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Postinfections and Autoimmune Glomerulonephritis: Are Cationic Antigens Involved?

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Summary. **This review provides a short historical introduction to the development of ideas on the pathogenesis of immune complex glomerulonephritis. The accent is placed on the formation of subepithelial immune deposits and involvement of cationic antigens. Recent data are presented on the role of histones in human and experimental lupus nephritis; this type of polycation could play a key role in the induction of glomerular injury and throw new light on the conundrum of lupus nephritis and DNA. In spite of intensive efforts a nephritogenic antigen from an infectious agent has not yet been identified with certainty. The difficulties connected with this task are discussed and an approach to tackling this problem is outlined.**

In the last four decades a mass of data on possible pathomechanisms of immunologically mediated glomerulonephritis (GN) have been gathered. With few exceptions the antigens involved in natural occurring nephritides are still unknown. Sophisticated techniques developed in the last decade have opened new possibilities and time may be ripe to re-tackle the problem of identifying nephritogenic antigens with more prospect of success.

THE BEGINNING OF EXPERIMENTAL RESEARCH ON IMMUNOLOGIC NEPHRITIS

The scenery of immunological injury in kidney disease dates back to the experimental work of Lindemann,[1)] though this nephrotoxic (anti-whole kidney) antiserum seems to have caused more tubular than glomerular lesions. It was Masugi's great merit to have established an experimental nephritis model in the rabbit resembling diffuse GN observed in man.[2,3)] Masugi convincingly showed that, in contrast to the belief of earlier scientists, the primary lesions caused by his nephrotoxic antiserum affected the vessels of the relevant organs.[3)]

Masugi nephritis and also the autologous form of anti-GBM GN[4)] are characterized by linear deposition of antibody along the GBM.[5)] Both models resemble Goodpasture's syndrome in man.

The nephritogenic antigen[5)] responsible for the induction of both the experimental anti-GBM glomerulonephritides is not yet identified but a globular domain (NC1) of type IV collagen[6,7)] is a strong contender as has been already shown for the Goodpasture antigen by Wieslander.[8)]

IMMUNE COMPLEX (IC) GLOMERULONEPHRITIS

Goodpasture's syndrome is a rather rare disease in man. The most common immunologically caused nephritis form is immune complex GN. In this case the nephritogenic antigen is in general not a structural component of the glomerulus but rather an extrinsic molecule. Either an exogenous bacterial, viral or parasitic antigen in postinfectious GN or an autologous antigen in the case of autoimmune associated GN. The recognition of postinfectious GN as an allergic entity dates back to the early part of this century when Schick and von Pirquet pointed to the striking similarities in the course of GN in scarlet fever and the nephritis observed in serum sickness.[9,10)] Leaning on the observation that nephritis is one of the most typical features of serum sickness the classical studies of Germuth.[11)] Dixon et al.,[12)] and Germuth and Rodriguez[13)] in rabbits and

rats have elucidated the critical qualities of soluble circulating immune complexes depositing in the GBM. Particularly immune complexes formed in antigen excess, which ramain small, soluble and able to activate complement are thought to be deposited in the glomerulus and cause the inflammatory process.[14] Using radio-labeled tracer antigen in the acute and chronic serum sickness model the careful studies of Dixon[15] and Germuth and Rodriguez[13] seemed to leave no doubt about the pathogenic role of circulating immune complexes. However, whether the same mechanisms were working in postinfectious ICGN remained uncertain. There are several weak points in the above concept that circulating immune complexes of distinct size are pathogenic. Firstly one has to administer antigen in amounts so huge, that they are unlikely to occur in the circulation during the course of an infectious disease. Secondly, independent of the ratio of antigen and antibody, intravenous injection of preformed antigen-antibody complexes does not lead to their deposition along the glomerular capillary wall, they regularly end up in the mesangium.[16] There is only one exception in the literature, reported by Germuth et al.[17] These authors observed subepithelial deposits after repeated injection of preformed ovalbumin-anti-ovalbumin complexes into mice. The immune complexes were formed in 80 fold antigen excess and the antibodies used were of low avidity. Analyzing the experimental conditions Mannik[18] came to the conclusion that the repeated injection of these complexes would create conditions of free antigen and antibody due to rapid loss of excess ovalbumin allowing local and even subepithelial IC formation. A similar situation of alternating antigen and antibody excess can be postulated in chronic serum sickness in those animals developing GN. This idea is supported by the observation of Fleuren et al[19]; who showed that only when the isolated kidney was perfused repeatedly and alternatively with antigen (BSA) and antibody (anti-BSA) could subepithelial deposits be observed. Perfusion with preformed soluble immune complexes did not result in capillary deposition. Adler et al.[20] repeating the experiment obtained subepithelial deposits only when cationized BSA was used instead of native BSA. This apparent discrepancy was resolved by the discovery that the antibody fraction employed by Fleuren et al. was in fact cationic (personal communication). Clearly one of the components of the IC had to be cationic; cationic molecules possess, as we will learn later, affinity for the negatively charged glomerular filtration barrier.

THE CONCEPT OF IN SITU IC FORMATION

Izui and co-workers[21] first proposed that antigens may be nephritogenic because they possess affinity for the GBM. They were also the first to propose in situ formation of glomerular immune deposits with such antigens. In situ formation of glomerular deposits as an alternative glomerular deposition of circulating immune complexes was favored by Couser and Salant[16] who pointed to the model of passive Heymann nephritis where such a mechanism could be demonstrated.[22,23] In passive Heymann nephritis the target structure for the anti Fx1A antibody, a 330kD glycoprotein (gp 330) is a constituent of the coated pits of glomerular epithelial cells.[24,25] The question arises whether a mechaism similar to that operating in passive Heymann nephritis can be assumed for membranous nephropathy in man or if it is not more likely that extrinsic antigens with special qualities are the initiating agents. Certainly till now there has been no convincing report on the existence of autoantibodies to an antigen, comparable to the gp 330 in the rat, in sera of patients with membranous nephropathy. Attempts to detect brush border antigen in membranous GN in man have usually been negative.[26] Let us turn to non-glomerular constituents. Here the experimental studies of the last decade have opened up new perspectives. Two classes of non-glomerular antigens have been shown to possess affinity for the GBM and to act as planted target for circulating antibody: lectin and cationic (cationized) proteins (Table 1).

Though in retrospective some variations of the nephrotoxic nephritis model[28,29] as well as the experimental design of Mauer et al[30] can be regarded as examples involving planted antigen (Table 1) they cannot give us exact information about the qualities which make an antigen nephritogenic. The notion of Izui et al[21] that DNA could act as planted antigen in Lupus nephritis certainly stimulated experimental studies, but we now know that DNA does not possess any marked affinity for the GBM when tested in vivo.

LECTINS

The first experimental nephritis model utilizing the principle of planted exogenous antigen was that of Golbus and Wilson[31] who employed the lectin concanavalin A, a glucose and mannose binding plant product as antigen. Perfusion of the left kidney with Con A followed by systemic injection of antibody induced unilateral proteinuria. The glomerular

Table 1. "Planted antigens" forming complexes *in situ*

Antigen	Authors	Reference	Remarks
Anti GBM-IgG	Hammer and Dixon, 1963	28	Subnephritogenic dose
Anti GBM-Fab	Vogt et al., 1965	29	
Aggregated IgG	Mauer et al., 1973	30	Mesangial localization
DNA	Izui et al., 1976	21	Mesangial localization, prior systemic application of endotoxin necessary
Concanavalin A	Golbus and Wilson, 1979	31	Intrarenal injection of antigen
Cationized ferritin	Batsford et al., 1980	41	Intrarenal injection of antigen
BSA	Fleuren et al., 1980	19	Repeated alternating (antigen, antibody) renal perfusion
Cationized BGG-Anti BGG	Gallo et al., 1981	54	Intravenous application
Cationized BSA	Border et al., 1982	53	Repeated intravenous injection of antigen
BSA	Barnes and Venkatachalam, 1984	62	Mediated by prior injection of polyethyleneimine
Histone and DNA	Schmiedeke et al., 1989	64	DNA deposition mediated by histone

From Cameron, 1983, (27) supplemented

immune complexes in this model are found predominantly on the endothelial side of the lamina densa,[32] but as Fries et al.[33] commented the electron microscopic pictures presented also revealed some subepithelial deposits. Whenever subepithelial deposits are present one should carefully consider if cationic proteins could be involved (see below). Working with another lectin, Helix pomatia agglutinin, Matsuo et al.[34] observed even more prominent subepithelial deposits. In their experiments the lectin was planted initially at the surface of the glomerular endothelial cells by perfusion of the left kidney with neuraminidase followed by the lectin. The interaction of subsequently infused antibody with the planted antigen initially caused granular immune deposits within the subendothelial space which transferred to the subepithelial region within 2 to 7 days. The movement of the immune complexes from the luminal to the subepithelial side is less surprising if one bears in mind that Helix pomatia consists of isolectins, the majority being cationic the degree depending on the source of the product.[35] In the case of Con A/anti-Con A nephritis another explanation can be proposed for the occurrence of subepithelial deposits. Con A is an anionic protein (pI 4.5-5.5). Here the transar from the endothelial to the epithelial side of the GBM may have been accomplished by the participathon of cationic mediators. We have learned from our studies with polycations that such a mechanism can operate.[36]

In the acute phase of the Con A/anti-Con A nephritis model myeloperoxydase is released by the infiltrating activated polymorphs and is intimately involved in the resulting kidney injury.[32] Myeloperoxydase is a highly cationic enzyme (MW 117,000; pI 10.5) and should be able to mediate the passage of Helix pomatia through the GBM. The filtration pressure alone, as originally proposed by Matsuo et al[34] may be not sufficient to explain the translocation of anionic endothelial immune complexes across the GBM.

An exclusively subendothelial localization of the glomerular deposits was seen when the intra-renally perfused Con A had been conjugated to ferritin. In spite of massive glomerular infiltration by granulocytes, no passage of the large Con A/ferritin complexes through the GBM was observed, the complex was clearly too big to penetrate the filtration barrier.

Many bacteria have lectins on their surface[37] and those possessing affinity for the GBM are certainly candidates for nephritogenic antigens.

QUALITIES OF CATIONIC PROTEINS WHICH PROVIDE AN AFFINITY FOR THE GBM

The important message which one can infer from Izui's work[21] and Golbus and Wilson's[31] experiment is that antigens may be nephritogenic because they possess an affinity for the GBM. In other words: if

one is interested in identifying nephritogenic antigens one should look for antigens which reveal affinity for the GBM. What qualities of the GBM could, apart from lectin receptors, serve to bind antigens? In the 1970's scientists working in nephrology realized that the laminae rarae of the GBM were highly negatively charged and that anionic and cationic molecules were handled differently by the filtration barrier.[38] The negatively charged structures of the GBM can be made visible with tracers like polyethyleneimine (Fig. 1). The key inspiration for us was the paper of Rennke et al.[39] published in 1975, where they showed that cationized ferritin is not excluded from the filtration barrier like the native, anionic molecule but instead can penetrate into all three layers of the GBM. This gave us the idea that cationic antigens could interact with highly anionic structures of both laminae rarae and thus serve as target for circulating antibody.[40–42] We started to test this concept in simple experiments by intravenously injecting rats with proteins of various size modified to different degrees. In contrast to unmodified proteins intravenously given cationized antigens accumulated along the glomerular capillary wall. The degree of accumulation and the persistence within the GBM are dependent on charge and size. The higher the pI and the larger the size the better the accumulation and the longer the persistence.[43] Working with polymers of lysozyme (crosslinked by dimethylsuberimidate), a naturally occurring cationic (pI 11) but rather small (14KD) molecule we could confirm and extend the conditions for binding to and persistence in the GBM. Monomeric and dimeric lysozyme had no tendency to accumulate within the GBM. Injection of tetrameric (57KD) and higher polymers led to impressive glomerular capillary deposition.[44]

Thus we were able to establish criteria for selecting nephritogenic candidates. The pI should be above 9.0 and the size larger than 40kD. The planted antigens are accessible for subsequently, injected antibody (Fig. 2). If the antigen is highly cationic and of large size, like ferritin, then the antibody can reach the antigen planted to the glomerular capillary wall even after an interval of 6 to 16 hours (Table 2). At the electron microscopic level the deposits are mainly located in the subepithelial region. Using cationized ferritin as antigen we could show that the glomerular deposits initially form at the subendothelial sites but are transferred with increasing time to the subepithelial region.[43] Because no large aggregates of ferritin molecules were seen at any time in the lamina densa we believe that the conjugates passed the lamina densa in dissociated form as free antigen and antibody molecules or as small conjugates to reform beyond the lamina densa. In recent immunoelectronmicroscopic studies, applying the immunogold technique Fujigaki et al.[45] were able to show that not free ferritin and free antibody molecules but rather fer-

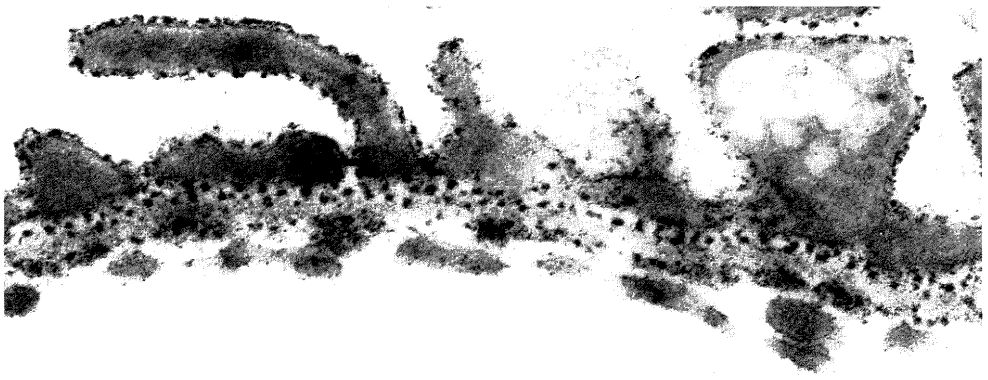

Fig. 1. Section of glomerular capillary wall. Distribution of negative charges is revealed by PEI staining. Note staining in the lamina rara interna, externa and on the epithelial surface coat.

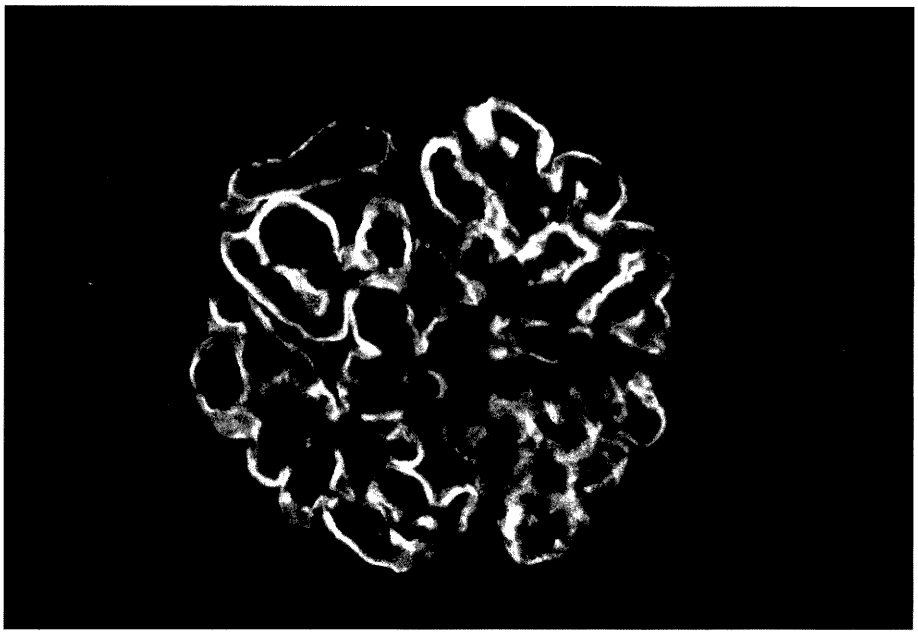

Fig. 2. Immunofluorescence micrograph of a glomerulus from a rat receiving cationized human IgG followed by rabbit anti-human IgG. Tissue taken one hour later stained for rabbit IgG.

Table 2. Accessibility of GBM bound cationized ferritin for circulating antibody

Interval (hours)	Immunofluorescent staining for	
	ferritin	rabbit IgG
0*	+++	0
1	(+)	+++
2	(+)	+++
4	(+)	+++
8	(+)	++
16	±	++
24	±	+
48	0	+
72	0	(+)

Per rat 5 mg highly cationized ferritin and 1.0 ml anti-ferritin from the rabbit were administered intravenously. Kidneys were removed 1 h after the injection of anti-ferritin
*Only ferritin was injected

ritin molecules associated with antibody molecules were present in the lamina densa. From these observations one can conclude that cationic immune complexes formed on the inner side of the filtration barrier can pass the lamina densa in small complexes and can reform to larger aggregates in the subepithelial region. As hinted at above all experimental data gathered till now supports the notion that the presence of subepithelial deposits is a strong indication for the involvement of cationic antigens. Using cationized human IgG or cationized ferritin planted, via the renal artery, in the left kidney followed one hour later by intravenously applied antibody we were able to cause a full blown unilateral nephritis with heavy proteinuria. Low amounts of antigen and antibody are sufficient to induce kidney damage.[46] Oite et al.[47,48] have also established an active model where the cationized antigen is given intravenously to a pre-immunized rat. Under these conditions a chronic ICGN can be achieved. The glomerular immune deposits in these models are predominantly, if not exclusively, located in the subepithelial area. The degree of subepithelial IC formation depends largely on epitope density as recent studies using haptenized cationic antigens have shown.[49,50] This is in concordance with earlier reports that precipitating antibodies[51] and polyvalent antigen-antibody interactions[52] are required for the formation of subepithelial immune deposits.

In the foregoing it was imputed that subepithelial deposit formation with cationic antigens is an in situ event. The first experiments studying the interaction of cationic antigens with the GBM and the local immune complex formation with the subsequently

given antibody used an in situ design. The experimental design is very convenient to study the affinity of antigens for the GBM and their nephritogenic potency.

But we are quite aware that in the natural course of ICGN other pathomechanisms can also be involved. In respect of cationic antigens the debate of in situ formation versus deposition of circulating immune complexes is a rather esoteric one. The latter, if cationic, possess affinity for the GBM, too. The data of Border et al.[53] obtained in the serum sickness model using cationized BSA instead of native BSA are compatible both with the assumption of in situ IC formation as well as glomerular deposition of soluble IC formed within the circulation. There is no reason to doubt that soluble immune complexes of which one or both components are highly cationic can fix to the anionic site of the GBM. In fact Gallo et al[54] injecting soluble preformed cationic immune complexes showed the same glomerular localization as with cationized ferritin or cationized human IgG in the in situ arrangement. The initial binding of the preformed immune complexes occurred subendothelially, with time the deposits changed to a subepithelial localization. Koyama et al.[55] also working with preformed immune complexes pointed to the fact that chemical cationization of antigen changes the characteristic of the antigen-antibody interaction resulting in low precipitating efficiency. They therefore believe that for subepithelial deposition of immune complexes not only charge but also avidity of the antibody involved is of relevance. Low avidity antibody favors dissociation of immune complexes and enhances the chance of antigen and antibody passing the size barrier of the lamina densa.[56] However, low avidity antibodies are not absolutely essential for subepithelial deposit formation. In experiments planting Helix fomatia agglutinin (68kD; pI 10.5) and avidin,[57] two native cationic antigens, to the GBM, followed by the corresponding hyperimmune antisera (certainly high avidity antibody) the glomerular deposits induced were finally located exclusively subepithelially.[34,57] The net charge of the antigen or of the immune complex and their size obviously determine their deposition in the glomerular capillary wall. Highly cationic compounds react strongly with the peripheral GBM. The more cationic the complexes the more they become localized subepithelially and the more nephritogenic they are.[33] Neutral and slightly cationized proteins and complexes are in contrast to real polycations deposited predominantly in the mesangium.[58] Glomerular persistence of the complexes depend largely on the degree of lattice formation.

Why cationic glomerular IC which reach the subepithelial space can, in contrast to the initially subendothelial located, persist there for a long time is not yet clear. Dissociation that is observed with the endothelial IC should also occur with the IC transferred to the epithelial region. Vogt et al.[59] have speculated that the persistence of glomerular IC may be prolonged due to covalent binding between glomerular structures and the IC and that this is more likely to happen in the vicinity of the epithelial cells than in the endothelial area.

MEDIATION OF GLOMERULAR DEPOSITION OF ANIONIC MACROMOLECULES BY POLYCATIONS

Polycation administration can not only increase the permeability of the glomerular filter for anionic macromolecules[60,61] but can also mediate the glomerular deposition of anionic macromolecules which then can act as planted antigen for circulating antibody[62,36] and probably also for circulating anionic IC. In this connection it may be of relevance that inflammatory cells, like polymorphs and macrophages, contain and when activated, release polycations which locally could reach concentrations high enough to permit charge neutralization or even charge conversion of the GBM. The occasional reports of subepithelial immune deposit formation after administration of soluble anionic immune complexes formed with low avidity antibodies[17,63] and after planting of Con A to the endothelial side of the GBM[31] may thus find an explanation.

In a recent study Schmiedeke et al.[64] have, following these preceding idea, presented a new pathomechanism for lupus nephritis. In their concept histone as a highly polycationic molecule and DNA as a strong polyanion and the corresponding specific antibodies are assumed to be involved. This concept is discussed in the following in more detail and may serve as an example of a cationic nephritogenic antigen playing the decisive role in the pathogenesis of an autoimmune immune complex glomerulonephritis.

THE PUTATIVE NEPHRITOGENIC ANTIGEN(S) IN LUPUS NEPHRITIS

Lupus nephritis is a common severe organ manifestation of systemic lupus erythematosus. Subepithelial deposits, which point to cationic antigens, are a frequent feature of this type of nephritis. A prominent

characteristic in SLE are the autoantibodies against nuclear antigens. These nuclear antigens and the corresponding autoantibodies are most likely the cause of this type of nephritis. It is generally accepted and you can still read it in the textbooks, that the glomerular deposits in lupus nephritis are composed of ds-DNA and anti-DNA antibody. For us, with experience of cationic antigens, it seems very unlikely that DNA, which is highly anionic, can be deposited in the GBM. Table 3 contains a list of autoantibodies found in the sera of lupus patients. Among the most frequent autoantibodies are those directed against histones. The prevalence is even higher than that of the assumed pathogenic anti-ds-DNA antibody (70 percent versus 40 percent). Histone is a DNA-binding and DNA packing protein which is highly cationic. There are 5 mammalian histone subfractions all with a pI of about 11. The size of the monomeric histones, 11 to 21kD, seems too small to endow high affinity for the GBM, but histones have the tendency to aggregate and can thus gain the critical size necessary to act as planted antigen. We proposed that histone may be the key antigen in the pathogenesis of lupus nephritis. Table 4 shows our concept which we arrived at after a long series of experiments. The extremely positive histones bind to the GBM, act as planted antigen and induce in situ IC formation leading to GN and perhaps chronic renal disease. A second, very intriguing mechanism, is that the polycation histone mediate the subsequent binding of polyanionic antigens, for example DNA. Also compatible with our concept is the idea, that small, soluble circulating complexes containing histone, DNA fragments plus antibody could become deposited in the GBM and act as target for further antibody and for example complement components.

Table 3. Autoantibodies found in high frequency in systemic lupus erythematosus

Antigen	Autoantibody frequency (%)
ds DNA	40
ss DNA	70
Histones	70
Sm	30
Nuclear RNP	32
SS-A/Ro	35
Heat-shock protein, Hsp 90	50

From Tan (65) shortened

Table 4. Suggested pathways of histone and DNA involvement in lupus nephritis

GBM + histone	+ Anti-histone
GBM + histone/DNA (circulating complex)	+ Anti-histone + Anti-DNA
GBM + histone/DNA/Anti-DNA (circulating complex)	+ Anti-histone
GBM + histone/DNA/Anti-histone/Anti-DNA (circulating complex)	

The + sign denotes a sequential series of events.
A combination of events is possible.
These ideas can be applied to other cationic and anionic antigens; the common principle is the involvement of a polycationic macromolecule as the primary event

Intrarenal perfusion of histone subpopulations into a rat resulted in massive glomerular deposits. When 200 μg of ^{125}I labeled histone subfraction were given between 6.6 and 15.8 percent of the injected material was found in the isolated glomeruli after 15 min, the histones H3, H2a/H4 and H2b revealing the highest affinity for the GBM[64] (Fig. 3). Significant histone deposits were still present after 8 hours. The planted histones were accessible for subsequently given antibody, resulting in in situ immune complex formation. Histones are highly conserved proteins, and therefore only weak immunogens. The antibody titers attainable by immunization of rabbits are low. This is a major obstacle in our attempts to induce nephritis with subsequent passive injection of anti-histone antibody. In this connection two considerations may be pertinent. Firstly, in the natural course of lupus nephritis there is a multiple sequence of antigen-antibody interactions in the circulation or at the glomerular capillary wall, rather than a single immunological event and secondly other antigen-antibody systems may be involved in addition. As already mentioned before polycations can mediate the glomerular deposition of anionic macromolecules.[36,62] There are repeated reports that eluates of isolated glomeruli from lupus kidneys contain anti-DNA activity.[66] The simplest explanation for the presence of anti-DNA-antibody is that it is complexed with DNA. DNA as we have shown does not possess an affinity for the GBM in vivo as renal perfusion with polydisperse ss- or ds-DNA does not produce localization in the glomeruli to a significant extent.[67] However, when histone has been previously planted in the GBM, massive glomerular DNA-deposition can be achieved. More than 30 percent of intrarenally perfused ^{125}I-labeled ds-DNA fragments were found in the isolated glomeruli when measured 15 min after administration,[67] binding was clearly mediated by the planted histone. Histone and DNA could be detected in a granular pattern along the capillary wall (Fig. 4). By electronmicroscopy

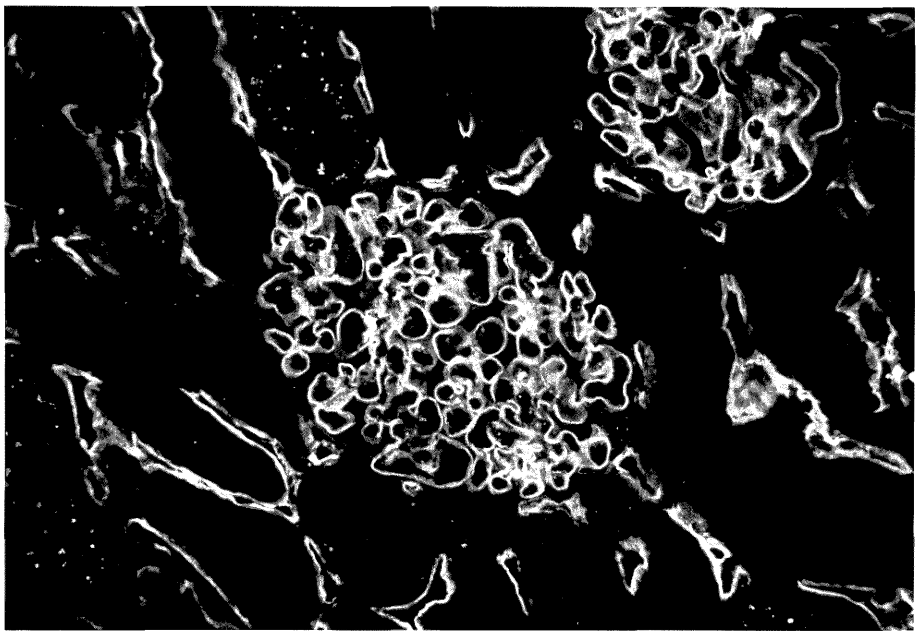

Fig. 3. Immunofluorescence micrograph of a glomerulus from a rat receiving histone H2a/H4 stained with rabbit anti-H2a/H4. Tissue removed after 1 h. Intense staining of the glomerular and peritubular capillaries is seen.

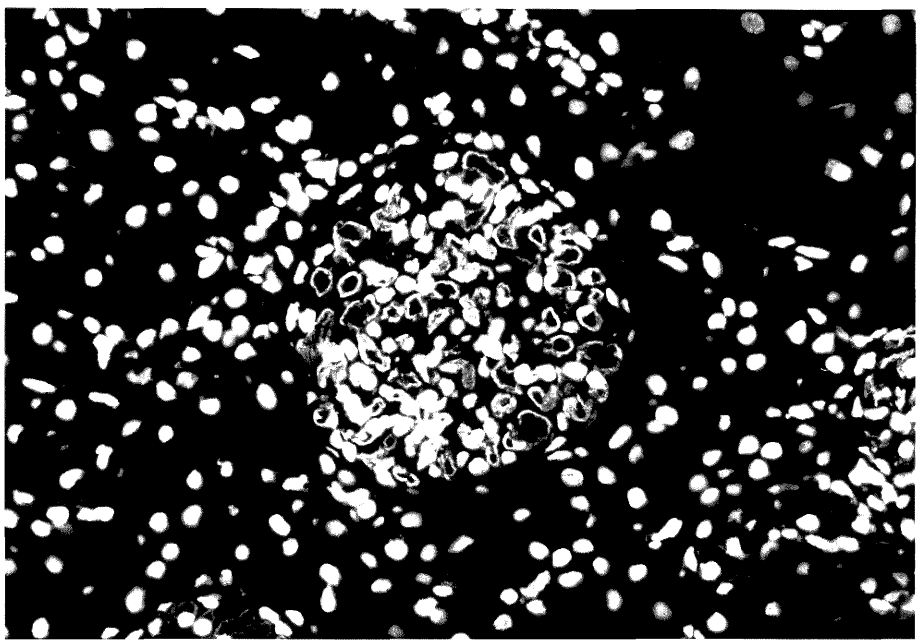

Fig. 4. Demonstration of glomerular deposition of DNA mediated by histone stained with the intercalating dye DAPI. Cell nuclei are stained in addition.

both components, ^{125}I labeled histone and subsequently given DNA, were located in all three layers of the GBM (Fig. 5). Elution studies with isolated glomeruli containing ^{125}I labeled DNA revealed that predominantly large DNA fragments of about 500 kD were present in the glomerular deposits (unpublished).

If histones are, as we believe, the initiating nephritogesic antigen in lupus nephritis, one should

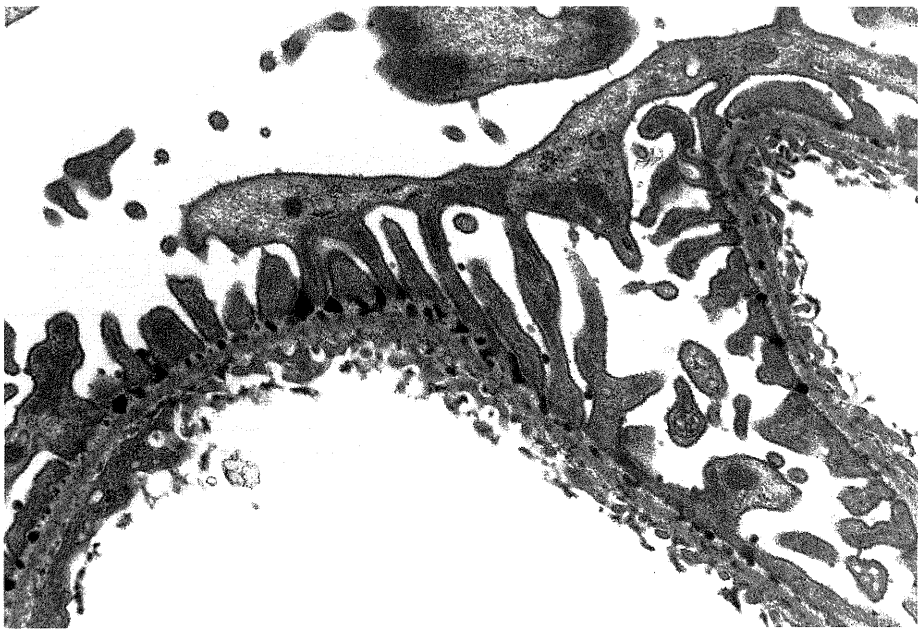

Fig. 5. Section of capillary wall showing electron dense DNA deposits in all layers of the GBM. The rat received 500 μg histone H3 followed by 320 μg ds-DNA fragments. Tissue removed 15 min later.

expect to find histones in the glomerular deposits in diseased kidneys. This is the case, as we were able to demonstrate recently. We have investigated kidneys of lupus mice (NZB/W and GvH) at different stages of disease by indirect immunofluorescence. Positive staining was seen along the capillary wall in a pattern similar to IgG in the proteinuric mice and in some mice not yet proteinuric but with IgG deposits.[68] In the meantime we have had the chance to investigate kidney biopsies from patients with lupus nephritis, where we were also able to find glomerular deposits of histones in about 70 precent of the cases (unpublished).

All the data gathered till now are in line with the notion that we have probably identified a nephritogenic antigen in lupus nephritis.

SEARCH FOR NEPHRITOGENIC ANTIGENS IN POSTINFECTIOUS ICGN

One of the commonest and best investigated forms of postinfectious ICGN is acute poststreptococcal GN (APSGN). Subepithelial deposits are a common feature in this form of GN and this encouraged us to search for cationic antigens. Group A streptococci, when grown at pI 5.9 in a suitable medium, produce a huge amount of cationic products, now known to be cleavage products of a cationic extracellular protease and the precursor zymogen. In 1982 we had the opportunity to investigate biopsies from cases with APSGN. We were able to demonstrate the extracellular cationic protease within the glomerular deposits.[69] Our expectation that we had, for the first time, identified a nephritogenic antigen from an infectious agent was unfortunately not fulfilled. Thus it shares the same fate as two other streptococcal antigens, also accused of being involved in the pathogenesis of APSGN, the nephritis strain associated protein (NSAP) of Zabriskie's group[70] and the endostreptosin of Lange's group.[71] None of these antigens possess significant affinity for the GBM. The cationic streptococcal protease (pI 9 to 9.2) does not bind to any worthwhile degree to the GBM, when injected intravenously into rats. In retrospective this failure is not surprising for the molecular mass size of the (monomeric) protease (30 kD) is rather small.

STRATEGY FOR IDENTIFYING CATIONIC NEPHRITOGENIC ANTIGENS

The search for nephritogenic antigens has been largely disappointing. Can one develop more promising strategies? Our failure with the streptococcal antigen and the success with histones in lupus nephritis can give some answers. We have to screen for antigens which possess an affinity for the GBM. If we think of cationic antigens, the candidate should have a pI of above 9 or even above 10 and be larger than 40 to 50

Table 5. A Strategy for identifying nephritogenic antigens

1. Isolation of cationic extracellular and intracellular proteins from infectious agents
2. Selection and enrichment of proteins with high affinity for heparin sepharose
3. Intravenous (intrarenal) injection of ^{125}I labeled antigen into a rat
4. Isolation of glomeruli and extraction with SDS
5. Separation in SDS-PAGE of
   a) injected material
   b) glomerular SDS extract
6. Autoradiography
7. Identification of proteins binding to glomeruli
8. Isolation of the identified proteins and production of antibody.
9. Evaluation of their capability to induce GN

kD. In the latter respect one should keep in mind that extremely highly charged molecules can have the tendency to form aggregates. Gel electrophoresis give a false impression, by de-aggregating the complexes. An easy and reliable method for predicting in vivo affinity for the GBM is the elution behavior of an antigen preparation from the polyanionic matrix heparin sepharose. Proteins which can be eluted only with high molarity NaCl or requiring even guanidine HCl are often both highly cationic and display a marked degree of aggregation. These promising fractions can be labeled with ^{125}I and tested for their in vivo affinity for the GBM in the rat. After intravenous injection one can isolate the glomeruli, extract the proteins fixed to the GBM and separate them in SDS-PAGE. Finally with the help of autoradiography, the proteins which stuck to the GBM can be identified.

Using this strategy (Table 5) we were able to identify cationic proteins with affinity for the GBM from two bacterial species: Yersinia enterocolitica and Staphylococcus aureus. Both fractions possess strong in vivo affinity for the GBM. The Yersinia protein is composed of a 19kD protein but occurs in dimeric and aggregated form with a size exceeding 65kD, as judged from gel filtration.[72] The staphylococcal protein migrates in SDS-PAGE as 31/32 kD bands. With the latter antigen we were able to induce proteinuria and kidney damage after a single intravenous injection of the antigen into immunized rats (unpublished data). The data so far obtained seem to support the concept of cationic antigens as candidate antigens of ICGN where subepithelial deposits are prominent. The strategy outlined above may enable us to identify some of them.

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