follow in many cases an upper respiratory infection and five of the 32 had frank flu-like illness.

On physical exam, hypertension with a diastolic above 110 mmHg was uncommon, but mild hypertension was the rule. Clinically detectable physical findings of anemia were often present. In particular, mucosal pallor is often

remarked upon as being unusual for the age group of the patient afflicted. Pulmonary signs were common including intra-alveolar rales or frank rhonchi, but pulmonary edema was uncommon.

Table VI

Clinical feature	Goodpasture's patients	Non-Goodpasture's patients
Presenting clinical illness		
hemoptysis	23/32 (72%)	0/18 (0%)
dyspnea	14/32 (44%)	2/18 (11%)
weakness	10/32 (31%)	7/18 (39%)
fever	5/32 (17%)	2/18 (11%)
gross hematuria	13/32 (41%)	8/18 (44%)
dysuria	2/32 (6%)	2/18 (11%)
oliguria, anuria	3/32 (9%)	2/18 (11%)
increased nocturia	6/32 (19%)	2/18 (11%)
nausea, vomiting, diarrhea, weight loss	14/32 (44%)	10/18 (55%)
edema	5/32 (17%)	8/18 (44%)
Antecedent infection		
sore throat, respiratory infection	14/32 (44%)	6/20 (30%)
flu syndrome	5/32 (17%)	2/20 (10%)
Past medical history		
pulmonary disease	2/29 (7%)	1/18 (6%)
renal disease/hypertension	4/29 (14%)	5/18 (28%)
diabetes	0/28 (0%)	1/18 (6%)
Family history		
renal or pulmonary disease	4/27 (15%)	2/18 (11%)
Physical findings		
fundal abnormalities	8/24 (33%)	5/17 (29%)
blood pressure 140/90 or greater	17/30 (57%)	10/19 (53%)
diastolic 110 or greater	5/30 (17%)	4/19 (21%)
pallor	22/29 (76%)	10/19 (53%)
rales-rhonchi	14/30 (47%)	3/19 (16%)
cardiomegaly	4/30 (13%)	5/19 (26%)
cardiac murmur	8/29 (28%)	9/19 (47%)
hepato-splenomegaly	6/30 (20%)	5/19 (26%)
abdominal or costovertebral angle tenderness	5/30 (17%)	2/19 (11%)
edema	8/30 (27%)	9/19 (47%)

Table VII

Clinical feature	Goodpasture's patients	Non-Goodpasture's patients
Laboratory investigations		
hemoglobin, mean	6.6 g%	8.5 g%
< 10.5 g-%	27/27 (100%)	9/14 (64%)
hematocrit, mean	20 vol%	23 vol%
31% vol or less	25/25 (100%)	15/18 (83%)
white blood cell count mean	12,200/mm^3	8,100/mm^3
10,000/mm^3 or more	14/22 (64%)	5/19 (26%)
>75% polymorphonuclear leukocytes	14/17 (82%)	3/15 (20%)
>4% eosinophils	2/17 (12%)	8/16 (50%)
reticulocytes>1%	9/11 (82%)	4/6 (67%)
platelet count<150,000/mm^3	4/18 (22%)	3/15 (20%)
throat culture positive for streptococci	0/14 (0%)	2/8 (25%)
antistreptolysin 0 titer>250	1/20 (5%)	4/14 (29%)
proteinuria	32/32 (100%)	19/20 (95%)
hematuria	32/32 (100%)	19/20 (95%)
abnormal urinary sediment	32/32 (100%)	19/20 (95%)
24-hour urinary protein excretion, mean	3.4 g	7.9 g
elevated blood urea nitrogen and/or creatinine	28/31 (90%)	18/20 (90%)
blood sugar>140 mg-%	3/16 (19%)	6/18 (33%)
bilateral radiographic chest infiltrates	23/26 (88%)	0/17 (0%)
sputum hemosiderin-laden macrophages	9/11 (82%)	0/2 (0%)
decreased serum complement	1/11 (9%)	1/8 (13%)
positive antinuclear antibodies (low titer)	1/21 (5%)	3/10 (30%)
liver functional abnormalities	3/19 (16%)	1/8 (13%)
clotting abnormalities	2/10 (20%)	1/6 (17%)

Laboratory data were helpful in discerning the presence of the disease. When the kidney was involved, a nephritic urinalysis was almost always encountered characterized by gross or microscopic hematuria and red cell casts (Table VII). An abnormal urinary sediment was found in all 32 cases of anti-GBM nephritis of Wilson and Dixon regardless of whether the kidney was felt to be initially involved. Proteinuria was common, but nephrotic range proteinuria was less so although when it occurred it was so spectacular that the mean level of protein excretion in the Wilson-Dixon series approached 3.4 grams. On chest x-ray, pulmonary intra-alveolar pulmonary infiltrates are characteristic when pulmonary hemorrhage is involved. Even in the absence of symptomatic hemoptysis, the combination of hematuria and pulmonary infiltrates is suggestive of Goodpasture's disease. An unexplained iron deficiency anemia without evidence of frank blood loss coupled to the chest x-ray is a useful clinical hint to the presence of anti-GBM nephritis. The usual laboratory evidence of immune complex disease or connective tissue disease is absent. ANA, anti-DNA, elevated ASLO titers, and serum complements are either absent or normal. The laboratory hallmark, of course, would be the detection by radioimmunoassay of anti-

nephritis. Occasionally, one can detect a familial propensity. There have been recognized not only sporadic cases but also epidemic outbreaks of the disease such as that reported in Auckland, New Zealand in 1974 and one reported from the northwest of England in 1982. Lastly, the cases seem to peak in spring and early summer and are not evenly distributed throughout the calendar. These features suggest an infectious agent may be partially involved in the pathogenesis of anti-GBM nephritis.

The two laboratory tests that most directly establish the diagnosis of Goodpasture's disease are the radioimmunoassay for the presence of anti-GBM antibody in plasma and the renal biopsy. Using the most sensitive assays developed by Wilson and his cohorts and Lockwood and his, one can detect circulating anti-GBM antibodies in as many as 90% of the patients at the onset of disease. The actual titer of the antibody does not closely correlate with the severity of the disease, but antibody titers are useful in therapy as will be discussed. Although circulating antibody levels are helpful in a confirmatory manner, the clinical hallmark of the disease is the detection of anti-GBM antibodies at the site of the pathologic intervention, the lung or the kidney. By light microscopy, Goodpasture's disease may have a range of morphologic abnormalities from focal and segmental glomerular hypercellularity to a frank diffuse proliferative nephritis with crescents (Figures 15 and 16).

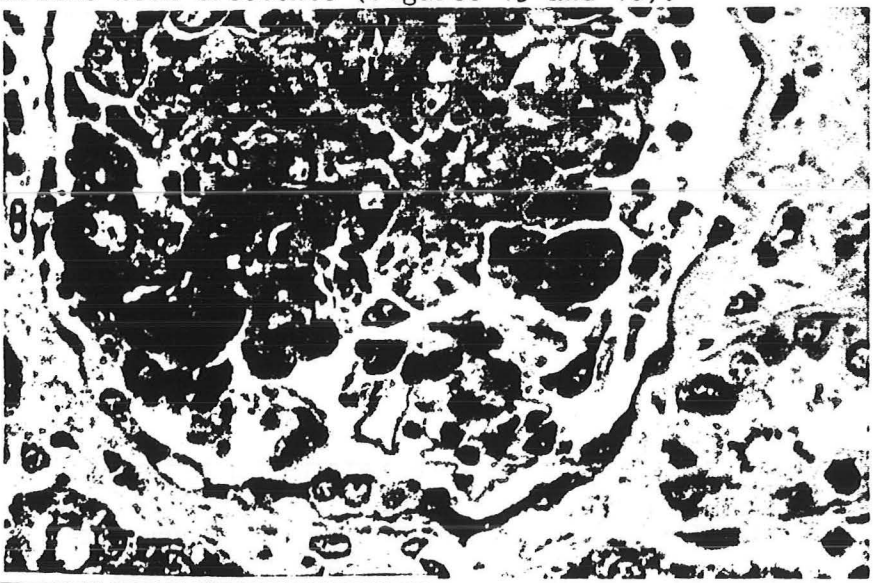


Figure 15

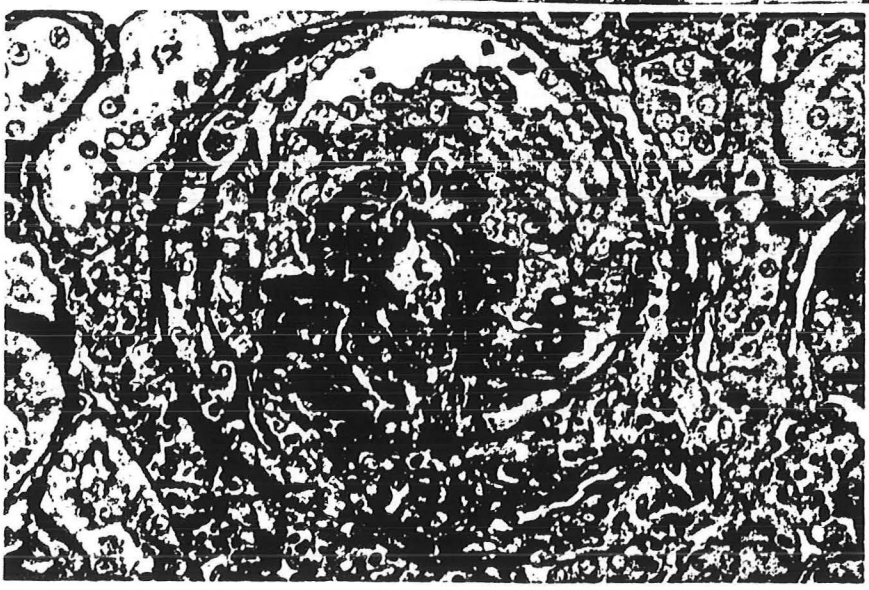


Figure 16

The circumferential crescents in more than 50% of the glomeruli correlates with the rapidly progressive course and often leads the clinician to search for anti-GBM antibodies. Again it must be pointed out that this crescentic glomerulonephritis is not pathognomonic of Goodpasture's disease, but only suggests that in the correct setting the pathologist should seek for the presence of anti-GBM antibody. It has been felt that the degree and extent of crescent formation may be prognostic, but the dire clinical outcomes for most patients in a brief time suggest that the clinician needs no additional prognostic indicators to truly determine the potential outcome for his patient. By electron microscopy, one visualizes findings which are corroborative of both light microscopy and immunofluorescence. Crescents seen by light microscopy are confirmed by EM. In evaluation of the pathogenetic etiology of crescentic GN, the EM of the basement membrane is an important place to start. The membrane is devoid of electron dense deposits which might be seen in other entities associated with a crescentic rapidly progressive GN when a vasculitis or immune complex disease is the pathogenetic mechanism underlying the clinical problem. Instead, in anti-GBM there is merely widening of the subendothelial space. By immunofluorescence, one has the classical finding described by Dixon initially, linear staining of immunoglobulin often overlaid with the third component of complement (Figure 17).

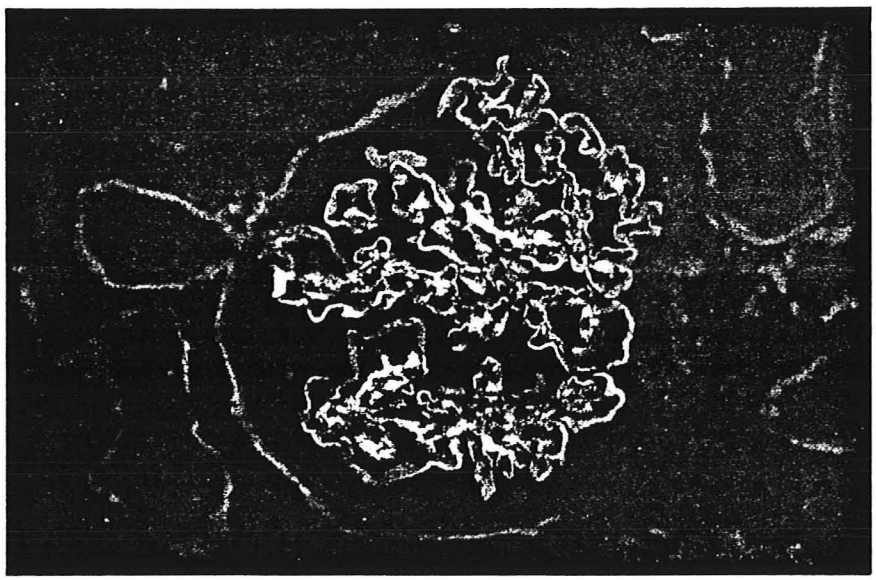


Figure 17

The linear staining of anti-GBM, in contrast to the "lumpy bumpy" pattern described by Dixon for classical serum sickness, was taken to be evidence for an antibody mediated nephritis in which the antibody reacts with a structural element of the basement membrane linearly displayed within the glomerulus. This hypothesis was clearly supported by McPhaul and Mullins when they demonstrated that antibodies eluted from the basement membrane of biopsy specimens from patients with presumptive anti-GBM disease were truly directed against the basement membrane and could refix to basement membrane material in index animals. Indeed, these studies fulfilled all of Koch's hypothesis in that the antibody detected in the disease kidney was directed against the glomerulus, was found in diseased kidneys and could reproduce the injury when superfused passively into

animals by binding to the glomerulus. The anti-GBM antibodies are not confined to the glomerulus and may be found directed against tubular basement membrane in a small but real fraction of cases. In these cases, the interstitial inflammatory component of the biopsy or kidney specimen is significant with edema, leukocyte infiltration, tubular dropout and atrophy. Interestingly, the biopsy of the lung when involved, by light microscopy reveals an extensive intra-alveolar hemorrhage with characteristic intra-alveolar hemosiderin laden macrophages present. A similar anti-GBM antibody can be detected along the alveolar capillary membrane by immunofluorescence as that found in the kidney. Bespeaking the commonality of the pathogenesis of the lung and kidney lesions is the fact that antibodies eluted from the lung often crossreact with glomerular basement membrane at the kidney when perfusion experiments were conducted by Koffler and Kunkle in 1969. These pathologic findings provide another important clue to the solution of our clinical mystery. The clue devolves upon the presence of an antibody directed against the basement membrane in the kidney and the lung but generally not in other organs of the body.

Although not particularly germane to the solution of our clinical mystery, it is impossible to leave the discussion of anti-GBM nephritis or Goodpasture's disease without commenting upon its treatment since major advances have been made in this direction which have altered the natural history of the disease. Patients with Goodpasture's disease from the 60's to the 70's, an historical era in which a wide range of therapeutic modalities were tried and abandoned, had a generally dismal outcome (Table VIII).

Table VIII

Author (year)	Number of cases	Number of deaths (%)	Number with surviving renal function (%)	Presenting plasma creatinines (mg/dl) of those with surviving renal function
Benoit (1964)*	52	50	2	Not stated
Proskey (1970)*	56	43	Not stated	Not stated
Wilson (1973)	53	25	6	Not stated
Whitworth (1970)	9	6	3	1.0; 0.8; 1.0
Beirne (1971)	29	17	5	1.8; 1.1; 1.1; 1.6; 2.3
Briggs (1979)	14	3	2	0.9; 0.8
Bergrem (1980)	7	4	0	None
Simpson (1982)	12	2	4	0.9; 1.0; 1.1; 1.4
Johnson (1985)	8	0	3	0.9; 1.0; 1.4

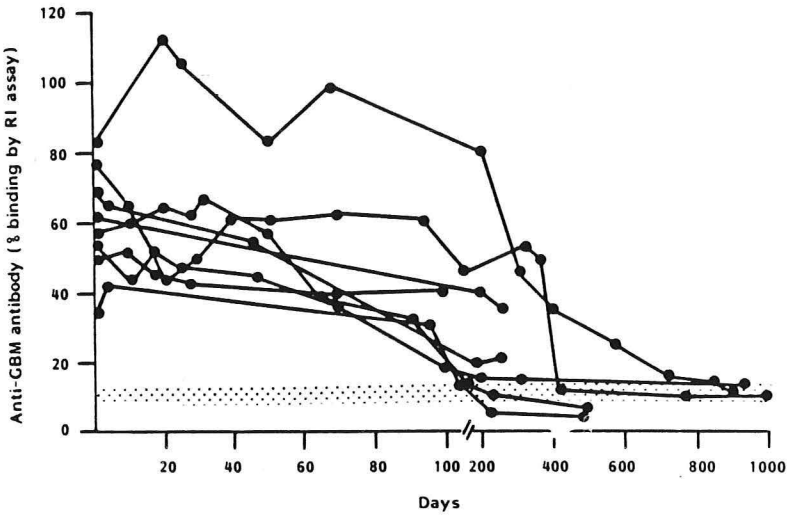
*Patients in these series were not immunopathologically defined but were diagnosed because of focal necrotizing glomerulonephritis and pulmonary hemorrhage.

Benoit reviewing the literature in 1964, a time when the pathogenesis of the disease was not clear and patients were not treated with a rational approach, found 50 of 52 deaths. When it became clear that the clinical picture of Goodpasture's disease was similar to that of the anti-GBM nephritis that Dixon and Wilson had described in animals, attempts were made to direct therapeutic approaches to antibody formation. One early approach was emergent bilateral nephrectomy to remove the antigen. This seemingly intelligent approach, rendered all patients in renal failure with the thought in mind to salvage life most often lost to pulmonary hemorrhage, exsanguination, and respiratory failure. In contrast to expectations, anti-GBM antibody titers actually rose when the antigen, which functioned as an eluting matrix, was removed. Clinically,

pulmonary disease could be caused or worsened when antibody titers rose in this fashion. Bilateral nephrectomies were rapidly abandoned. Anecdotal successes aside, there has been no convincing series that directly demonstrates the clinical efficacy of steroid and/or cytotoxic drug therapy. The penchant for high dose so-called bolus or pulse therapy for immune mediated disease led to the test of this approach with again anecdotal success in aborting pulmonary hemorrhage. Even in this setting there was little amelioration of the rapidly progressive glomerulonephritis.

What seems to have revolutionized the treatment approach to this disorder has been intense plasma exchange introduced by the Hammersmith group led by C.M. Lockwood. With the advent of sensitive radioimmunoassays, the goal of therapy became the removal of the antibodies which can be shown in experimental studies to be directly pathogenetic and cause the disease. Several features of anti-GBM nephritis lend themselves to successful therapy with plasma exchange. The first of these features is that the production of an autoantibody directed against the anti-GBM antigen(s) seems to be of short-lived duration. For example, antibody titers in eight untreated patients became undetectable one year after the disease had been discovered (Figure 18).

Figure 18

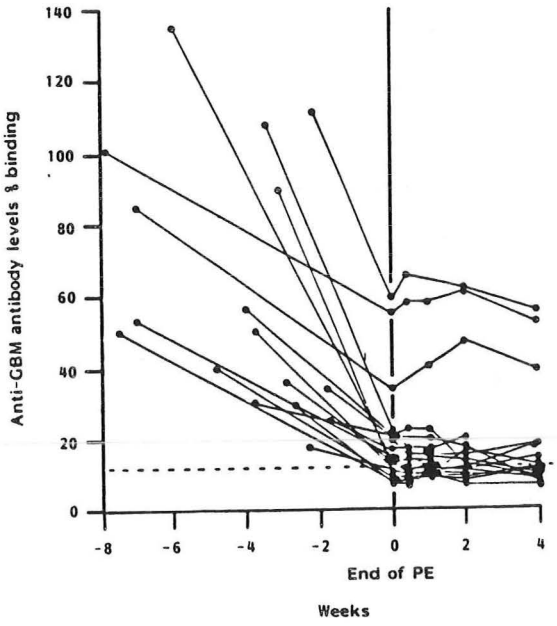


LOCKWOOD AND PUSEY 1987

The working hypothesis to explain the genesis of Goodpasture's disease centers around an incitement in susceptible individuals such as that induced by an appropriate infection which leads to a secondary production of an autoantibody directed against lung and kidney basement membrane antigens. When the inciting event is over the immune system will ultimately return to baseline. In that setting the antibody formation dwindles and ultimately returns to zero. Unfortunately, tissue damage in the relatively short period of time during which antibodies are made is often severe enough to either lead to the death of the patient or to the "death" of the kidneys. It is reasonable to believe, then, that the removal of the antibodies before the destruction of lung and kidney has

ensued may abort the disease. Intensive plasma exchange can then be accomplished for a finite period of time after which antibody formation will have ceased and the patient will have been "cured". It was quickly learned by the Hammersmith group, that plasma exchange could not be utilized alone to accomplish the goals of removal of anti-GBM antibody. Shortly after the cessation of the exchange therapy, one can appreciate a rapid rebound in the formation of the antibody titers many fold greater than the initial titer associated with an important relapse in the clinical signs and symptoms. Cytotoxic drug therapy was added to plasma exchange in order to attempt to abort the rebound of antibody formation with the recrudescence of clinical disease successfully (Figure 19).

Figure 19



Despite the initial enthusiasm for plasma exchange regimens associated with cytotoxic regimens, all investigators repeating the Lockwood experiments did not have as salutory clinical results. Some used plasma exchange regimens without cytotoxic drugs. In that setting, one can explain the negative results on the basis of failure to prevent rebound formation of anti-GBM antibody. Others repeated the precise regimen of the Hammersmith deriving results which led to less enthusiasm for plasma exchange. Lockwood and Pusey then conducted a prospective randomized trial of plasma exchange with cytotoxic drugs against drug therapy alone. They were able to discern two groups, some were responders and some were nonresponders based on the initial presentation. Of 44 patients studied, those with reasonable renal function on admission (17/44:creatinine less that than 5.5 mg/dl) were improved or cured by plasma exchange (Table VIII). If patients presented oliguric or in frank renal failure with creatinines greater than 5.5 mg/dl, response to plasma exchange was rare. The investigators explained these results by turning to animal models of anti-GBM nephritis and arguing that when frank renal failure was present indicated by severe reduction in glomerular filtration rate and/or urine flow rate, removal of antibodies from plasma space could no longer reverse the serious and irreversible tissue damage

that had ensued so that one could not expect reversal of renal failure.

Table VIII

Presentation		Outcome		
		Improved	No response	Death
Oligo-anuric	22	0	16	6
Creatinine >600 µmol/l	5	1	4	0
Creatinine <600 µmol/l	17	15	1	1
Lung haemorrhage			Controlled 30/34	

If intervention could be undertaken prior to final destruction of kidney or lung tissue then removal of antibody from blood salvaged the organs from further assault and potentially allowed damaged areas to heal. Suffice it to say that with the advent of plasma exchange, the first rational approach to therapy is available that holds out considerable hope to patients with a disorder once felt almost universally fatal.

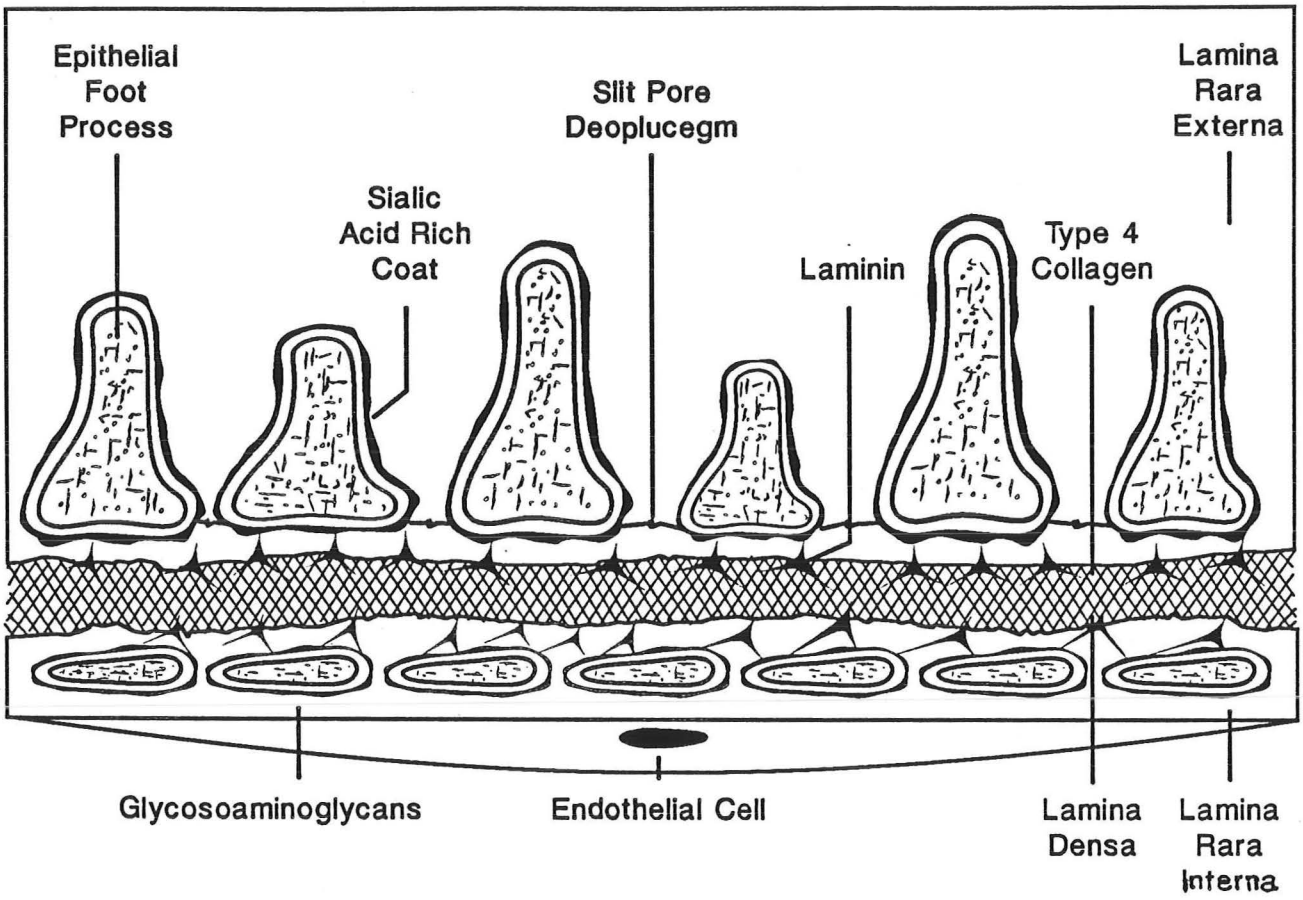
I have already shown that Alport's disease represents a defect in normal basement membrane. It is now reasonable to pose the question of precisely what biochemical or structural defect underpins Alport's disease. The explication of Goodpasture's disease has provided us with our **second clue**, an antibody directed to the basement membrane. We now have the essential features of each of the two disparate diseases that have come together in our family of transplant recipients. Our attention has been focused now directly to the basement membrane as the potential site of the solution of the clinical dilemma. We can focus our questions more tightly at that structure and begin to pose certain questions the solution of which will end our mystery. We need to now know what the structure of the normal basement membrane is and what structure or structures serve as the antigen in Goodpasture's disease. When we have learned about the composition of the normal basement membrane, the antigens that induce the anti-glomerular basement membrane nephritis, and the defects present in Alport's, I believe we will understand how and why three patients with Alport's disease could lose their transplants to anti-GBM nephritis.

Clue #3 - The Basement Membrane

To obtain our third and last clue we need to begin with a description of the normal basement membrane. Martinez-Hernandez and Amenta define a basement membrane as "a ubiquitous extracellular matrix found at the boundary between cells and the connective tissue stroma...visualized uniquely by an electron microscope and containing a lamina densa with one or more laminae rarae...demonstrated to contain at least laminin and type 4 collagen". This

inclusive definition involves the morphologic localization of the structure, its ultrastructural appearance, and its biochemical makeup (Figure 20).

Figure 20



This particular basement membrane expands the extracellular region between the endothelial cell which lines the filtering capillary and the foot process of the epithelial cell which lines the urinary space of the glomerulus. This unique basement membrane is comprised of the essential lamina densa and two laminae rarae, a lamina rara interna and a lamina rara externa. As demonstrated by Farquhar and her coworkers, then by Cotran and Renke, the epithelial foot process bears a strong negative charge, the result of a glycoprotein coat rich in sialic acid residues. The basement membrane itself is highly negatively charged not only owing to sialic acid bearing peptides but also to glycosoaminoglycans (GAGS) rich in heparan sulfate. The structural backbone of this basement membrane is a series of interlacing alpha helices of that unique collagen found in basement membranes, type 4 collagen. Other structures are found which brace this structural backbone mostly made up of the glycopeptide called laminin. I would like to examine in more detail each of the individual compositional elements of the basement membrane.

The basement membrane of the kidney is comprised of at least 4 and perhaps 5 intrinsic structural elements (Table IX).

Table IX

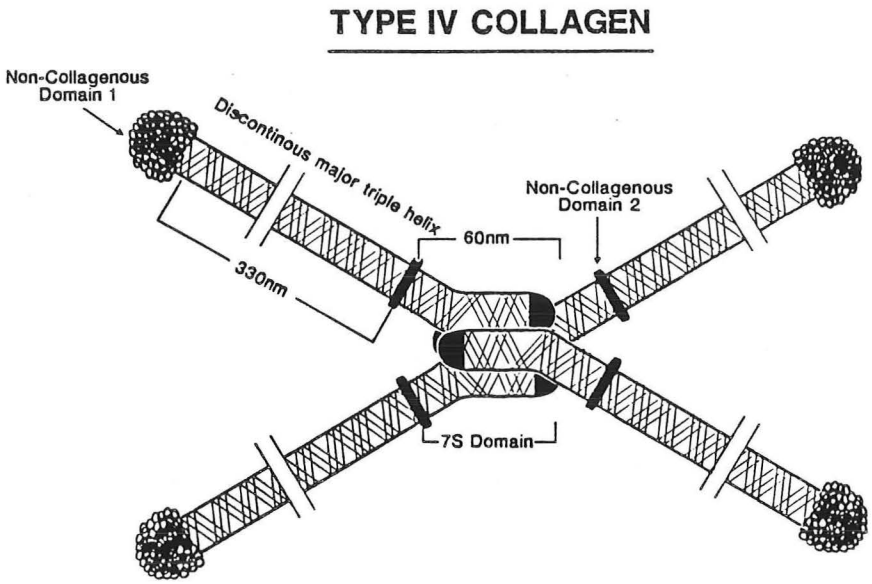
BIOCHEMICAL MAKEUP OF THE BASEMENT MEMBRANE

Type 4 Collagen	2 Unique alpha chain helices; rich in 3 hydroxyproline
Laminim	900Kd glycopeptide with one longarm and three short
Gags	Rich in heparan sulfate, strong neg charge
Fibronectin	Traces only in filtering BM, some believe extrinsic
Entactin (?)	Recently described 150Kd peptide, may not be in kidney

The backbone, and the bulk of the basement membrane is comprised of the unique material, type 4 collagen. At least 5 structural collagens have been defined, each one of which has a basic biochemical structure; 3 polypeptide alpha chains intertwined as an alpha helix with a series of repeating polymers of the form glycine-X-Y where the Y is frequently either hydroxyproline or hydroxylysine. Type 1 through 3 collagens are designated "interstitial collagens" and are found in bone, skin, vessels, cartilage and muscle. They are secreted as procollagens with an initial non-helical extension cleaved finally into the alpha helix position by a procollagen protease after secretion. Type 5 has recently been discovered by use of monoclonal antibodies directed against what was previously felt to be type 4 collagen. Its role in the structural basement membranes has yet to be completed elucidated. Type 4 collagen is found exclusively in basement membranes. As such it is of particular interest to these Rounds. The figure demonstrates the unique morphologic features of the type 4 collagen (Figure 21). Each of the strands of collagen molecules exist as discontinous triple alpha helices with 4 such structures bound together through a stable 7S domain. The helical structures are interrupted by <u>non</u>-glycine-X-Y sequences permitting the type 4 collagen to be digestible by unique enzymatic reactions. Furthermore there are end terminal glycopeptide domains about which more will be said during the discussion of the antigen in Goodpasture's disease. Two other differences exist between type 4 and other collagens. Firstly, when hydroxyproline forms the Y of the glycine-X-Y sequence, 3 hydroxyproline rather than 4 hydroxyproline is to be found. Secondly, the molecule is secreted intact without a procollagen

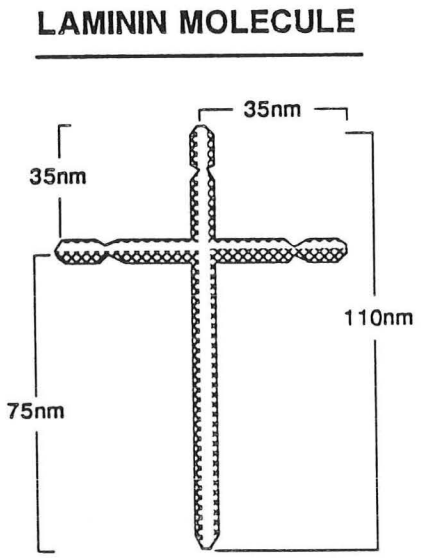
form so that extracellular processing by proteolytic cleavage is not required.

Figure 21



A number of noncollagen elements also comprise the basement membrane, the best characterized of which is laminin, a 900 kd rotary, crosslike structure composed of one long and three short arms as shown (Figure 22).

Figure 22



Laminin is a glycoprotein with an amino acid composition clearly different from a similar material, fibronectin, but not yet completely understood. Monoclonal antibodies directed to laminin localize within the basement membrane but are found in no other structures.

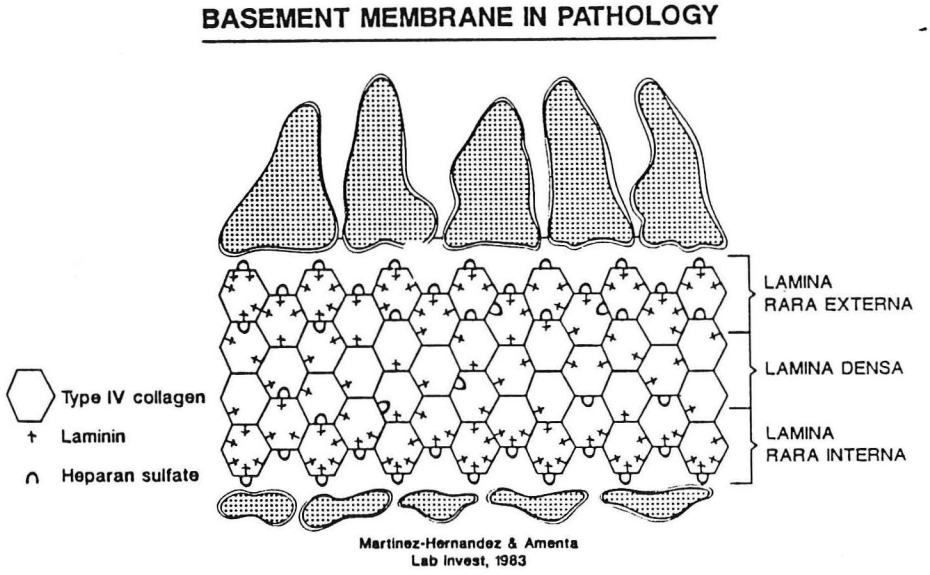
Glycosoaminoglycans (GAGS), rich in heparan sulfate, are important determinants of the basement membrane in that they confer a strong negative charge important for the sieving properties of the membrane. A wide variety of molecular weights exist for these structures ranging from 14,000 to 750,000 daltons.

Fibronectin is a peptide generally ascribed to plasma and tissue ground substance which has recently been found to be present only in the basement membrane of the filtering organs such as the kidney. Some have argued that the presence of fibronectin within the kidney basement membrane is contamination by filtering protein; others, that small amounts of basement membrane fibronectin are structurally important to the kidney itself. Fibronectin exists as identical 220 kd subunits held together by disulfide bonding.

An unusual or rare element in some basement membranes is the recently described Type V collagen discerned by variant reactions to monoclonal antibodies initially thought to be directed against Type IV structures. Although Type V collagen exists in some basement membranes, there is a healthy debate as to whether this structure is found in the basement membrane peculiar to the glomerulus. A protein molecule derived from a cancer line, intactin, a highly sulfated material of 150 kd, may also exist in the glomerular basement membrane, at least in rodents. Its function and presence in the human kidney has yet to be described.

One can redraw schematically the basement membrane placing the elements the biochemical elements within the membrane accounting for its supermolecular organization (Figure 23).

Figure 23



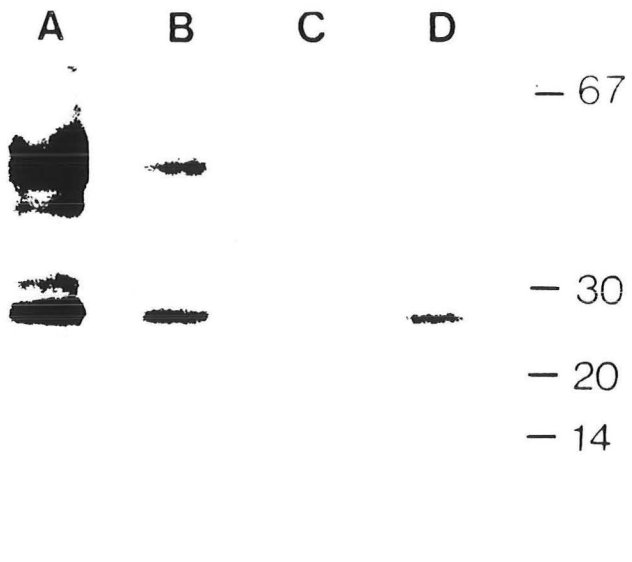
Not shown in the figure or the previous table is the fact that there are a number of unidentified noncollagen proteins which may form up to 20% of the basement membrane and which may be important for understanding anti-GBM nephritis.

Identifying the precise Goodpasture's antigen has not been easy. For more than 30 years it has been clear that collagen itself did not serve as the antigen

that induces nephrotoxic serum nephritis. Antibodies raised against collagen by Rothhard and Watson were intrinsically poor nephrotoxins. Although one could argue that these antibodies raised by Rothbard and Watson may not have been directed against Type IV collagen, absorption of nephrotoxic sera containing the putative anti-glomerular basement membrane antibody with collagen also failed to abrogate nephrotoxicity. Marquardt, Wilson, and Dixon demonstrated that collagenase digested basement membrane, which destroys the Type IV collagen leaving behind the noncollagen peptide elements of the membrane, was a superb antigen in inducing anti-GBM antibodies and disease. In the modern era of monoclonal antibody analysis, Fish demonstrated the pattern of reactivity of human anti-GBM antibodies to be distinguished from those observed with antisera directed against Type IV collagen, Type V collagen, heparan sulfate proteoglycans, laminin, or fibronectin.

The precise nature of this noncollagen glycoprotein present in the kidney basement membrane which serves as an antigen to develop anti-GBM antibodies has yet to be completely elucidated. Using the techniques of immunoblotting and 2-D gel electrophoresis, Fish and co-workers were able to identify unique highly cationic glycoproteins in collagenase digests of the human glomerular basement membrane which could be immunoblotted by binding to anti-GBM autoantibodies. These antigen structures were generally between 25 and 50 kd in size and presumptively were not present in a conformational form that permitted direct binding of antibody to tissue structures since similar structures are not identified by immunohistochemical techniques of tissue sections directly. Pusey and colleagues at the Hammersmith developed a range of mouse anti-human monoclonal antibodies directed against variant antigen structures in collagenase solubilized human basement membranes, one of which was most instructive for our understanding of the Goodpasture's antigen. By Western blot analysis of collagenase digested human GBM, he could demonstrate that one monoclonal antibody developed by the research team crossreacted with human anti-GBM antibody and identified a small molecular weight protein in the 26 and in the 54 kd region respectively, almost identical to the material first described by Fish (Figure 24).

Figure 24



LEGEND:

Lane A = MOAB against Goodpasture's antigen
B = Anti-GBM antibody staining of Good-pasture's antigen
C = MOAB against Good-pasture's antigen after absorption with anti-GBM anti-sera
D = Irrelevant MOAB

It remains to be seen whether this Goodpasture's antigen is found as a unique structure within the basement membrane or is the noncollagen, peptide domain associated with Type IV collagen (Figure 23). The latter interpretation of the data would explain the continuous, non-discrete presentation of the antigen and thus account for the linear immunofluorescence of anti-GBM antibody. More difficult to explain would be the failure of direct identification of the Goodpasture's antigen in tissue structures since these domains should be available for binding to antibody unless protein confirmation does not allow the epitopes to the readily exposed in tissue section. Pusey then screened basement membranes from various organs for the presence of the antigen that would react with the monoclonal antibody to the Goodpasture's antigen. One could find the Goodpasture's antigen in structures in the kidney (Bowman's capsule, distal tubule, the glomerulus), the alveolar basement membrane, the cochlea of the ear, the choroid plexus, the lens capsule and the retina of the eye. Basement membranes of almost all other screened tissues did not have the putative Goodpasture's antigen. Isn't it interesting that the very organs which might be diseased in Alport's syndrome, the eye, the ear, the kidney, are structures which contain the Goodpasture's antigen? Herein lies a powerful clue to the solution of our clinical mystery.

It is time to return to Alport's syndrome. Recall that the morphologic lesion which is almost pathognomonic for this hereditary disorder of kidney, ear, and eye, is a thickened basement membrane with fracturing or splitting of the structure. McCoy and colleagues made an astounding finding in 1982 when they attempted to use anti-GBM antisera to perform a simple descriptive study of the composition of the basement membrane in patients with Alport's syndrome. The idea was that the antibodies would identify the Goodpasture's antigen and could be used by that various immunochemical staining techniques to better understand the nature of the laminations and the lucent regions within the basement membrane in Alport's. To the surprise of the investigators, anti-GBM antisera failed to bind to any region of the basement membrane of the patients with Alport's syndrome, however disordered. One could absorb the anti-GBM activity against normal kidney so that when tested against a glomerular section, anti-GBM activity could be lost. Absorption of anti-GBM antisera by Alport's kidney failed to remove the anti-GBM reactivity of the sera. The absence of binding of anti-GBM antibody to Alport's GBM was confirmed by at least 6 other workers. The Hammersmith team, having developed the mouse monoclonal antibody directed specifically against the Goodpasture's antigen, examined the problem of the nature of the basement membrane structural abnormality in Alport's syndrome. They found that the monoclonal antibody against Goodpasture's antigen failed to bind to a single one of 10 patients with known Alport's syndrome who had strong electronmicroscopic evidence of the structural defect of that entity.

It is now reasonable to establish the pathogenesis of Alport's syndrome as the genetic absence of or the presence of a structurally defective polypeptide which normally exists within the basement membrane. This polypeptide probably is a monomeric polypeptide of the noncollagen globular domain of Type IV collagen which is intensely antigenic and can lead to the formation of an antiglomerular basement membrane antibody responsible for Goodpasture's disease. This unique peptide is confined to the ear, the eye, and the kidney, the absence of which leads not only to the morphologic evidence of rarifaction and splitting with lamellation of the structure, but also to a range of abnormalities clinically

observable including hematuria, proteinuria, renal failure, lenticonis, retinal abnormalities, and deafness. The solution to our clinical mystery is now elementary.

Conclusion

Because a normal protein of the basement membrane is either absent or biochemically altered in Alport's Disease, it is reasonable to propose that provision of that normal protein could function as a strong antigenic signal to induce an immune response. The renal transplant from a non-Alport's patient bears within the glomerulus the Goodpasture's antigen as a normal element of its structure. The transplanted kidney, then, serves as both the antigen and the target of an immunization, the transplant surgical event. Placing the normal kidney in the patient with Alport's leads to the formation of anti-GBM antibodies reactive to the Goodpasture's antigen which is not recognized as a self structural protein because of its genetic absence in the patient with Alport's syndrome. These anti-GBM antibodies bind to the Goodpasture's antigen in the transplant leading to a crescentic glomerulonephritis, hematuria, proteinuria, a reduction in the glomerular filtration rate which perforce cannot be amenable to anti-rejection therapy. Is this hypothesis or is this solution of our mystery farfetched? McCoy and colleagues actually described in 1982 the detection of circulating anti-GBM antibodies and crescentic glomerulonephritis in a young boy with Alport's syndrome five months after a transplant. Milliner, screening 11 consecutive patients with Alport's syndrome, demonstrated crescentic GN with linear IgG staining in 2 of the 3 failed allografts in these patients. In Paris at the Necker 5 cases of crescentic GN with a rapidly progressive course of renal failure associated with linear fixation of IgG were described in patients in renal failure from Alport's Sydnrome The hypothesis advanced is correct. Our mystery is solved.

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