JCI The Journal of Clinical Investigation

Immunofluorescent Localization of Antihemophilic Factor Antigen and Fibrinogen in Human Renal Diseases

John R. Hoyer, Alfred F. Michael, Leon W. Hoyer

Research Article

Tissue localization of antihemophilic factor (AHF, factor VIII) antigen and fibrinogen by immunofluorescent microscopy was determined in 146 specimens of normal and diseased kidneys. AHF antigen was present in the endothelial cells of glomeruli, peritubular capillaries, arteries, and veins of normal kidneys; a distribution similar to that in other tissues. In scleroderma and malignant hypertension, deposition of AHF antigen and fibrinogen was limited to the markedly thickened endothelial layers of arteries. More extensive intense deposition of both AHF antigen and fibrinogen in glomeruli and in arterial walls were present in hyperacute renal homograft rejection, hemolyticuremic syndrome, postpartum renal failure, and in some cases of acute homograft rejection. In contrast, deposition of fibrinogen was observed in glomerular epithelial cresents in severe proliferative glomerulonephritis, but AHF deposition was not present in these lesions. Glomerular deposition of fibrinogen without increased AHF standing was also detected in renal tissue from patients with anaphylactoid purpura nephritis and in recurrent macroscopic hematuria with focal glomerulonephritis. Increased staining of peritubular capillaries with anti-AHF was seen in diseased kidneys irrespective of etiology. Immunofluorescent localization of AHF, a participant in the intrinsic coagulation pathway, offers a new way by which to analyze the mechanisms responsible for fibrinogen deposition in disease.

Find the latest version:

https://jci.me/107686/pdf

Immunofluorescent Localization of Antihemophilic Factor Antigen and Fibrinogen in Human Renal Diseases

John R. Hoyer, Alfred F. Michael, and Leon W. Hoyer

*From the Department of Pediatrics, University of Minnesota School of Medicine,
Minneapolis, Minnesota 55455 and the Department of Medicine, University
of Connecticut School of Medicine, Farmington, Connecticut 06032*

ABSTRACT Tissue localization of antihemophilic factor (AHF, factor VIII) antigen and fibrinogen by immunofluorescent microscopy was determined in 146 specimens of normal and diseased kidneys. AHF antigen was present in the endothelial cells of glomeruli, peritubular capillaries, arteries, and veins of normal kidneys; a distribution similar to that in other tissues. In scleroderma and malignant hypertension, deposition of AHF antigen and fibrinogen was limited to the markedly thickened endothelial layers of arteries. More extensive intense deposition of both AHF antigen and fibrinogen in glomeruli and in arterial walls were present in hyperacute renal homograft rejection, hemolytic-uremic syndrome, postpartum renal failure, and in some cases of acute homograft rejection. In contrast, deposition of fibrinogen was observed in glomerular epithelial cresents in severe proliferative glomerulonephritis, but AHF deposition was not present in these lesions. Glomerular deposition of fibrinogen without increased AHF standing was also detected in renal tissue from patients with anaphylactoid purpura nephritis and in recurrent macroscopic hematuria with focal glomerulonephritis. Increased staining of peritubular capillaries with anti-AHF was seen in diseased kidneys irrespective of etiology. Immunofluorescent localization of AHF, a participant in the intrinsic coagulation pathway, offers a new way by which to analyze the mechanisms responsible for fibrinogen deposition in disease.

INTRODUCTION

Immunopathologic studies have identified glomerular and vascular fibrinogen deposition in a number of human and experimental renal diseases (1–10). In addition, fibrin degradation products have appeared in the blood and urine of patients with glomerulonephritis and in renal transplant recipients (11, 12). How fibrinogen is deposited and fibrin degradation products are produced in renal disease have not yet been established; and the precise role of coagulation proteins in the pathogenesis of renal failure is still unknown. The tissue distribution of antihemophilic factor (AHF, factor VIII)[1] antigen (13) and fibrinogen in human renal diseases has been determined in the present study to obtain additional information about the possible involvement of the coagulation system in their pathogenesis. These immunohistologic studies of human kidney biopsy specimens indicate that fibrinogen may be deposited in human renal diseases through multiple pathways.

METHODS

The tissues which were examined included 100 consecutive renal specimens obtained during a 3-mo period in 1972–1973 at the University of Minnesota Hospitals. Renal tissue obtained during 1970–1973 from an additional 46 patients was also studied. Specimens in the second group were selected to include specific pathologic entities not represented in the first group; in most instances fibrinogen had been demonstrated in a previous immunofluorescent study. Diagnosis in all diseases was based on typical clinical features and morphologic characteristics by light microscopy. The intensity and patterns of staining for fibrinogen and other proteins in these specimens were unchanged from those observed at the time of initial immunofluorescent study. Specific informed consent for evaluation of renal tissue was obtained before removal of any specimens.

Portions of tissue from percutaneous and open renal biopsies and from bilateral nephrectomy and autopsy specimens were fixed in buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin, periodic acid-Schiff, and azocarmine. Other portions of tissue were placed on a cellulose sponge and rapidly frozen in iso-

[1] *Abbreviation used in this paper:* AHF, antihemophilic factor (factor VIII).

TABLE I

AHF and Fibrinogen Deposition in Human Renal Diseases

Staining pattern	Disease	Patients studied	AHF		Fibrinogen	
			Glomeruli‡	Arteries§	Glomeruli‡	Arteries§
I	Scleroderma	2	0	+ +(E)	0	+ +(E)
	Malignant hypertension	1	0	+ +(E)	0	+ +(E)
II	Hyperacute homograft rejection	3	+ + +	+ + +	+ + +	+ + +
	Acute homograft rejection*	5	+ to + + +	+ + to + + +	+ to + +	+ + to + +¯+
	Hemolytic-uremic syndrome	2	± to + + +	+ + to + + +	± to + +	+ + to + +_+
	Postpartum renal failure	1	+ + +	±	+ + +	±
III	Glomerulonephritis with cresent formation	8	0	0	+ +(C)	0
	Anaphylactoid purpura	2	0	0	+ +	0
		1	+	0	+ +	0
	Focal glomerulonephritis with	1	0	0	+ +(M)	0
	recurrent macroscopic hematuria*	1	0	0	+	0
		1	+	0	+	0

The staining of tissues was graded as follows: 0, no detectable staining (fibrinogen) or normal endothelial cell staining (AHF); ±, minimal or very focal staining; + to + + +, increased staining.
* Specimens from two additional patients had no AHF or fibrinogen deposition.
‡ Glomerular staining was predominantly endothelial. Specific localization in crescents (C) or in the mesangium (M) is noted.
§ Arterial staining involved all layers of the vessel except where noted as being limited to endothelial cells (E).

pentane prechilled in liquid nitrogen and stored at −70°C until sectioned. 4-μm thick sections were cut in a cryostat and stored at −20°C for a period no longer than 3 mo before staining. Sections were stained with fluorescein-conjugated monospecific antisera prepared in our laboratory
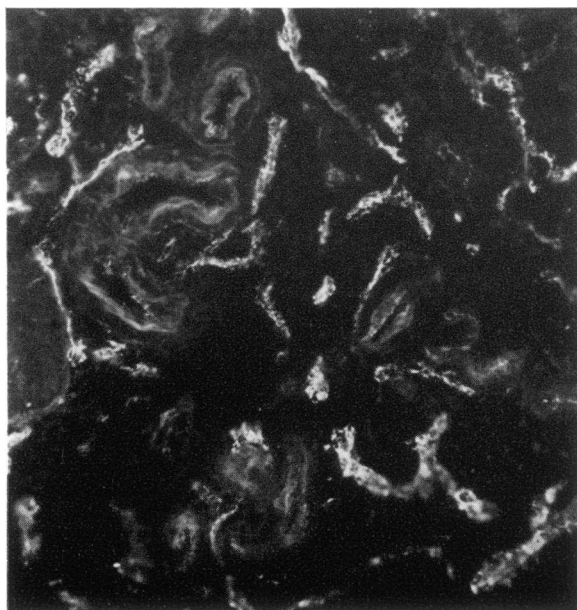

FIGURE 1 The intense staining for AHF in peritubular and interstitial capillaries demonstrates the vascularity of this "end-stage kidney" (× 120).

to human IgG, IgM, IgA, C3 (β-1C globulin), fibrinogen, and albumin by the direct technique as previously described (14, 15). Sections were stained with rabbit antihuman AHF and rabbit antihuman α-2 macroglobulin by the indirect technique using fluorescein-conjugated monospecific goat antirabbit IgG as previously described (13). This latter antiserum had been absorbed previously with human IgG and reacted only with rabbit IgG. Specific staining of normal and pathologic human tissues with the rabbit anti-AHF was blocked by absorption of the antisera with highly purified AHF (16) or with an ethanol concentrate of normal human plasma, but not by an ethanol concentrate of plasma from a patient with von Willebrand's disease or with human fibrinogen (13). The antiserum to α-2 macroglobulin was purchased from Behring Diagnostics (Woodbury, N. Y.). Most specimens were examined by transmitted fluorescence using a Zeiss microscope (Carl Zeiss, Inc., New York) equipped with an HBO 200 W light source and a BG12 excitation filter, or, in some instances, an FITC interference filter. More precise cellular localization of positive staining in some sections was obtained using phase contrast immunofluorescence with indirect fluorescence illumination in a Zeiss Ultraphot II microscope. This was particularly helpful in identifying endothelial cells and/or mesangial cells of glomeruli.

RESULTS

Normal tissues

The distribution of AHF antigen in normal renal tissue was identified in specimens obtained at the time of transplantation from 25 living, related kidney donors. Each specimen showed staining within the endothelial cells of glomerular capillaries, arteries, arterioles, veins,

and peritubular capillaries. Both finely granular and interrupted linear staining were observed within glomerular endothelial cells. Staining of endothelial cells of peritubular capillaries, arterioles, and veins was predominantly granular whereas staining of the luminal surface of larger arteries appeared linear and was limited to a single endothelial cell layer. Staining intensity varied between and within specimens but positive staining was seen in each tissue section. No staining was observed within tubules or within vessel lumens. These findings are identical with the pattern of AHF antigen staining seen in all other tissues that have been studied (13). Fibrinogen deposition was not detected in sections from normal renal tissue.

Abnormal renal tissues

Three distinct patterns of abnormal AHF and fibrinogen staining were identified in glomeruli and arteries of kidney tissue from patients with renal diseases (Table I). In addition there was a common feature, increased intensity of granular staining for AHF within endothelial cells of peritubular capillaries (Fig. 1). Peritubular capillary staining with anti-AHF was intense and widely distributed in tissues which showed evidence of severe injury and architectural distortion when examined by light microscopy. The staining was present in specimens from patients with both acute and chronic renal diseases. A similarly increased intensity of granular AHF staining of a single layer or arterial endothelial cells was also seen in the majority of chronically diseased kidneys. Complete loss of glomerular AHF staining was seen in hyalinized glomeruli and in areas of glomerular sclerosis.

AHF deposition in diseased kidneys was limited to endothelial cells and vascular deposits. In contrast, fibrinogen was sometimes noted within tubular casts and was occasionally identified in a granular pattern within tubular cells, particularly in specimens from patients with marked proteinuria. AHF antigen was rarely observed in casts and was not detected within renal tubular cells.

The three patterns of AHF antigen and fibrinogen deposition were:

AHF and fibrinogen deposition within arteries. Marked endothelial proliferation was noted in renal biopsy specimens from two patients with scleroderma and one patient with malignant hypertension (Table I, pattern I). It was apparent on light microscopy that the endothelial lesions completely occluded the lumens of some vessels. These arteries stained strongly for AHF and fibrinogen within the endothelial layers (Fig. 2D), although a single well-organized thrombosed artery in one specimen was negative. Abnormal AHF staining and fibrinogen deposition were not detected in glomeruli of these sections and both arteries and glomeruli were
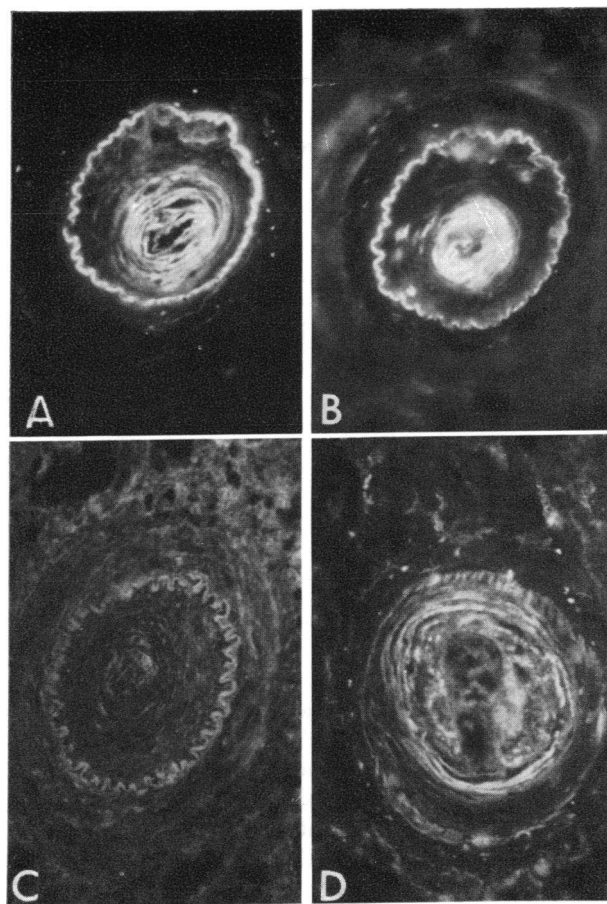

FIGURE 2 Endothelial proliferative lesions in arteries of renal biopsy tissue from a patient with hemolytic-uremic syndrome and malignant hypertension and a patient with scleroderma. (A) Intense AHF deposition in the inner endothelial layers (hemolytic-uremic syndrome, patient J. R.). The elastic lamina demonstrates autofluorescence rather than AHF staining ($\times$ 320). (B) Fibrinogen deposition in the same artery ($\times$ 320). (C) Artery in same tissue section as A and B stained with anti-α-2 macroglobulin. There is no positive staining of endothelial layers ($\times$ 320). (D) AHF deposition in endothelial layers of artery from a patient with scleroderma ($\times$ 280).

negative when stained for IgG, IgM, IgA, C3, and albumin.

AHF antigen and fibrinogen deposition in glomeruli and arterial walls. More widespread staining for AHF antigen and fibrinogen was identified in specimens from patients with several acute renal diseases (Table I, pattern II). To a variable extent, specimens from each of these patients showed parallel deposition of fibrinogen and AHF antigen in glomerular capillaries, endothelial cells, and arteriolar walls. This pattern was most consistently seen in kidney tissue from three patients with hyperacute renal homograft rejection (Table II). In each instance, the kidney was removed within 24 h of

TABLE II

Protein Deposition in Glomeruli and Arterial Walls

	Fibrinogen		AHF		IgM		C3		IgG		IgA		Albumin		α-2 Macroglobulin	
	G	A	G	A	G	A	G	A	G	A	G	A	G	A	G	A
Hyperacute homograft rejection																
Patient C. G.	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	0	0	0	±	0	±	0	0	±	±
Patient J. G.	+ + +	+ + +	+ + +	+ + +	+ +	+ + +	±	0	±	0	0	0	0	0	±	±
Patient J. T.	+ + +	+ + +	+ + +	+ + +	+ +	+ + +	0	0	0	0	0	0	0	0	±	0
Hemolytic-uremic syndrome																
Patient J. R.																
Biopsy 1	±	+ + +	±	+ + +	+	+ +	0	+ +	0	0	0	0	0	0	0	+
Biopsy 2	+ +	+ + +	+ +	+ + +	+	+ +	0	+ +	0	0	0	0	0	0	0	0
Patient J. F.	+ +	+ +F	+ + +	+ +F	+ +	+ +F	0	0	±	0	0	0	0	0	±	±
Postpartum renal failure																
Patient L. B.	+ + +	+F	+ + +	+F	+ + +	+F	0	0	±	0	0	0	—	—	—	—

Localization of staining: G, glomerular; A, arterial or arteriolar; F, focal.
Staining of tissues was graded as follows: 0, normal staining pattern; ±, minimal or very focal staining; + to + + +, increased staining; —, not evaluated.

grafting. Intense deposition of AHF was present throughout arteriolar walls and along glomerular capillaries of each specimen (Figs. 3A–C). In specimens from two of the three patients, AHF deposition was identified within the intima, media, and adventitia of larger arteries. The distribution of fibrinogen and IgM was identical to that of AHF in these specimens (Fig. 3D). In contrast, staining for other serum proteins was barely detectable or absent.

A similar but less regularly observed pattern was seen in specimens obtained at the time of open biopsy or nephrectomy 1–3 mo after renal transplantation in seven patients with acute homograft rejection (Table III). Each patient was azotemic (serum creatinine of 3 mg/100 ml or greater). All specimens had evidence of homograft rejection by light microscopy with interstitial edema and/or fibrosis, lymphocyte and plasma cell infiltration, and endothelial proliferation within arteries. Intense staining with anti-AHF and antifibrinogen was observed throughout the walls of one or more arteries and in thrombosed vessels of four specimens and within the thickened arterial endothelium of a fifth specimen (Table III). Glomerular capillary staining for AHF and fibrinogen was more focal and less intense in most instances. Intense IgM staining with the same distribution as that of AHF and fibrinogen was observed in only one of these five specimens (patient L. D.). Other specimens contained nodular deposits of IgM (and C3) in the outer layers of arterial walls without similar AHF or fibrinogen deposits. AHF and fibrinogen deposition was not present in the biopsy specimen of patient J. B. whose subsequent transplant nephrectomy specimen (1 mo

TABLE III

Protein Deposition in Glomeruli and Arterial Walls in Acute Homograft Rejection

Patient	AHF		Fibrinogen		IgM		C3		IgG		IgA	
	G	A	G	A	G	A	G	A	G	A	G	A
J. B.												
Biopsy 1	0	0	0	0	0	+ +N	0	+ +N	0	0	0	0
Biopsy 2	0	+ + +	0	+ +	0	+N	0	+ +N	0	0	0	0
L. D.	+ + +F	+ +F	+ +F	+ +F	+ +F	+	0	+ +N	0	0	0	0
J. S.	±	+ +	±	+ +	0	0	0	0	0	0	0	0
J. R.	+	+ +	+	+ +	+ +F	+N	+F	+ +N	0	0	0	0
M. W.	+ +	+ + +E	+ +	+ + +E	0	+F	0	+N	0	0	0	0
L. A.	0	0	0	0	0	0	0	+N	—	—	0	0
M. G.	0	0	0	0	0	0	0	0	—	—	—	—

Localization of staining: G, glomerular; A, arterial or arteriolar; F, focal; N, nodular; E, markedly thickened endothelial layers.
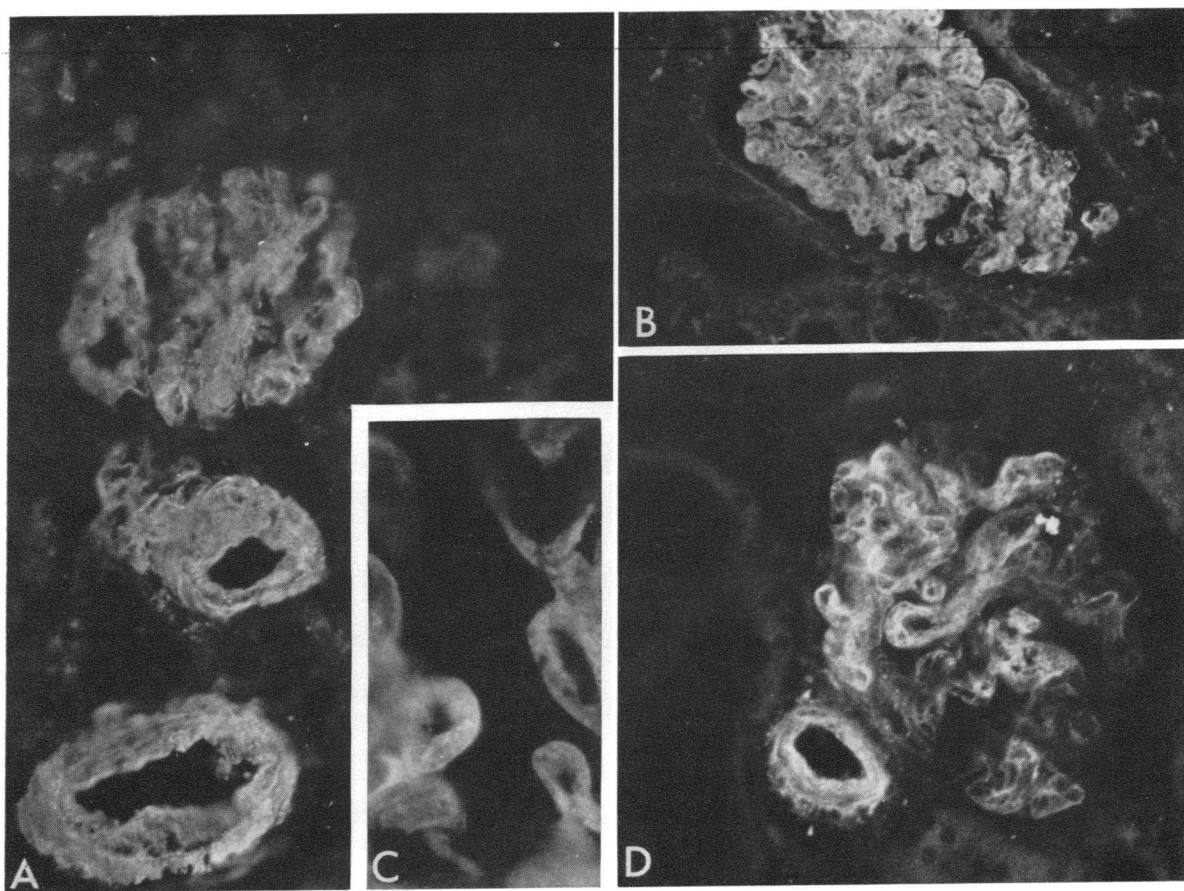Staining of tissues was graded as in Tables I and II.

FIGURE 3 Hyperacute rejection of renal homografts. (A) AHF deposition along glomerular capillary loops and throughout the walls of two arteries (patient C. G.) (× 160). (B) Similar AHF deposition in glomerulus from patient J. G. (× 200). (C) High power view of glomerulus (patient J. T.) demonstrating dense AHF deposition along capillary wall (× 640). (D) Fibrinogen deposition along capillary loops of the glomerulus and throughout the wall of the afferent arteriole (patient J. T.) (× 240).

later) showed intense deposition of both proteins in the walls of thrombosed arteries.

Similar findings were also observed in tissue from two patients with hemolytic-uremic syndrome and in a woman with postpartum renal failure (Table II). Specimens from both patients with the hemolytic-uremic syndrome were obtained 2 wk after onset of the disease. Intense deposition of AHF, fibrinogen, and IgM was seen along glomerular capillary walls, within glomerular capillary thrombi, and throughout arteriolar walls (Fig. 4), 3 mo later, one patient's kidneys were removed because of severe hypertension not controlled by hemodialysis. Arterial endothelial proliferation with an onion-skin appearance was apparent by light microscopy of the nephrectomy specimens, and intense staining for AHF and fibrinogen was regularly present in the thickened endothelial layers of these vessels (Figs. 2A and B). Only one of these lesions had positive staining for

IgM and none of these arteries showed positive staining for α-2 macroglobulin. It should be noted that the positive staining of arteries in these specimens with anti-C3 was granular and did not correspond in distribution with that of AHF and fibrinogen.

Renal tissue from a patient with postpartum renal failure after preeclampsia and abruptio placentae was obtained 2 wk after delivery. All glomeruli showed intense staining for AHF, fibrinogen, and IgM, but not for other proteins (Fig. 4D). Whereas some arteriolar walls had focal positive staining for coagulation proteins and IgM, no AHF or fibrinogen deposition was identified within large arteries.

Fibrinogen deposition in the absence of increased AHF staining. In contrast to the close correspondence between the deposition of AHF and fibrinogen in diseases described above, there was a clear dissociation of fibrinogen deposition from that of AHF in severe proliferative

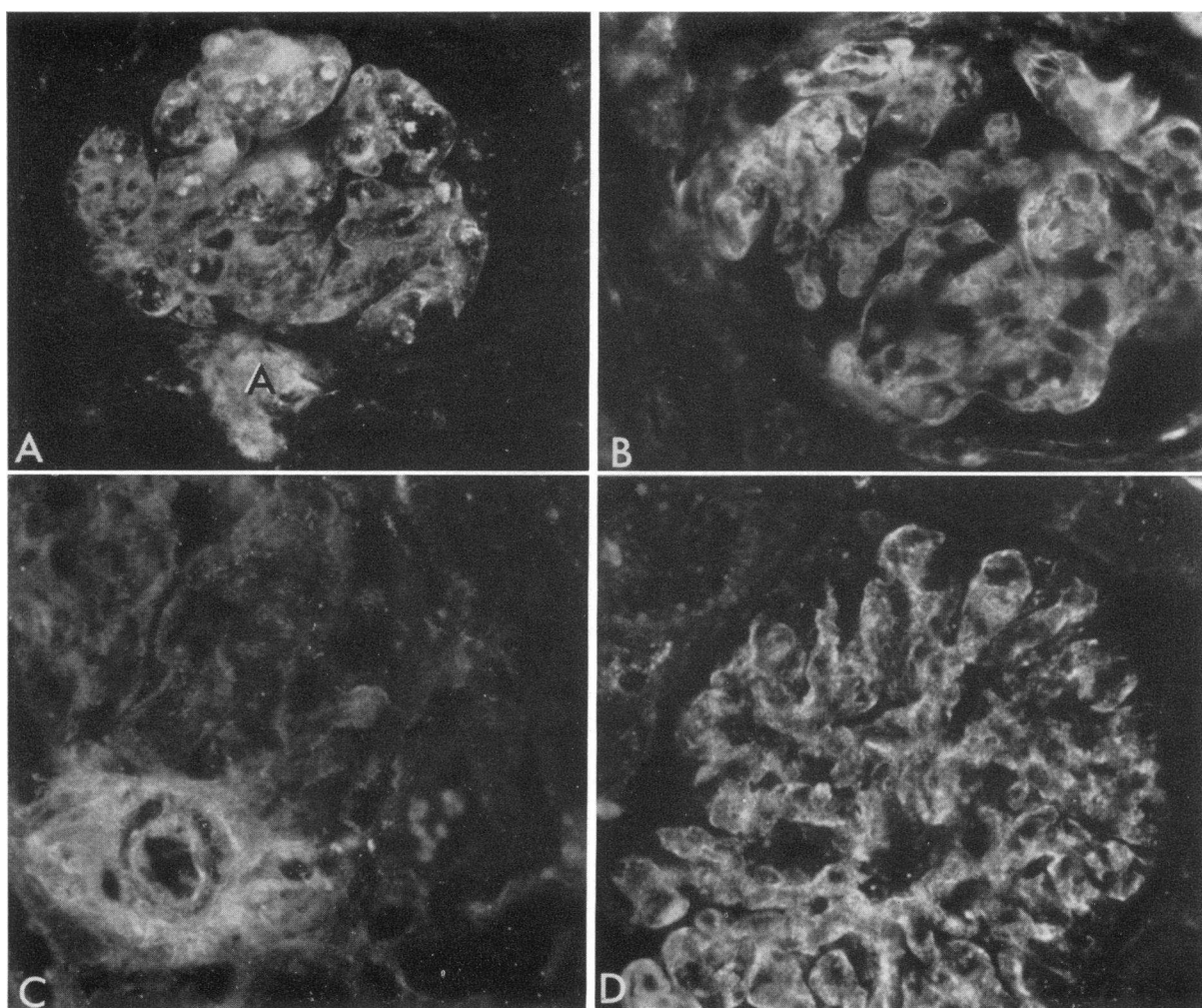*AHF Antigen and Fibrinogen in Renal Disease* 1379

FIGURE 4 Hemolytic-uremic syndrome and postpartum renal failure (A) AHF deposition in glomerulus and in afferent arteriole (A) in hemolytic-uremic syndrome (patient J. F.) ($\times 270$). (B) Fibrinogen deposition in glomerulus. Same renal biopsy as in A ($\times 450$). (C) Intense AHF deposition in an afferent arteriolar wall in hemolytic-uremic syndrome (patient J. R.). There was no significant glomerular AHF deposition in this specimen ($\times 480$). (D) AHF deposition along glomerular capillary loops in renal biopsy tissue from woman with postpartum renal failure. Fibrinogen deposition was seen in a similar distribution ($\times 500$).

glomerulonephritis with crescent formation. The eight patients with crescentic glomerulonephritis studied included two patients with Goodpasture's syndrome, two with Wegner's granulomatosis, two with rapidly progressive glomerulonephritis with granular immunoglobulin and complement deposition, one with hypersensitivity angiitis, and one with systemic lupus erythematosus nephritis. In each of these specimens, intense staining with antifibrinogen was present within the epithelial crescents of glomeruli (Figs. 5A and C). In these same glomeruli, positive staining for AHF was present within the glomerular capillary tufts but never in the crescents (Figs. 5B and D).

Dissociation of glomerular deposition of fibrinogen from that of AHF was also apparent in renal tissue from some patients with anaphylactoid purpura nephritis as well as patients with focal glomerulonephritis with recurrent macroscopic hematuria (Table I). Although precise glomerular localization was often difficult to establish, staining for fibrinogen in the specimens from patients with anaphylactoid purpura appeared to be predominantly within endothelial cells and along peripheral capillary loops. In one of the patients with recurrent macroscopic hematuria, glomerular fibrinogen staining was primarily within the mesangium; there was no AHF deposition in this site.
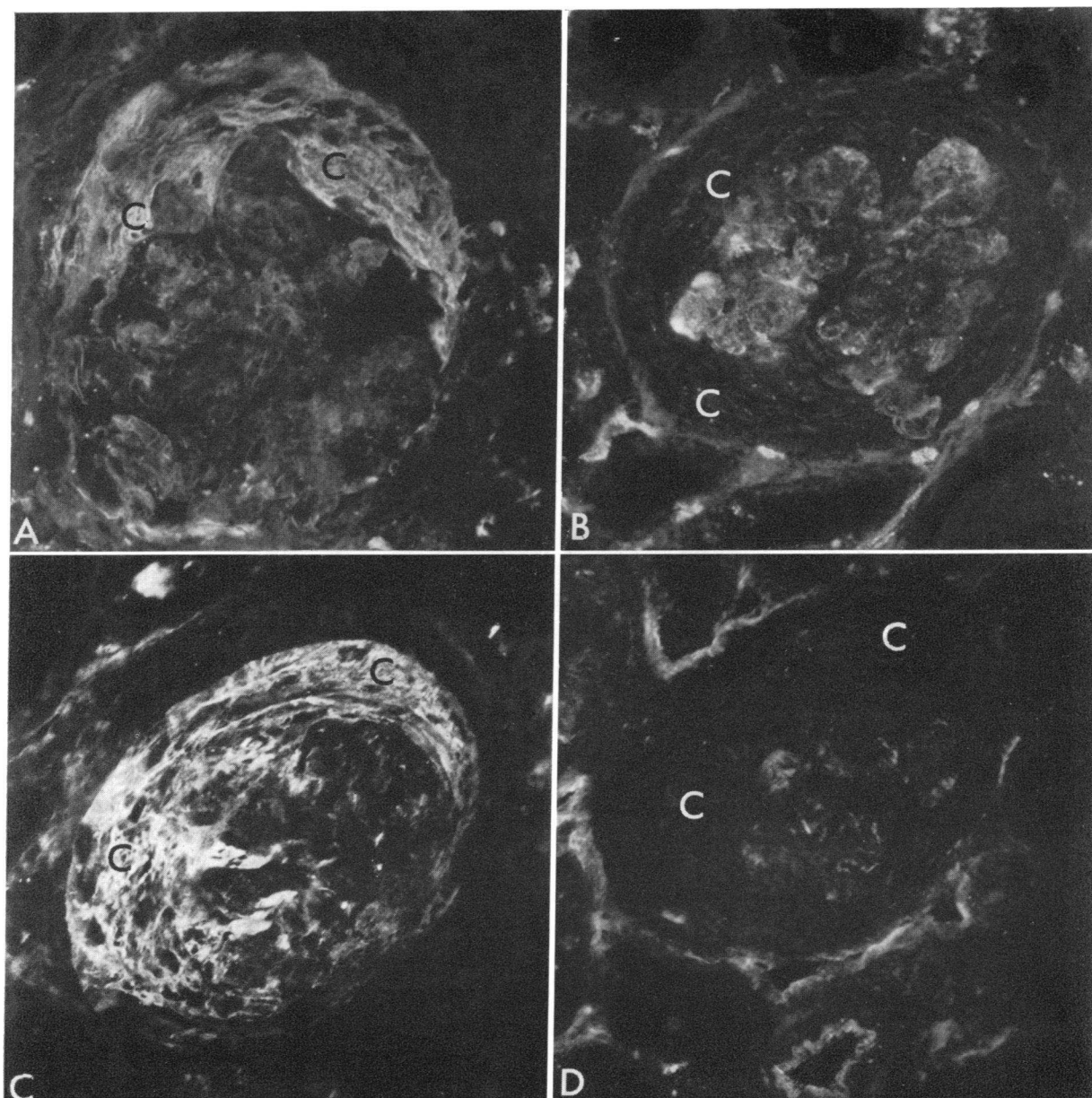
FIGURE 5 Glomerular crescents in proliferative glomerulonephritis. (A) Fibrinogen deposition in glomerular crescent (C) of a patient with rapidly progressive glomerulonephritis (× 260). (B) No deposition of AHF within the crescent of the same specimen (× 260). (C) Intense fibrinogen deposition in circumferential crescent (C) from a patient with Goodpasture's syndrome. Positive staining extends into spaces between lobules of the glomerular tuft (× 260). (D) Same glomerulus as in C when stained with anti-AHF. There is no positive staining within the crescent (C) (× 260).

Other diseases

In contrast to the patterns described above, no deposition of fibrinogen, or very weak staining compared with that of other serum proteins, was observed in glomeruli and arteries of 89 additional specimens. Most of these specimens were examined during the prospective study of 100 consecutive specimens received in our laboratory to gain further information about the specificity of altered AHF staining. Table IV lists specimens for which diagnoses could be established and in which there was normal or reduced staining of glomerular endothelial cells for AHF. Increased AHF staining of glomerular

TABLE IV

Renal Specimens without Increased AHF Antigen in Glomerular Endothelial Cells

Disease	Number of patients studied
Membranoproliferative glomerulonephritis	5
Membranous glomerulonephropathy	3
Steroid-resistant idiopathic nephrotic syndrome	3
Steroid-responsive idiopathic nephrotic syndrome	2*
Congenital nephrotic syndrome	5
Systemic lupus erythematosus	6
Amyloidosis	2
Renal dysplasia	3
Diabetes mellitus	2
Pyelonephritis	2
Fabry's disease	1
Nail-patella syndrome	1
Medullary cystic disease	1

* Two additional patients had increased AHF staining of glomerular endothelial cells without evidence of fibrinogen deposition.

endothelial cells, without fibrinogen deposition, was limited to renal tissue from three patients with acute glomerulonephritis and in two of four patients with steroid-responsive idiopathic nephrotic syndrome. Even though there was distortion of glomerular architecture, as in amyloidosis and chronic membranoproliferative glomerulonephritis, AHF staining was limited to peripheral capillary loops in these specimens.

DISCUSSION

Evidence that coagulation mechanisms and fibrin deposition play a role in renal disease is derived from several sources and has recently been reviewed in detail (1). The mechanism by which fibrinogen is deposited in renal disease has not been established, however, and the precise role of this protein in the pathogenesis of renal failure is unknown. Studies of other coagulation proteins may give insight into these mechanisms. Since antihemophilic factor is an essential component of the intrinsic coagulation pathway, evidence for participation of the coagulation system in the pathogenesis of renal diseases has been sought through immunohistologic study of AHF antigen.

The preparation of monospecific rabbit antisera to highly purified human antihemophilic factor has made it possible to define more clearly the physiologic regulation and synthesis of this coagulation protein (13, 17–20). In one of these studies, we have used immunofluorescent microscopy to identify AHF antigen in endothelial cells throughout the body (13). Tissue culture studies have verified this observation and have demonstrated AHF antigen synthesis by endothelial cells (20). Definition of antiserum specificity is essential for the interpretation of these studies and detailed evidence has been presented which demonstrates the monospecificity of the rabbit anti-AHF (13, 18). The protein which reacts with this antiserum can also be characterized by its two functional properties. Both antihemophilic factor procoagulant activity and "von Willebrand's factor activity" are inactivated by the rabbit antiserum (17, 18, 21). This inactivation, taken together with additional studies of the immunologic properties of anti-AHF (22), strongly suggest that this antiserum identifies the tissue localization of both AHF and von Willebrand's factor in these immunofluorescent studies (13).

The localization of AHF antigen in renal tissue has been compared with that of antigens which react with rabbit antihuman fibrinogen. Although antifibrinogen reacts with a single protein in normal human plasma, it does not differentiate this protein from other molecules which share antigenic determinants with fibrinogen (fibrin and the fibrinolytic degradation products of fibrinogen and fibrin). In this report these deposits are designated fibrinogen, the immunizing antigen, but the imprecise nature of this designation is recognized.

Intense staining with anti-AHF in a distribution similar to that of antifibrinogen was observed in glomeruli and/or arteries of renal tissue from patients with hyperacute homograft rejection, acute homograft rejection, hemolytic-uremic syndrome, postpartum renal failure, scleroderma, and malignant hypertension (Table I, patterns I and II). Proteins other than IgM were infrequently found in these tissues and then only in small quantities. It is possible, therefore, that the similar patterns of AHF and fibrinogen antigens reflect the involvement of the coagulation sequence. The basis for the IgM deposition (Table II), similar in distribution to that of AHF antigen and fibrinogen, is not known. It is possible that IgM initiates endothelial injury in these diseases and that the localization of coagulation proteins follows from this immunologic reaction. An alternative possibility, entrapment of large plasma proteins (IgM and AHF) within the fibrin coagulum, should also be considered. However, a protein with similar dimensions, α-2 macroglobulin, was not detected in these lesions. The rarity of IgM deposition in another site of AHF and fibrinogen deposition, arterial endothelial proliferative lesions, also supports the conclusion that AHF localization is not due to entrapment.

The precise mechanisms leading to the patterns of abnormal AHF antigen deposition have not yet been established. AHF localization may reflect the participation of this molecule in the coagulation sequence since the antigenic properties of the molecule are preserved

during coagulation despite loss of procoagulant function (17, 18). Breakdown in vascular integrity and subsequent diffusion of plasma AHF antigen into (and fixation within) extravascular sites could also lead to abnormal AHF antigen localization. For example, this mechanism is consistent with other features of the pathophysiology of hyperacute homograft rejection. Injury to the vascular endothelium, and thus the endothelial cells which are responsible for AHF antigen synthesis, might also lead to tissue deposition of AHF antigen. This sequence may be responsible for AHF localization in renal diseases in which there is marked endothelial cell hyperplasia.

In contrast to the parallel AHF and fibrinogen deposition in diseases with staining patterns I and II (Table I), the glomerular epithelial cell crescents of severe proliferative glomerulonephritis were intensely stained by antifibrinogen but not by anti-AHF. Although the mechanisms by which proteins with fibrinogen antigens are deposited in crescents are not known, the absence of AHF antigens in this site suggests that a different process, unrelated to coagulation, may be involved. More precise characterization of the fibrinogen-related proteins deposited in crescents is essential for a better understanding of the nature of these lesions. Antisera which specifically identified the degradation products of fibrinogen and fibrin (23) will be useful in future immunohistologic studies of crescents and other glomerular lesions in which fibrinogen antigen deposition is seen without AHF deposition. The presence of platelets in lesions may also be important since immunohistologic studies cannot distinguish fibrinogen intimately associated with the platelet surface from plasma-derived fibrin or its degradation products (24).

Another distinct immunofluorescent pattern, increased peritubular capillary staining with anti-AHF (Fig. 1) without fibrinogen deposition, was the most frequent finding in this study. This pattern was detected in a wide variety of renal diseases and there is no evidence that it is related to coagulation, per se. Although the studies reported here have been primarily directed toward an understanding of the role of the coagulation sequence in renal disease, they provide, as an associated finding, evidence that rabbit anti-AHF is a useful reagent for the identification of endothelial cells in normal tissues and in disease states.

Several recent studies, including the demonstrations that collagen and vascular basement membrane activate Hageman factor (25, 26), suggest that the coagulation sequence may be involved in some renal diseases. Endothelial injury, in some instances the result of immunologic reactions, may expose portions of glomerular basement membranes and other collagen-like proteins in arterial walls to the plasma protein, Hageman factor,

and thereby initiate coagulation through the intrinsic pathway. The patterns of AHF antigen and fibrinogen deposition described here in diseases characterized by endothelial injury suggest that coagulation mechanisms participate in the pathogenesis of some human renal diseases. Although a preliminary classification has been made possible by immunofluorescent studies, more precise definition of the pathophysiology remains a goal for future investigation.

ACKNOWLEDGMENTS

REFERENCES

1. Vassalli, P., and R. T. McCluskey. 1971. The pathogenetic role of the coagulation process in glomerular diseases of immunologic origin. Adv. Nephrol. 1: 47.
2. Vassalli, P., and R. T. McCluskey. 1964. The pathogenic role of the coagulation process in rabbit Masugi nephritis. Am. J. Pathol. 45: 653.
3. Vassalli, P., and R. T. McCluskey. 1964. The pathogenic role of fibrin deposition in immunologically induced glomerulonephritis. Ann. N. Y. Acad. Sci. 116: 1052.
4. Koffler, D., and F. Paronetto. 1965. Immunofluorescent localization of immunoglobulins, complement and fibrinogen in human diseases II. acute, subacute, and chronic glomerulonephritis. J. Clin. Invest. 44: 1665.
5. Koffler, D., and F. Paronetto. 1966. Fibrinogen deposition in acute renal failure. Am. J. Pathol. 49: 383.
6. McCluskey, R. T., P. Vassalli, G. Gallo, and D. S. Baldwin. 1966. An immunofluorescent study of pathogenic mechanisms in glomerular diseases. N. Engl. J. Med. 274: 695.
7. Vassalli, P., R. H. Morris, and R. T. McCluskey. 1963. The pathogenic role of fibrin deposition in the glomerular lesions of toxemia of pregnancy. J. Exp. Med. 118: 467.
8. Urizar, R. E., A. Michael, S. Sisson, and R. L. Vernier. 1968. Anaphylactoid purpura. II. Immunofluorescent and electron microscopic studies of the glomerular lesions. Lab. Invest. 19: 437.
9. Starzl, T. E., H. J. Boehmig, H. Amemiya, C. B. Wilson, F. J. Dixon, G. R. Giles, K. M. Simpson, and C. G. Halgrimson. 1970. Clotting changes, including disseminated intravascular coagulation, during rapid renal-homograft rejection. N. Engl. J. Med. 283: 383.
10. Roy, L. P., A. J. Fish, R. L. Vernier, and A. F. Michael. 1973. Recurrent macroscopic hematuria, focal nephritis, and mesangial deposition of immunoglobulin and complement J. Pediatr. 82: 767.
11. Stiehm, E. R., and C. W. Trygsted. 1969. Split products of fibrin in human renal disease. Am. J. Med. 46: 774.

12. Braun, W. E., and J. P. Merrill. 1968. Urine fibrinogen fragments in human, renal allografts. A possible mechanism of renal injury. *N. Engl. J. Med.* **278**: 1366.

13. Hoyer, L. W., R. P. de los Santos, and J. R. Hoyer. 1973. Antihemophilic factor antigen: localization in endothelial cells by immunofluorescent microscopy. *J. Clin. Invest.* **52**: 2737.

14. Michael, A. F., Jr., K. N. Drummond, R. A. Good, and R. L. Vernier. 1966. Acute poststreptococcal glomerulonephritis: immune deposit disease. *J. Clin. Invest.* **45**: 237.

15. Gajl-Peczalska, K. J., A. J. Fish, H. J. Meuwissen, D. Frommel, and R. A. Good. 1969. Localization of immunological complexes fixing β_{1c} (C3) in germinal centers of lymph nodes. *J. Exp. Med.* **130**: 1367.

16. van Mourik, J. A., and I. A. Mochtar. 1970. Purification of human antihemophilic factor (factor VIII) by gel chromatography. *Biochim. Biophys. Acta.* **221**: 677.

17. Zimmerman, T. S., O. D. Ratnoff, and A. E. Powell. 1971. Immunologic differentiation of classic hemophilia (factor VIII deficiency) and von Willebrand's disease. *J. Clin. Invest.* **50**: 244.

18. Hoyer, L. W. 1972. Immunologic studies of antihemophilic factor (AHF, factor VIII). IV. Radioimmunoassay of AHF antigen. *J. Lab. Clin. Med.* **80**: 822.

19. Bennett, B., and O. D. Ratnoff. 1972. Changes in antihemophilic factor (AHF, factor VIII) procoagulant activity and AHF-like antigen in normal pregnancy and following exercise and pneumoencephalography. *J. Lab. Clin. Med.* **80**: 256.

20. Jaffe, E. A., L. W. Hoyer, and R. L. Nachman. 1973. Synthesis of antihemophilic factor antigen by cultured human endothelial cells. *J. Clin. Invest.* **52**: 2757.

21. Weiss, H. J., L. W. Hoyer, F. R. Rickles, A. Varma, and J. Rogers. 1973. Quantitative assay for a plasma factor, deficient in von Willebrand's disease, that is necessary for platelet aggregation. Relationship to factor VIII procoagulant activity and antigen content. *J. Clin. Invest.* **52**: 2708.

22. Hoyer, L. W. 1973. Specificity of precipitating antibodies in immunologic identification of antihaemophilic factor. *Nat. New Biol.* **245**: 49.

23. Plow, E., and T. S. Edgington. 1973. Immunobiology of fibrinogen. Emergence of neoantigenic expressions during physiologic cleavage in vitro and in vivo. *J. Clin. Invest.* **52**: 273.

24. Gokcen, M., and E. Yunis. 1963. Fibrinogen as part of the platelet structure. *Nature (Lond.).* **200**: 590.

25. Wilner, G. D., H. L. Nossel, and E. C. LeRoy. 1968. Activation of Hageman factor by collagen. *J. Clin. Invest.* **47**: 2608.

26. Cochrane, C. G., S. D. Revak, B. S. Aikin, and K. D. Wuepper. 1972. The structural characteristics and activation of Hageman factor. *In* Inflammation: Mechanisms and Control. I. H. Lepow and P. A. Ward, editors. Academic Press, Inc., New York. 119.