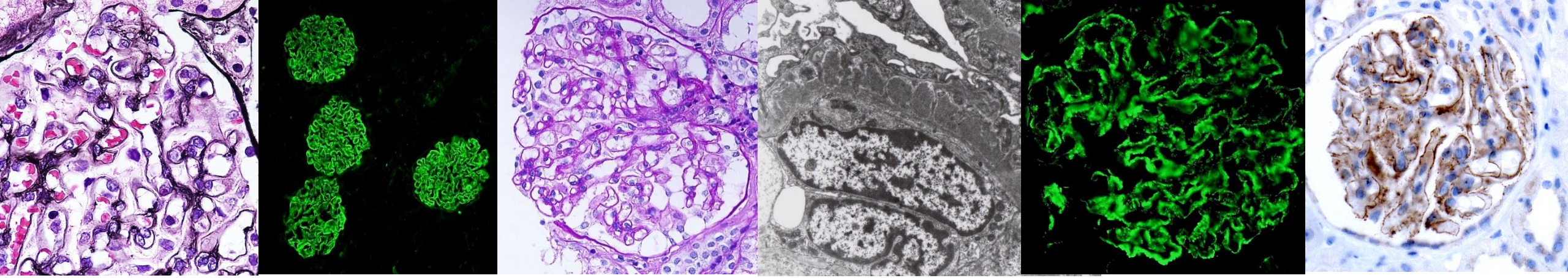
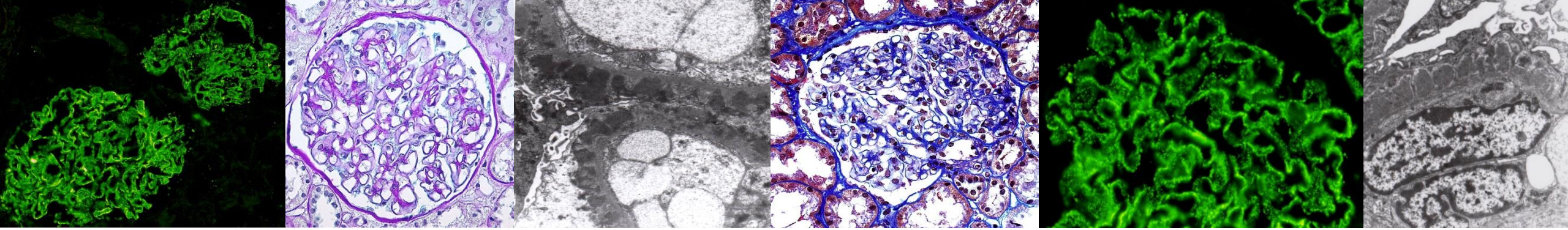


Membranous Nephropathy: from Heymann nephritis to the Human Disease

Eva Honsova
Institute for Clinical and Experimental Medicine
Pathology Department
Prague, Czech Republic

eva.honsova@ikem.cz

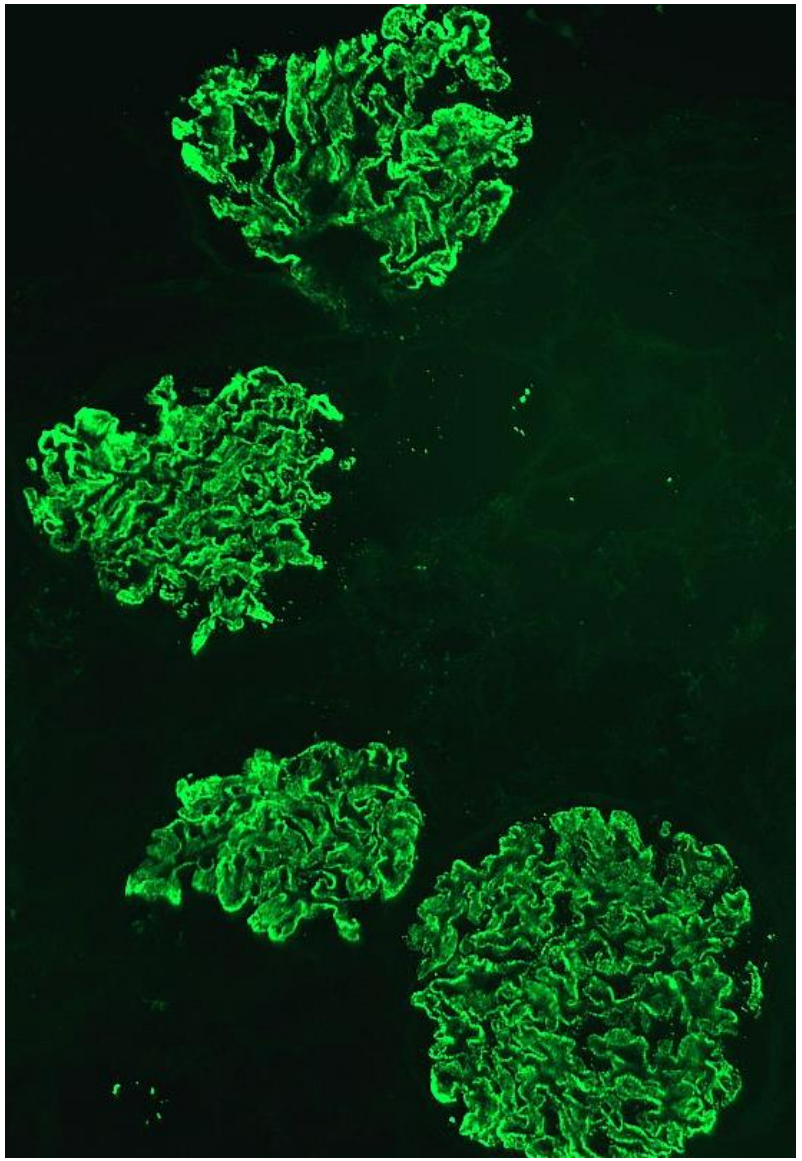


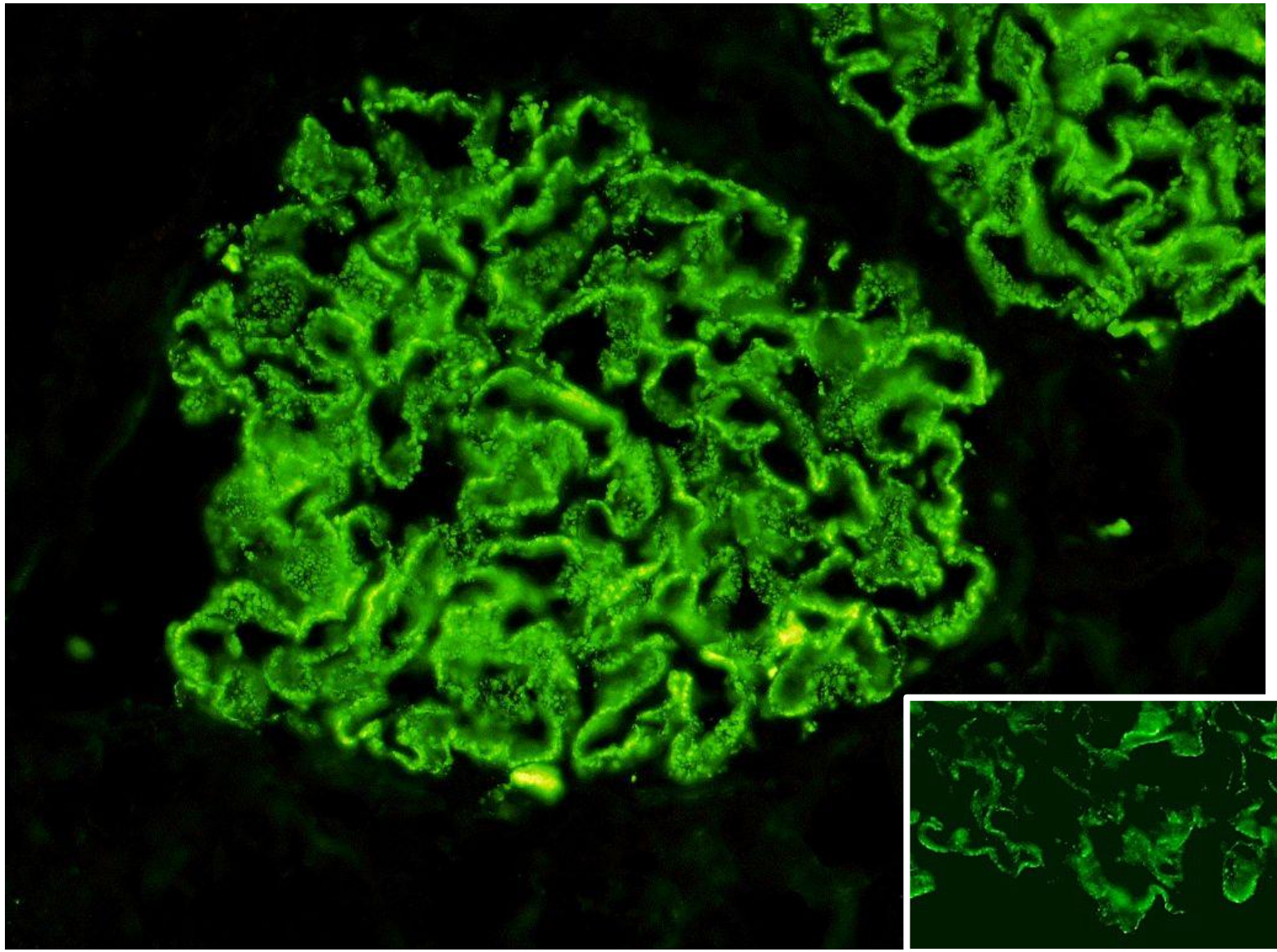
- **Membranous GN** (MGN) is an immunologically mediated disease with immune complex deposits in the subepithelial space

- MGN is one of the most common cause of NS in adults, accounting for about 20% of cases
- MGN is seen in all ethnic and in both sexes (more common in white males)
- **Primary (autoimmune)** associated with anti-PLA_2R and or THSD7A in serum and or in biopsy sample (70-80%)
- **Secondary** to various clinical conditions (infection, SLE, drugs, ca)
- The clinical course: variable
- 30% spontaneous remission, 30% persistent PU, progressive decline in renal function to ERDS

Membranous GN: morphology, IF

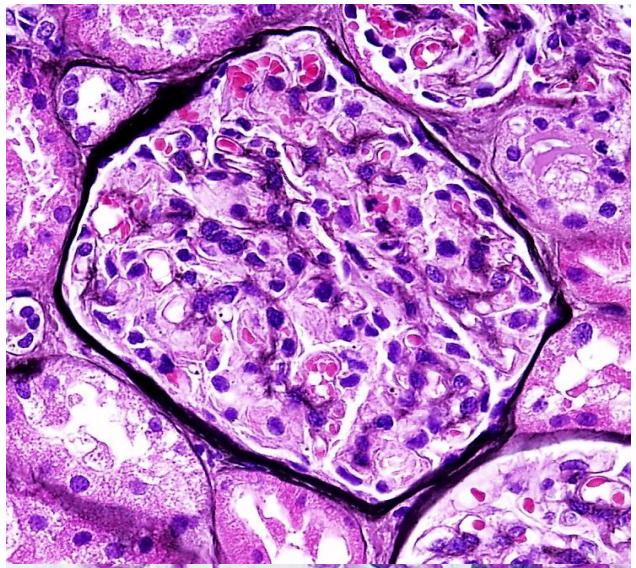
IgG, (IgG4, IgG1) C3, kappa, lambda

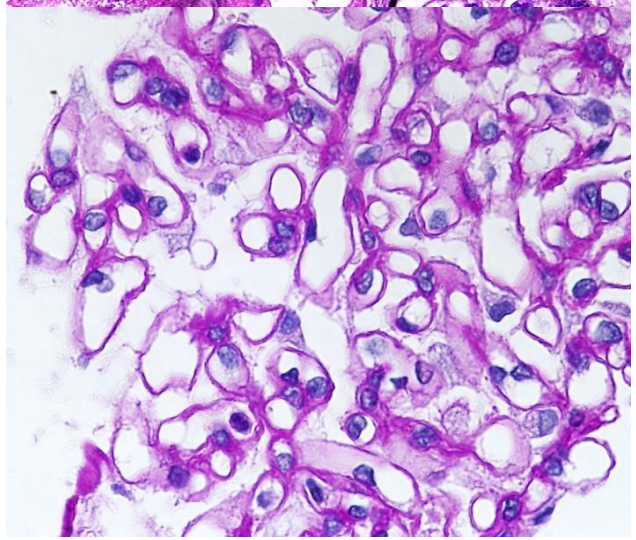


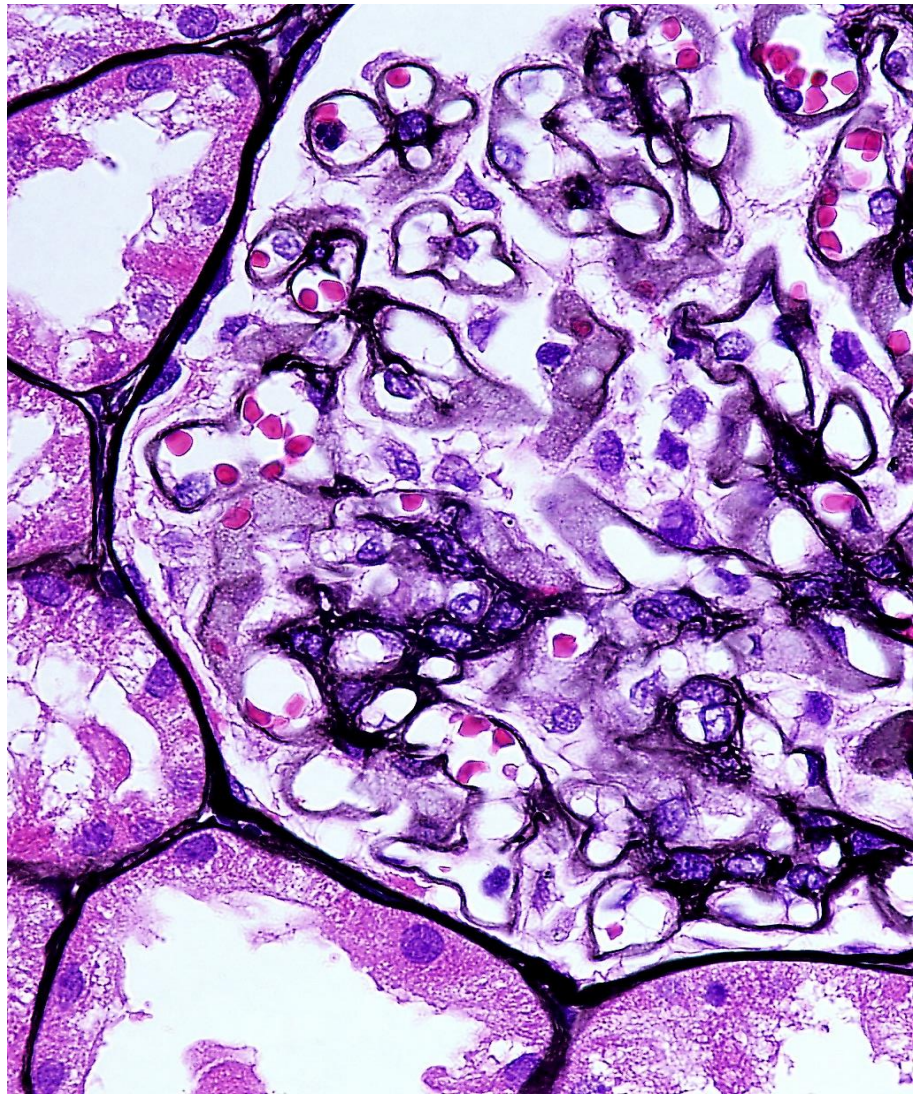


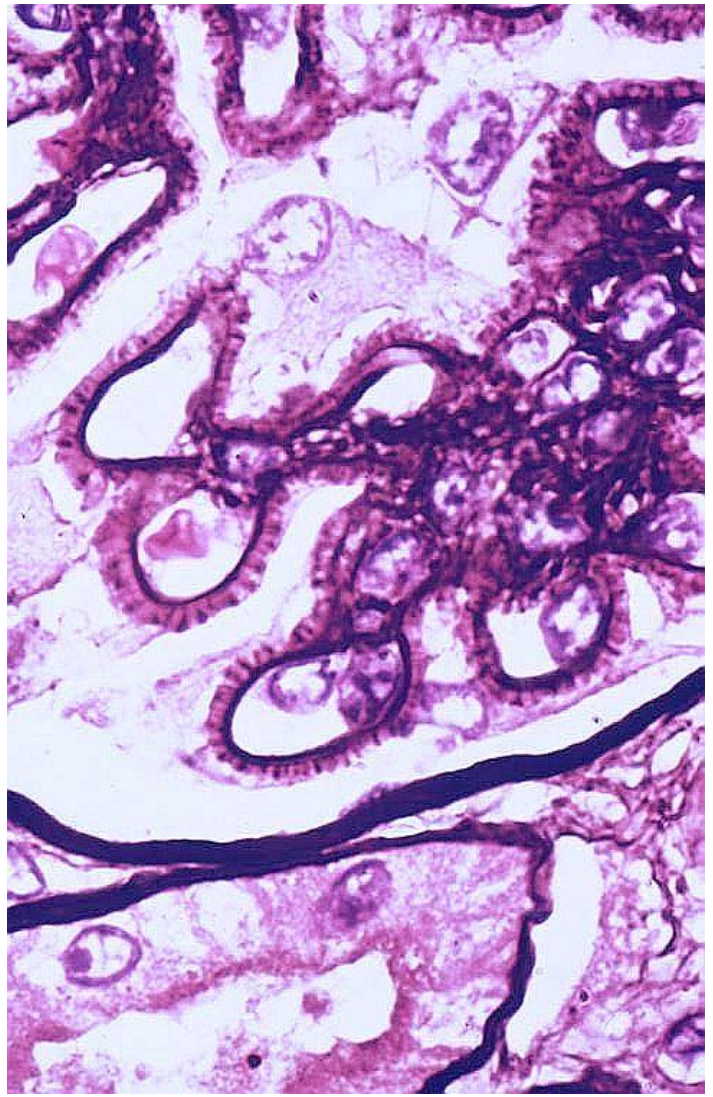
Membranous GN: morphology, light microscopy

Within one biopsy sample the morphological changes are homogeneous

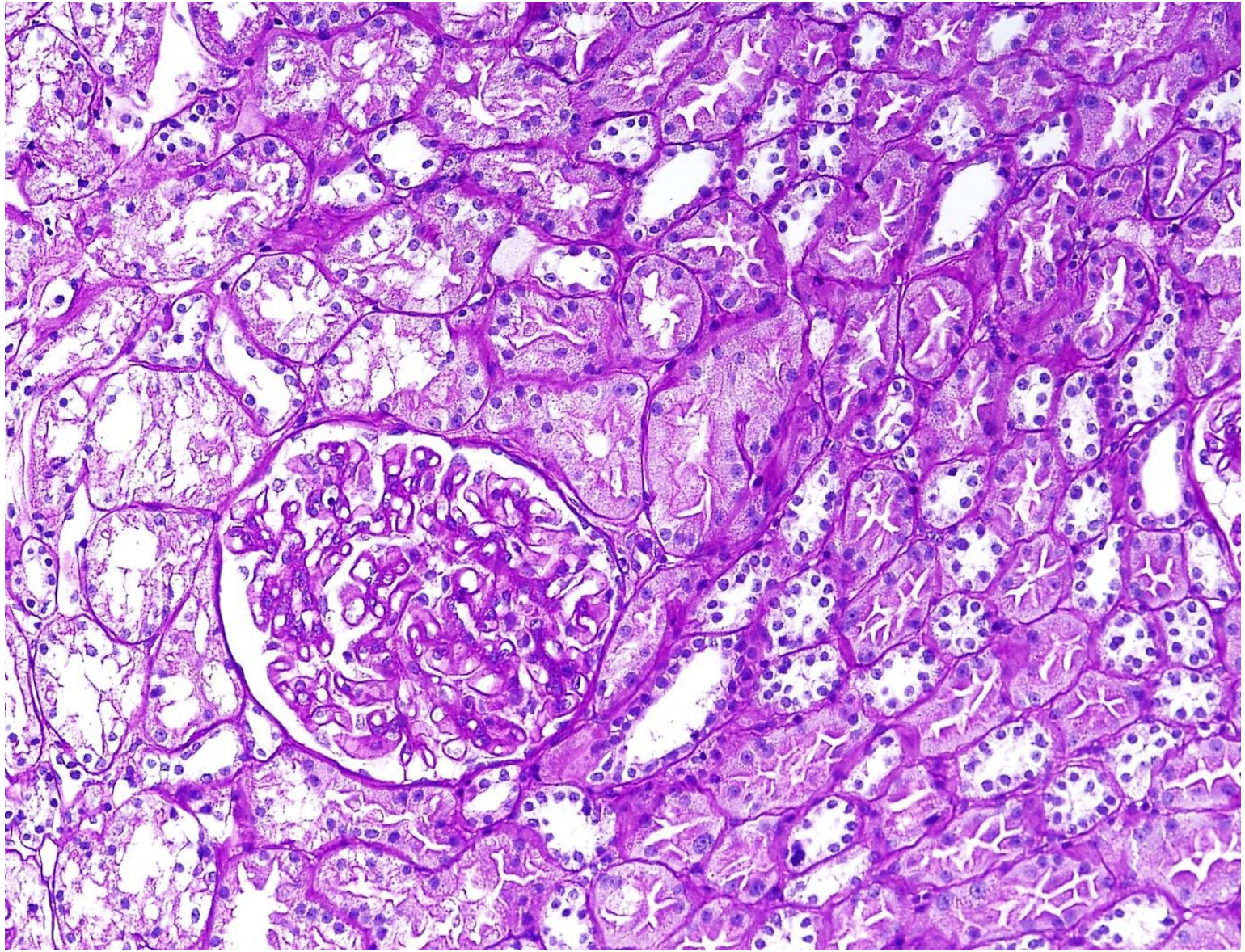


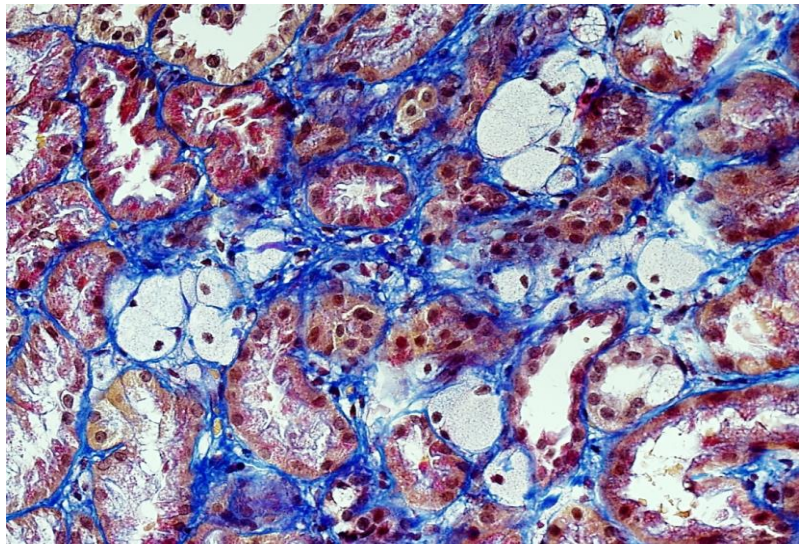


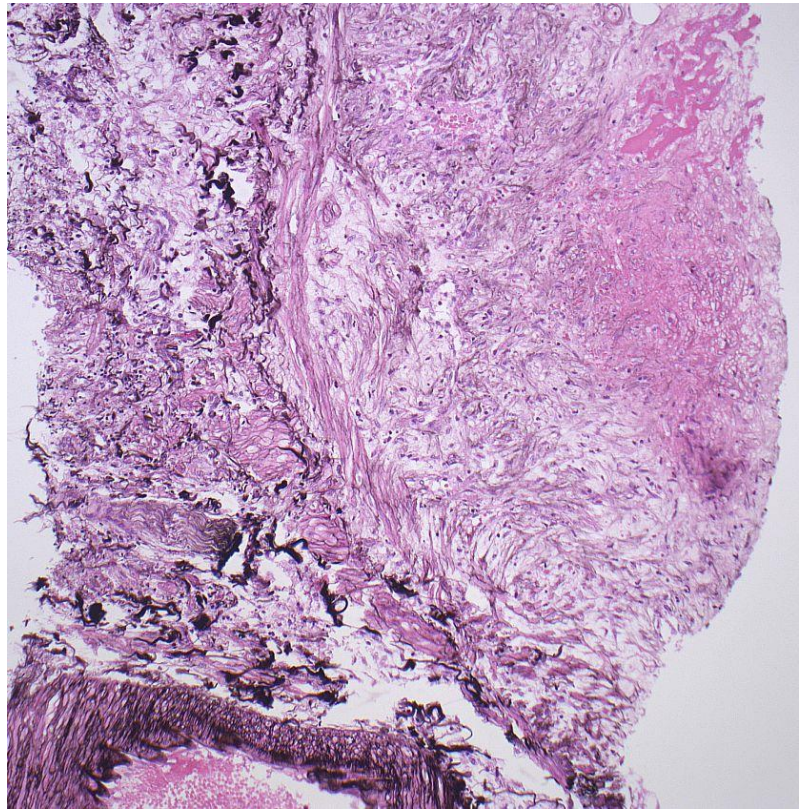




Membranous GN: morphology
LM: intersticium







Membranous GN

- The term MGN was first used by Bell in 1946

Term: disease with hidden onset and marked PU and edema

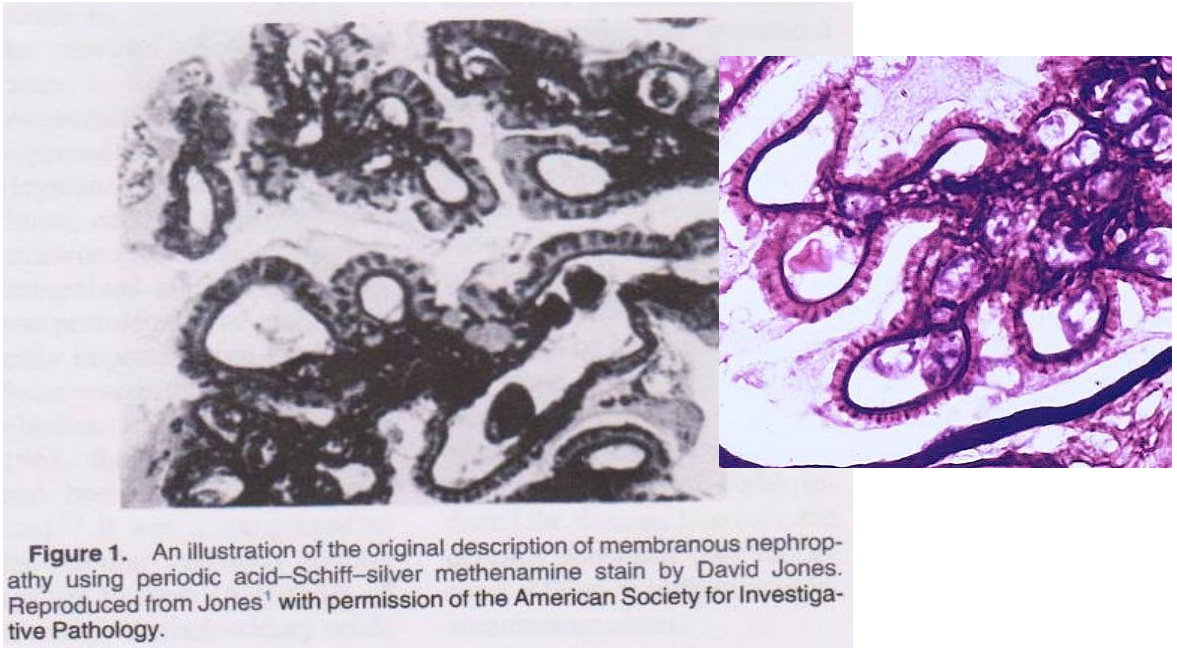


Figure 1. An illustration of the original description of membranous nephropathy using periodic acid–Schiff–silver methenamine stain by David Jones. Reproduced from Jones[1] with permission of the American Society for Investigative Pathology.

- First separated as a distinct entity by David Jones in 1957 (using silver stain, Jones stain)

  Nephrotic glomerulonephritis. Am J Pathol. 1957; 313-329.

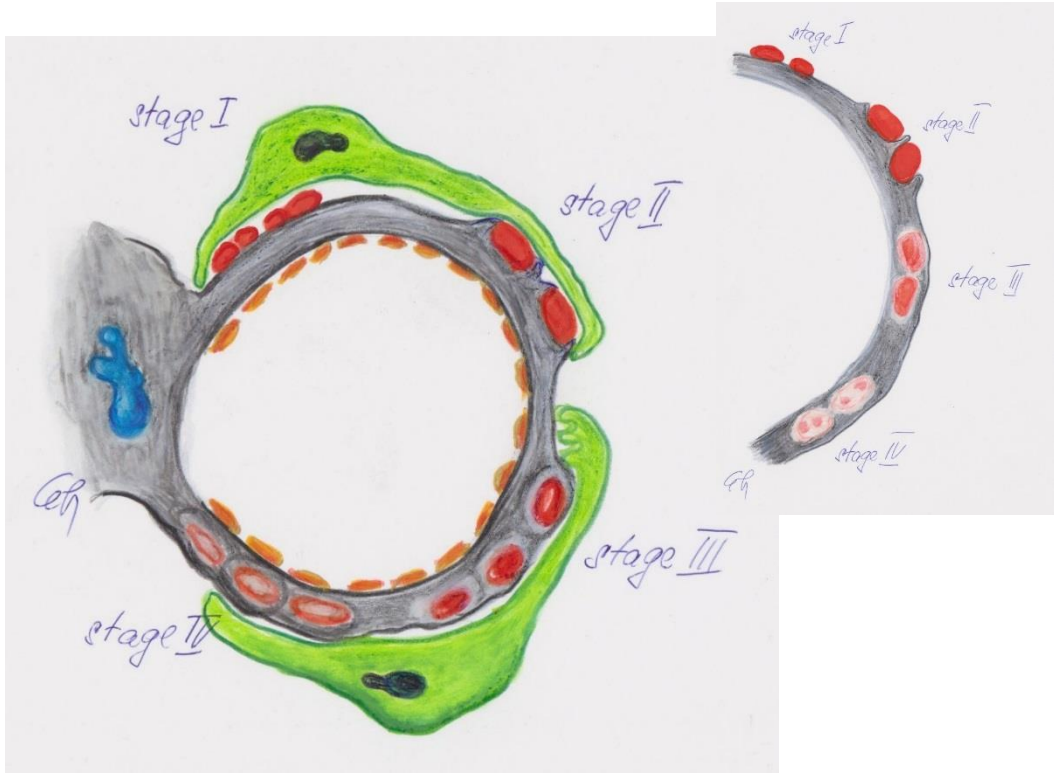
- IF: 1957 presence of immunoglobulin in the deposits (Mellors)

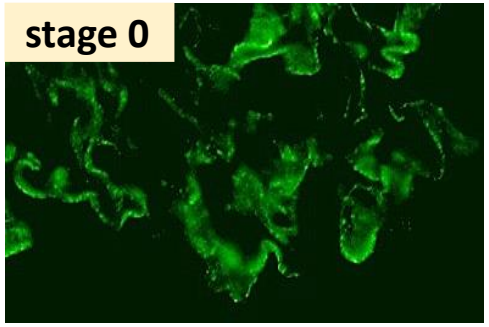
  Role of gammaglobulins in pathogenesis of renal lesions in systemic lupus erythematosus and chronic membranous glomerulonephritis. J Exp Med. 1957;1065:191-202.

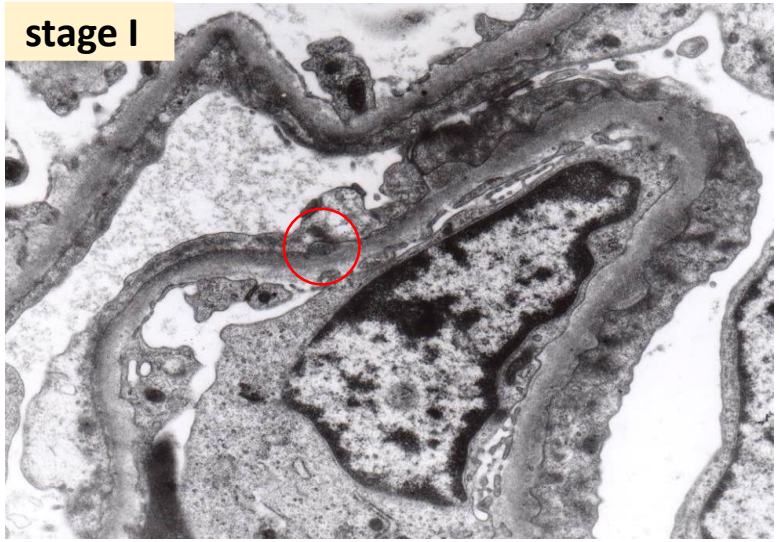
- EM: 1959 location of deposits (Movat, Mc Gregor)

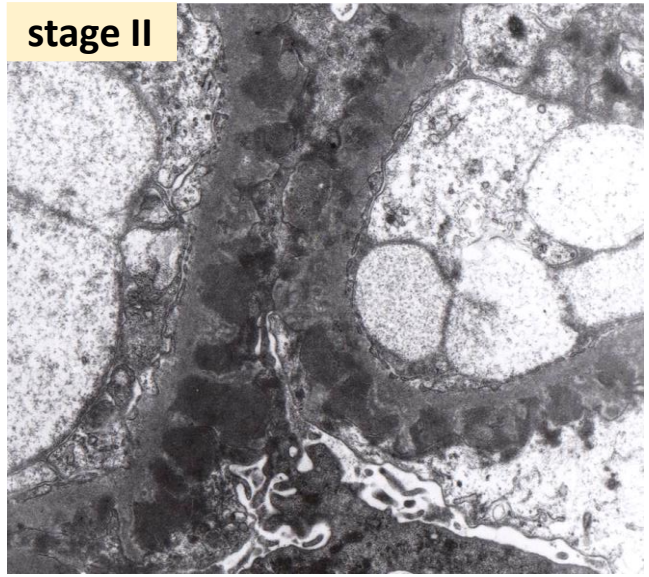
  The fine structure of the glomerulus in membranous glomerulonephritis in adults. Am J Clin Pathol. 1959; 100-127.

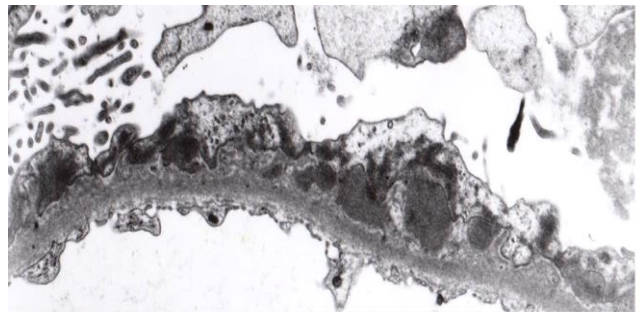
Membranous GN: EM











The MGN stages based on EM appearance were described by Ehrenreich and Churg in 1968

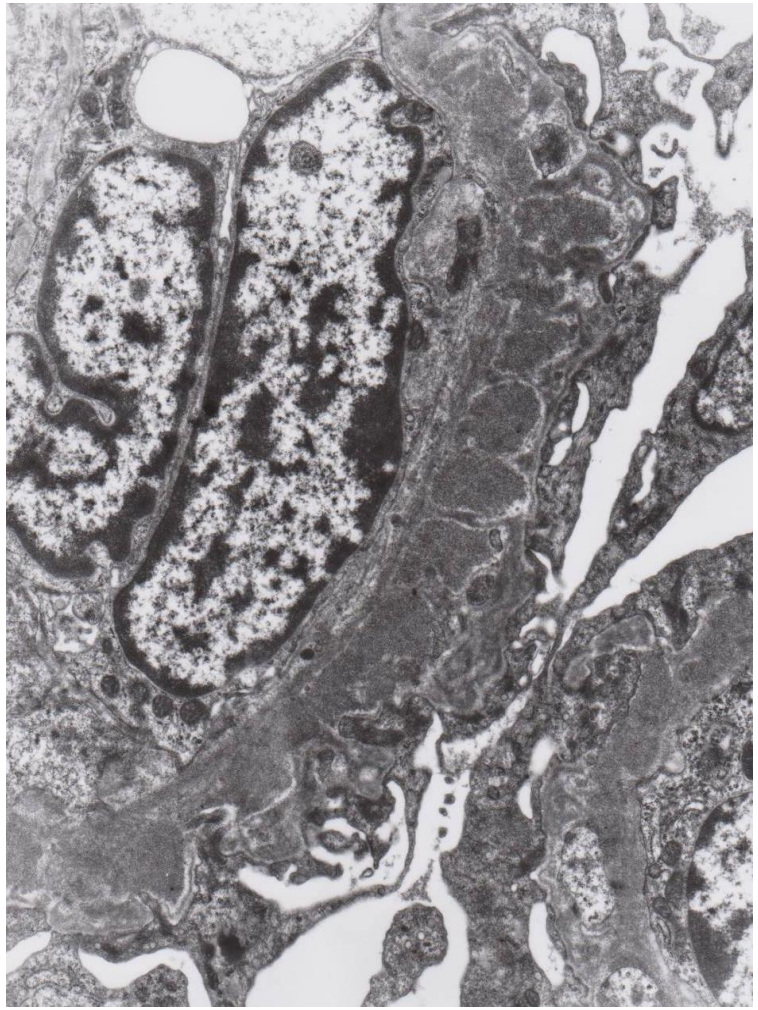
The original 4 stages described morphological features of deposits, the reaction to them

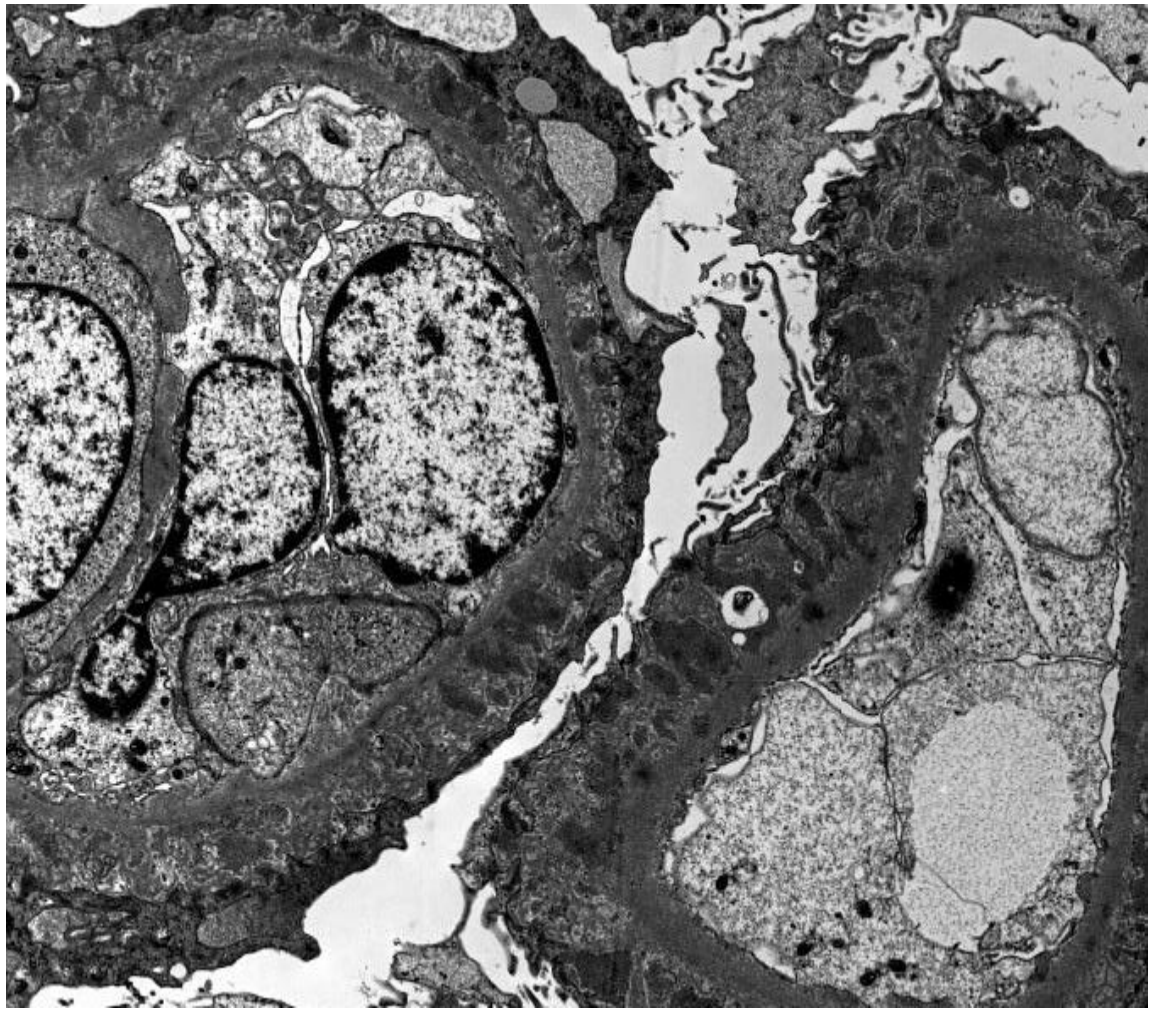
- **Stage 0**: *Absence deposits in EM*

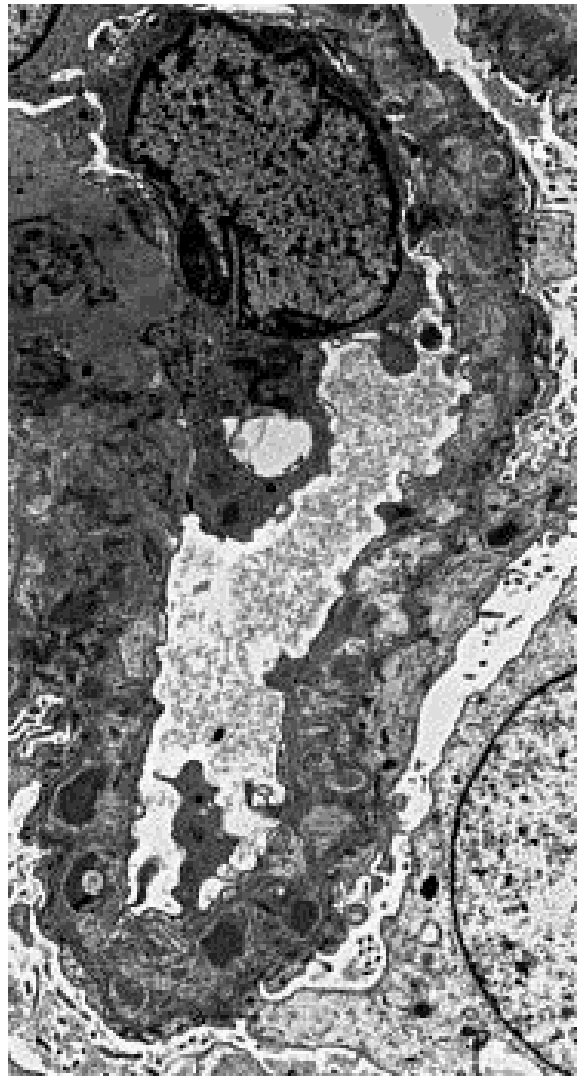
(Cornell LD et al. The pathology and clinical features of early recurrent membranous glomerulonephritis. Am J Transplant 2012; 1029-38.)

- **Stage I:** without GBM reaction
- **Stage II**: GBM reaction and spikes formation

EM (stage III and IV)

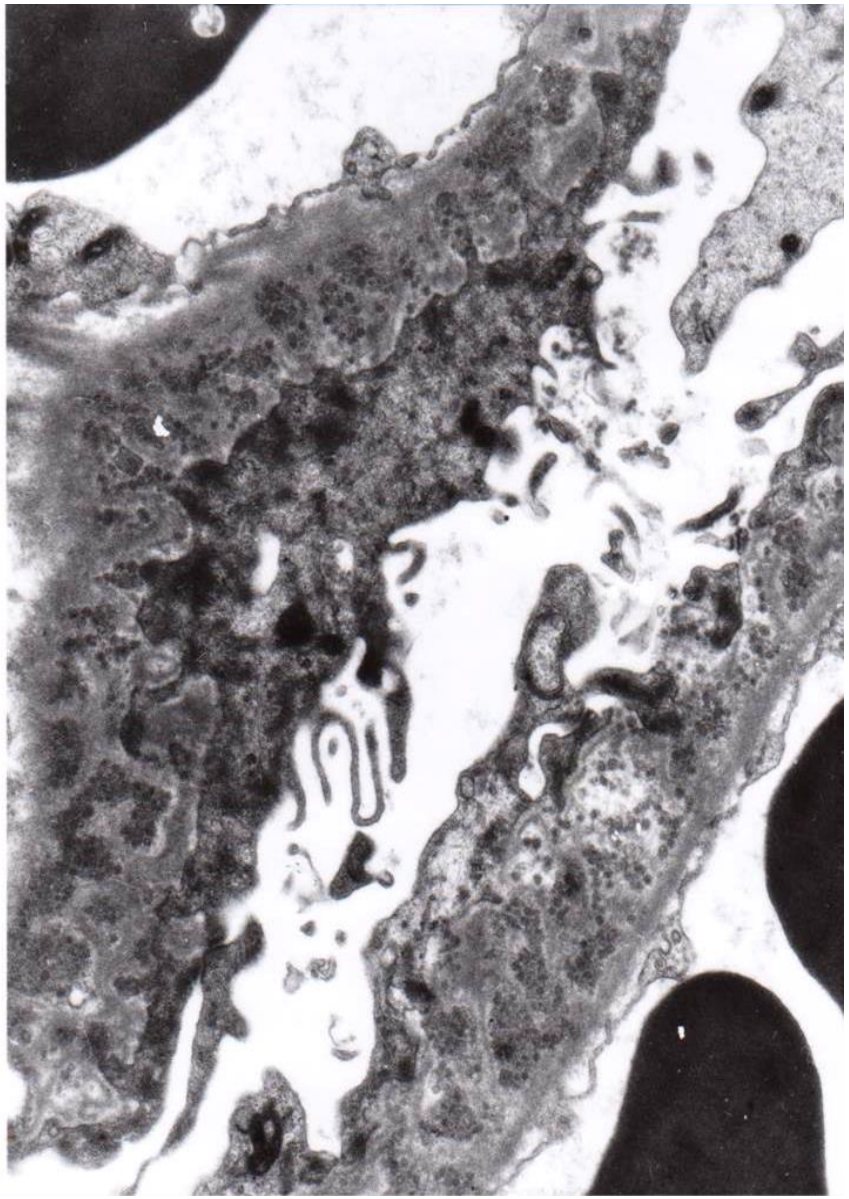




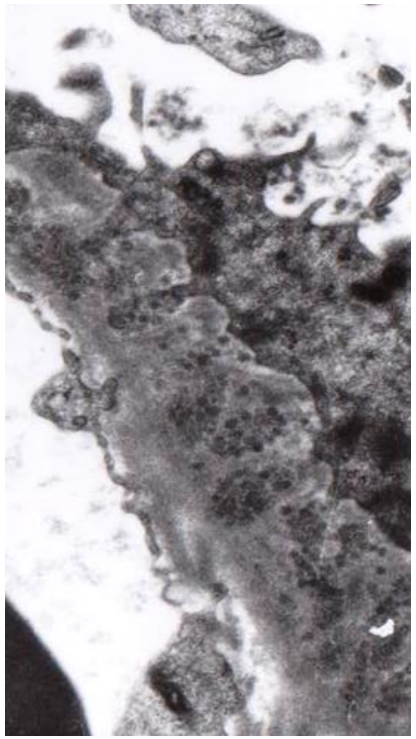


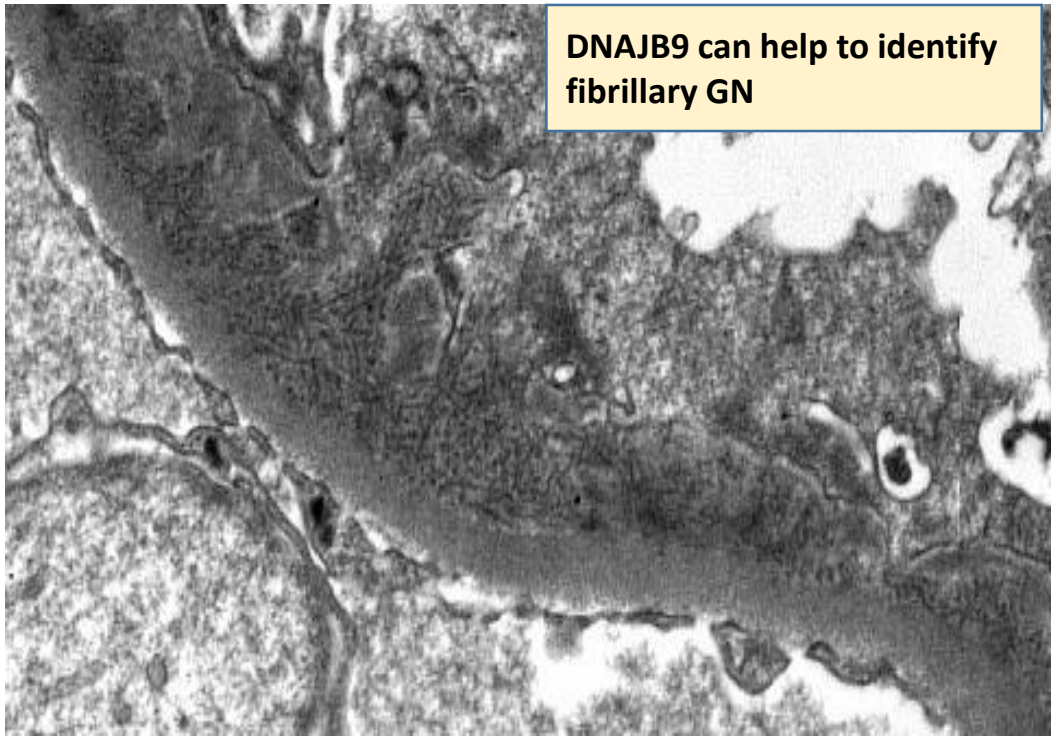
- **Stage III**: deposits are intramembranous, surrounded by material of GBMs
- **Stage IV**: remodeling of GBM with electron-lucent intramembranous areas (reabsorbed deposits)

EM: substructural deposits



- Sometimes deposits show substructural organization
- ***Cryoglobulins and SLE should be excluded***
- Neonatal form with NEP mutation shows microspherular substructure.





DNAJB9 can help to identify fibrillary GN

MGN: pathophysiology

Considerable progress has been made in the understanding of pathophysiology of MGN in humans (several endogenous antigens on podocytes; target for antibodies)

- same time as the dg. criteria: **animal model** of MGN so-called **Heymann nefritis** was described
- it was believed to be caused by autoimmunity
- the autoantigen, called **megalin** (Kerjaschki 1980s), is present ***in rats*** in the brush border and also in the glus at the podocyte surface (**endogenous antigen**)
- ***in humans***, megalin is present in the brush border of the proximal tubules, a crucial role in reabsorbing of proteins and light chains
- megalin is expressed in human podocytes

(Prabakaran T. et al. Receptor-mediated endocytosis of alpha-galactosidase A in human podocytes in Fabry disease, PLoS One , 2011, vol. 6 pg. e25065

Lisa Gianesello et al. Albumin uptake in human podocytes: a possible role for the cubilin-amnionless (CUBAM) complex, 2017 *https://www.nature.com › scientific reports › articles)*

- there is *no evidence that megalin is involved in human MGN (not detected in deposits, no Abs in serum)*

MGN: an autoimmune disease

endogenous antigen in human

The **first human antigen**: in neonates borne with MGN (H. Debiec, P. Ronco)

Human counterpart to Heymann nephritis

Early occurrence of MGN

Mother became immunized during pregnancy and Abs were transferred to the fetus

They detected IgG Abs against neutral endopeptidase (NEP)

Mothers had high level of Abs & did not show renal disease (deficient in NEP)

Mothers from 5 families and all mothers were NEP deficient

Genetic homogeneity and phenotypic variability,

Subclasses of IgG: non-complement fixing IgG4 predominant subclass

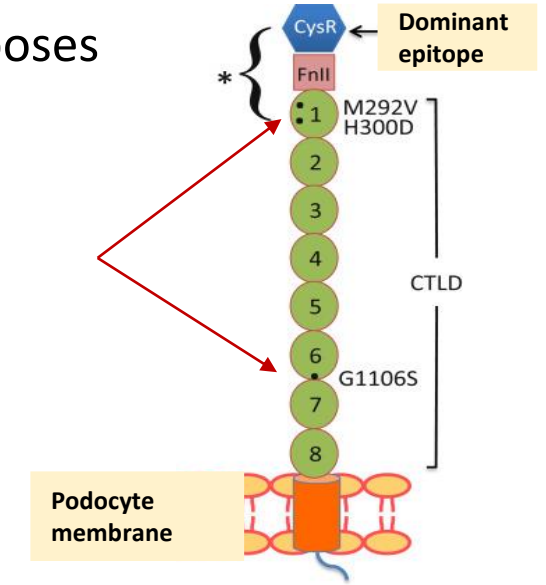
IgG subclass modulate disease severity, mother Abs IgG1 are associated with more severe disease

Identification of NEP proof concept that a podocyte antigen could serve as a target for circulating Abs

MGN: an autoimmune disease

endogenous antigen in human

- In 2009, Beck with colleagues discovered another antigen: the type M phospholipase A2 receptor (**PLA_2R**)
- Abs against PLA_2R: 70% idiopathic MGN
- Transmembrane receptor that is highly expressed on podocytes
- **IgG4 -** predominant humoral response leads to production of auto-Abs
- ***Anti-PLA_2R Abs in serum*** can be used clinically for diagnostic and monitoring purposes

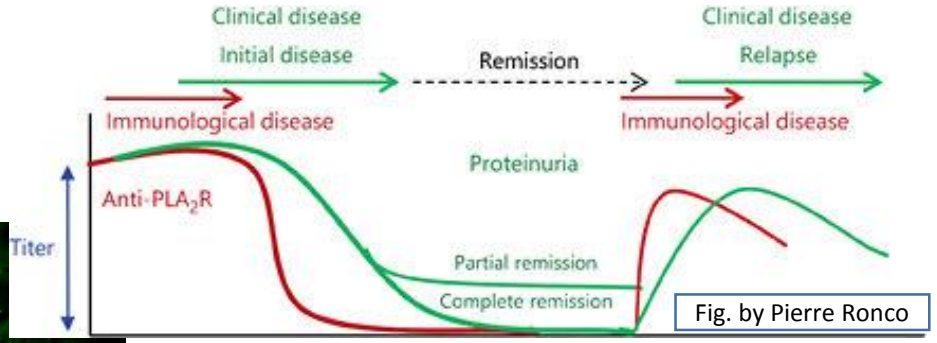


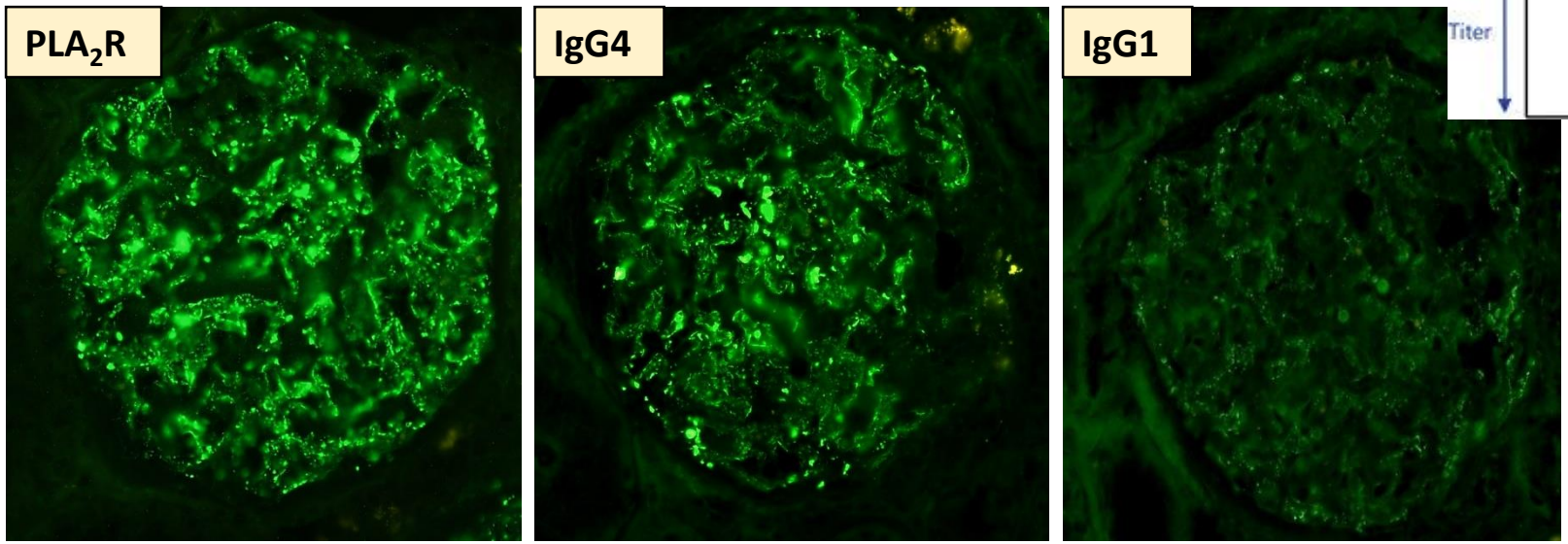
- 3 distinct humoral epitope-containing domains in the N-terminus of PLA_2R
- Immuno-dominant epitope: recognized in 100 % of patients
- ***Epitope spreading***
- Abs to 1 dominant epitope: mild disease
- Abs to all 3 epitope-containing domains: more severe disease, risk of ESRD
- **Genetic background**: HLA-DQA1 and PLA_2R1 risk alleles, carrying risk alleles had an additive effect

MGN: an autoimmune disease

endogenous antigen in human

- **Anti-PLA_2R IgG autoantibodies consist predominantly of IgG4** with a **smaller component of IgG1**.
- ***Anti-PLA_2R Abs in serum***; **PLA_2R in biopsy samples** (even retrospectively from paraffin-embedded specimens)
- **"Secondary" MGN**: IgG3 and IgG1, IgG2 (mainly SLE)



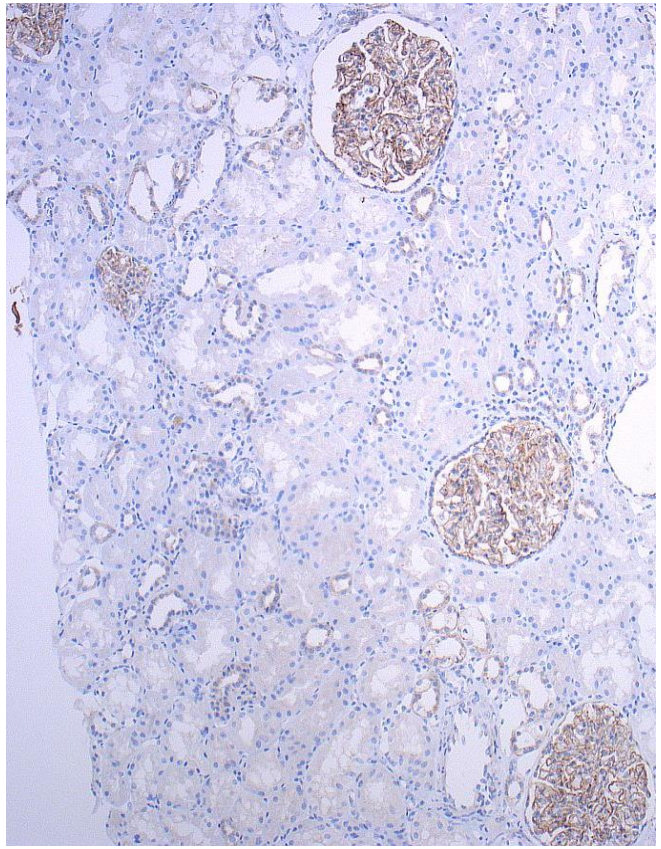


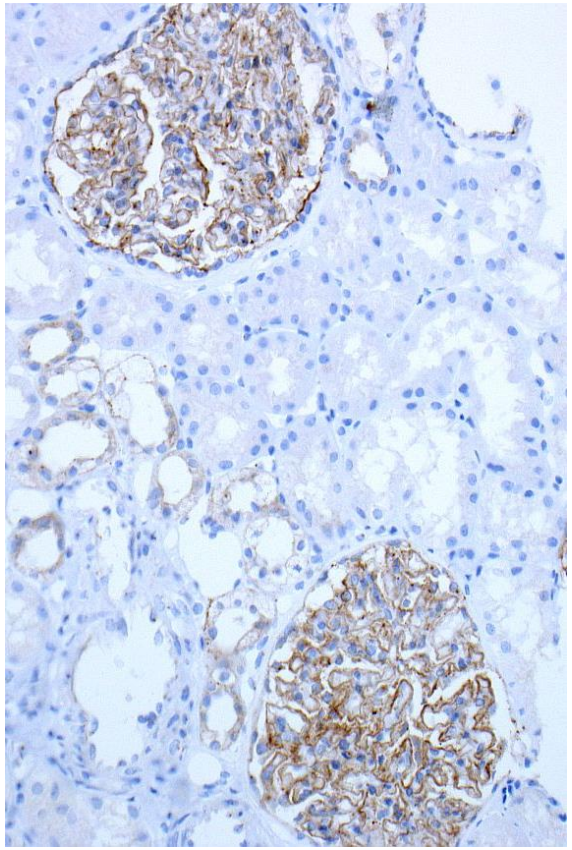
Circulating Abs are not always associated with deposits of PLA_2R antigen
PLA_2R in deposits can uncover "seronegative" patients

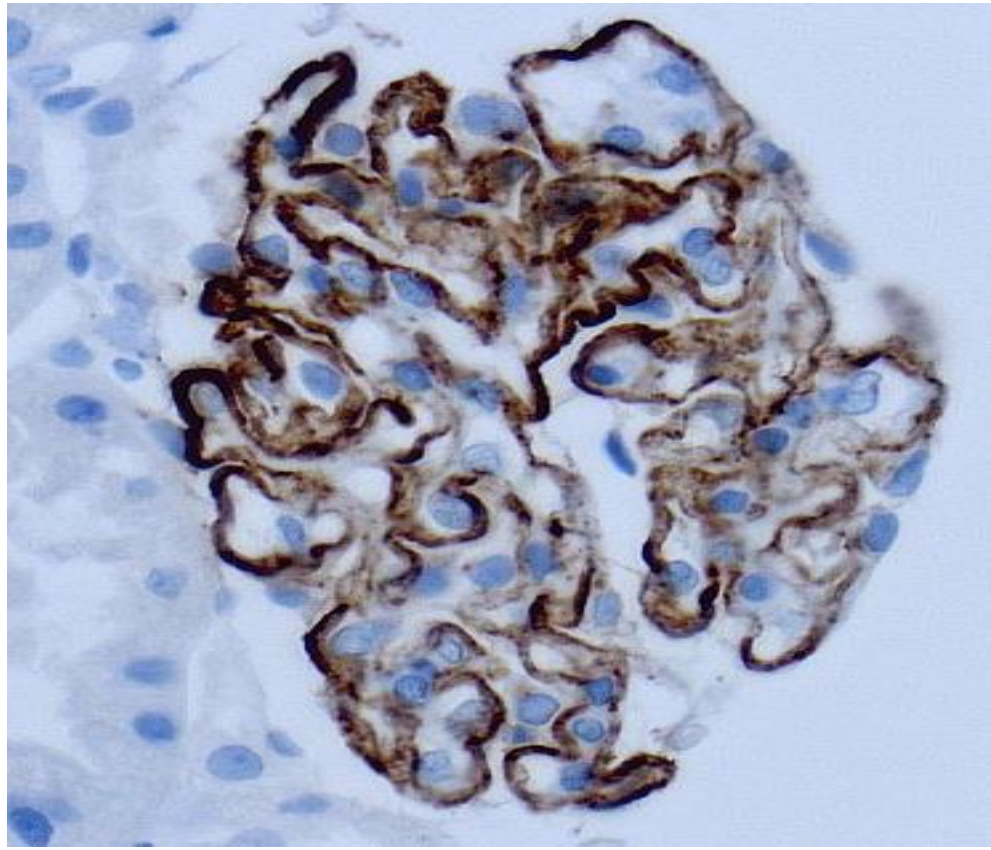
MGN: an autoimmune disease

other *endogenous antigen in human*

- THSD7A (thrombospondin type-1 domain-containing 7A), transmembrane protein expressed on podocytes
- May be the responsible antigen in up to 3-5% of patients with MGN (10 % who are PLA_2R negative);
- Predominant Abs IgG4







MGN: an autoimmune disease

endogenous antigen in human

Similarity of the phenotypes:

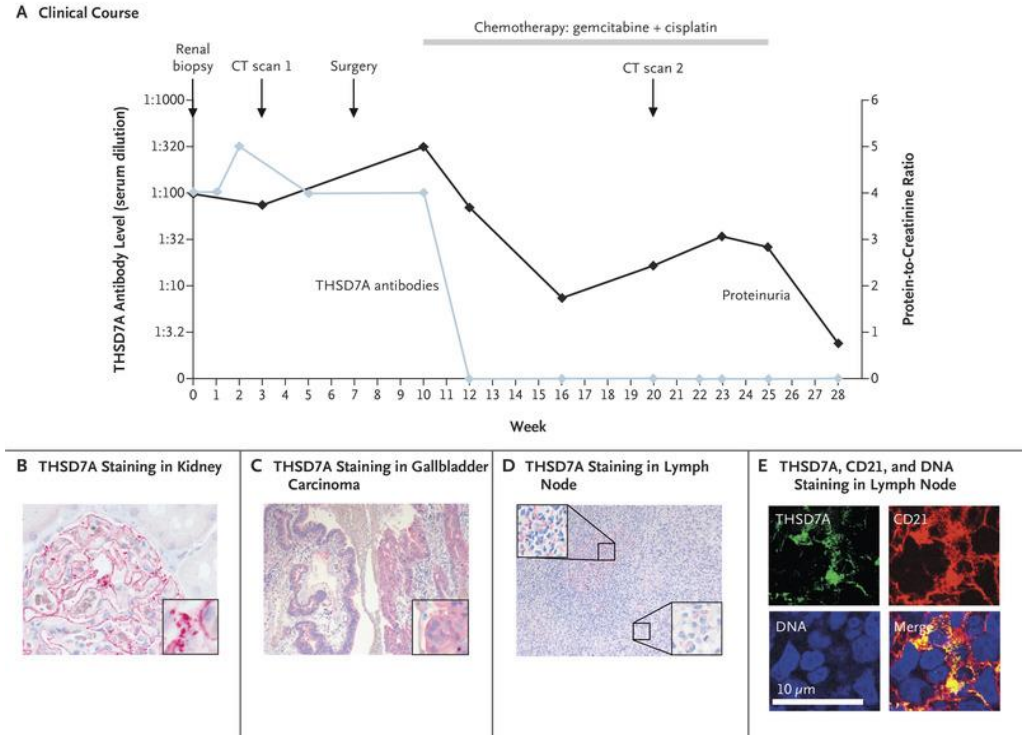
- Unexplained ***female predominance***

(Our study 114 pts with MGN: THSD7A 8 % in PLA_2R negative MGN, 65% women)

- THSD7A may also be involved in the pathogenesis of some cases of ***malignancy-associated*** MGN

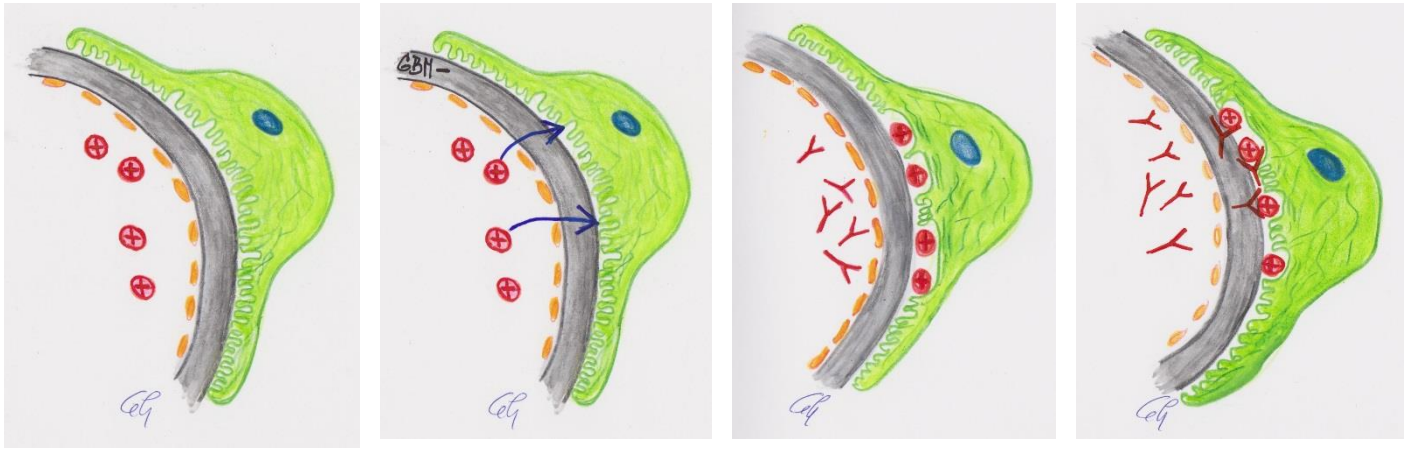
Hoxha E et al**. A mechanism for cancer-associated membranous nephropathy.** NEJM 2016;19;374(20):1995-6.

Sharma S & Larsen C. **Tissue staining for THSD7A in glomeruli correlates with serum antibodies in primary membranous nephropathy: a clinicopathological study.** Modern Pathology 2018; 31, 616–622.



MGN: an autoimmune disease

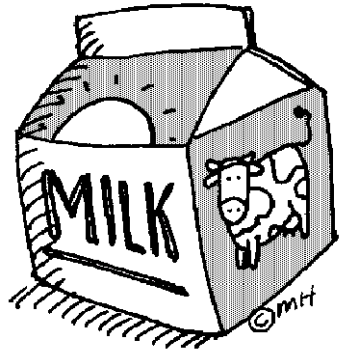
exogenous antigens in animal model (alternative model to Heymann nephritis)



- Alternative model to Heymann nephritis first described in rabit
- The injections of repeated doses of **cationized bovine serum albumin (BSA)**
- ***Hypothesis***: antigen charge could be a key factor for deposit formation
- Only rabbits immunized with cationic BSA developed subepithelial deposits
- Anionic and neutral BSA: animals developed mesangial deposits
- Model of so-called **planted antigen**

MGN: an autoimmune disease

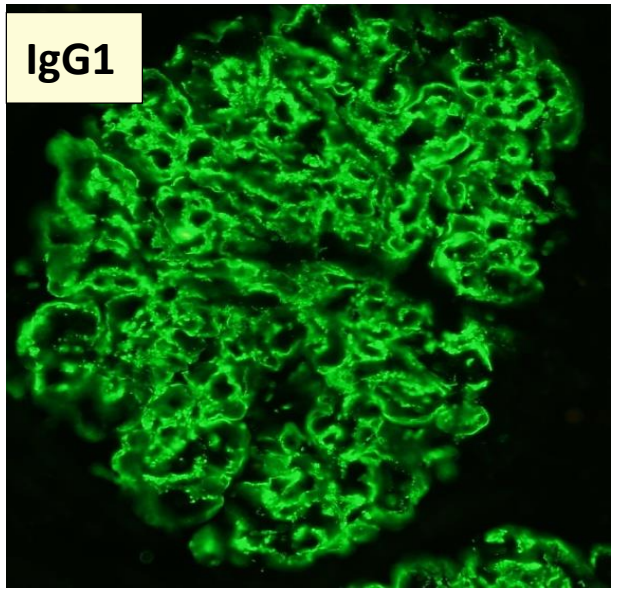
exogenous antigens in human

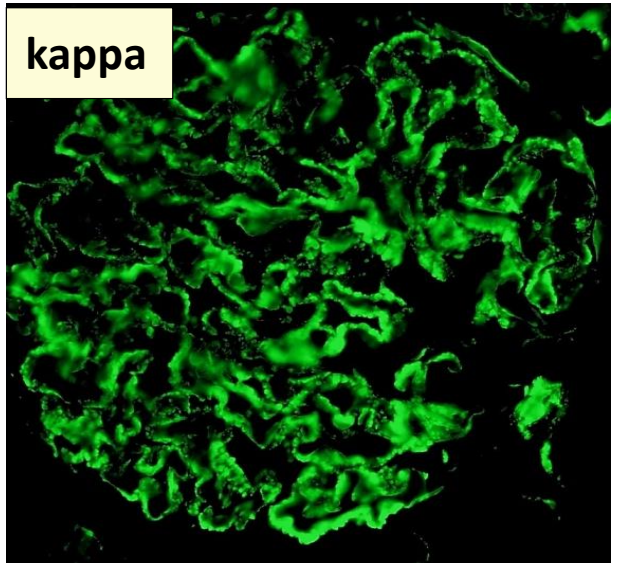


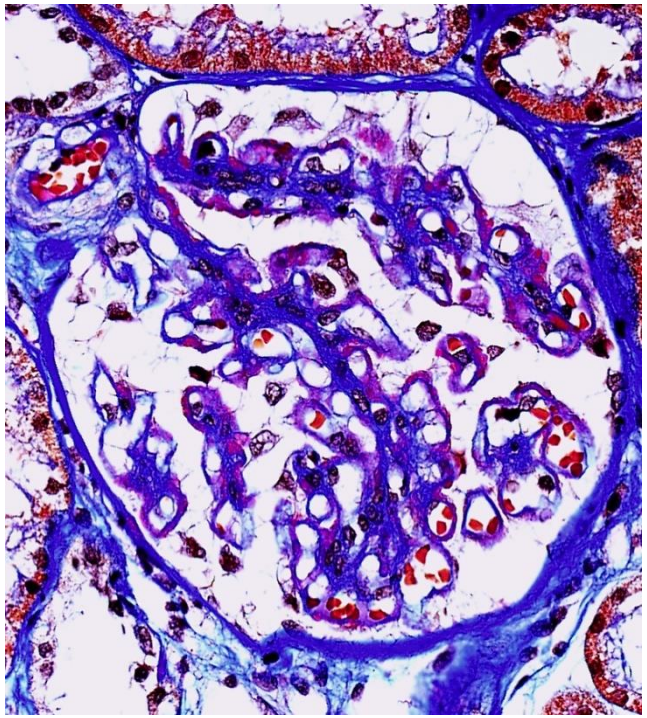
- the relevance of the animal model to human pathology was confirmed
- Some patients with iMGN had high-titer anti-bovine serum albumin antibodies
- They also identified **BSA in the glomerular deposits** in the absence of PLA_2R
- a subset of MGN is caused by immunization against an exogenous food antigen

- Food antigens as a cause of iMGN: **an important breakthrough in the field of kidney diseases.**
- Elimination the protein from the diet

MGN: morphological variant of GN with monoclonal deposits





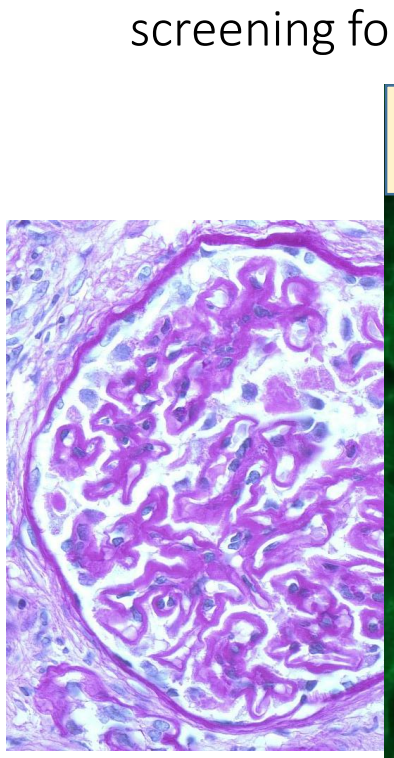


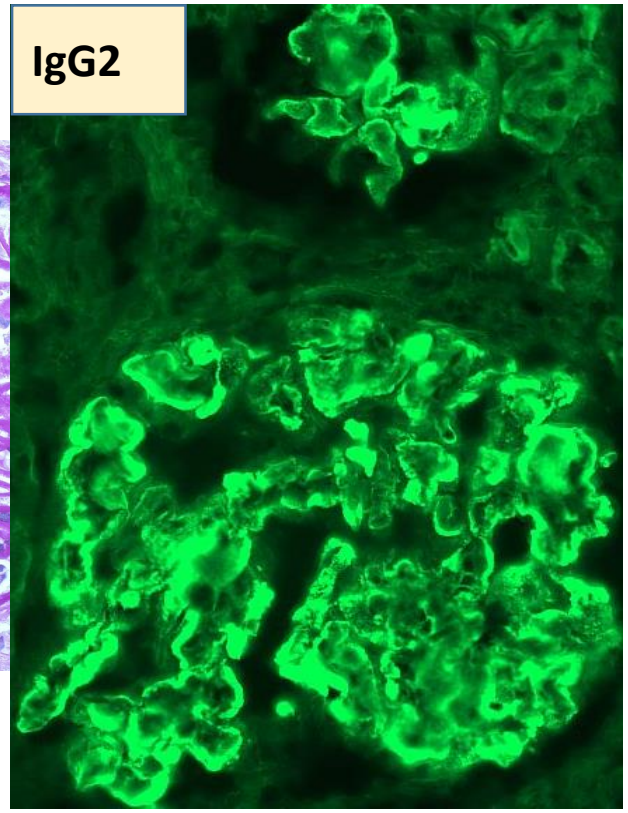
- **IgG1**, **kappa**
- **C3**: usually positive, **C1q**: sometimes positive staining
- **Restriction of light chain** (positive is only kappa or only lambda)
- Some monoclonal IgGs with unusual charge characteristics may bind to anionic sites in the subepithelial space and induce development of MGN
- Anti-PLA_2R Abs may rarely be monoclonal
- May be PLA_2R negative and or positive

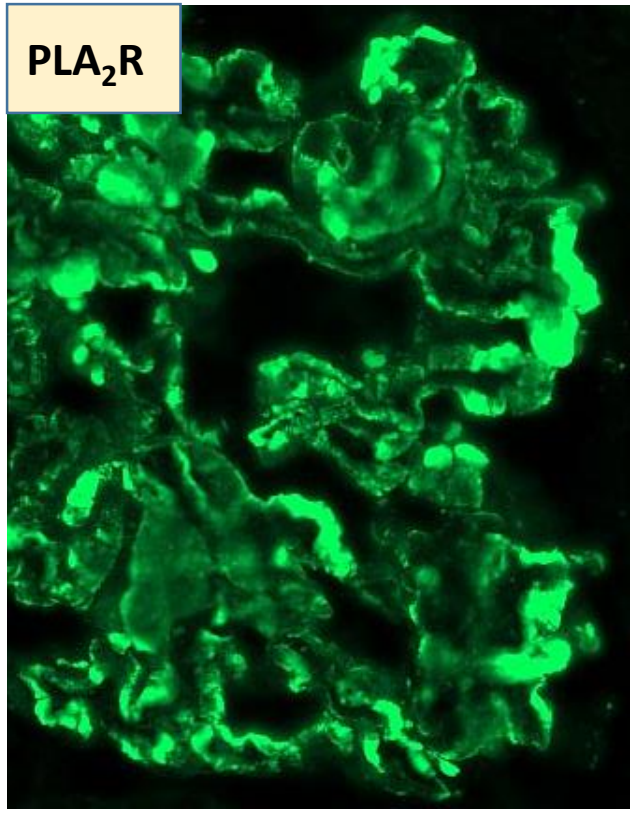
Rocha AB and Larsen CP. Membranous glomerulopathy with light chain-restricted deposits: A clinicopathological analysis of 28 cases. Ki Reports; 2017, 1141-1148.

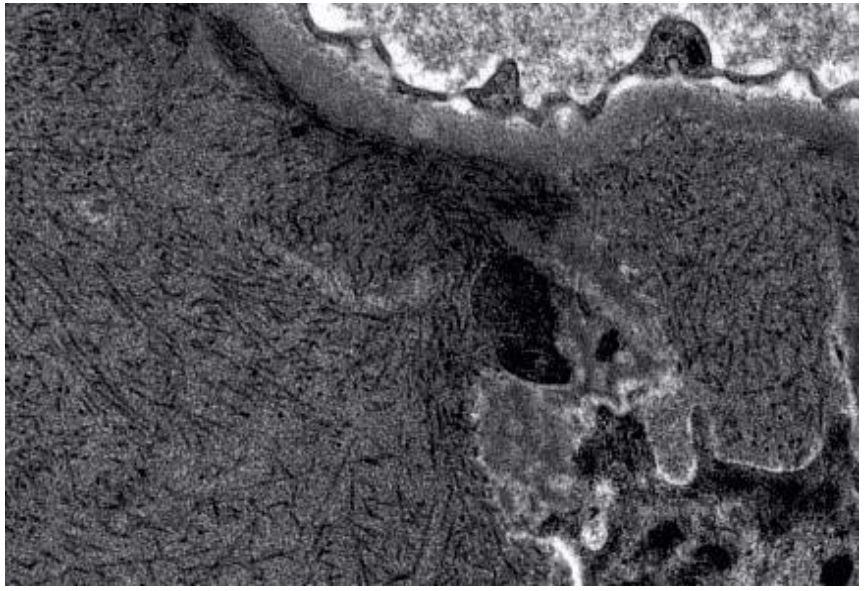
MGN: morphological variant of GN with monoclonal deposits

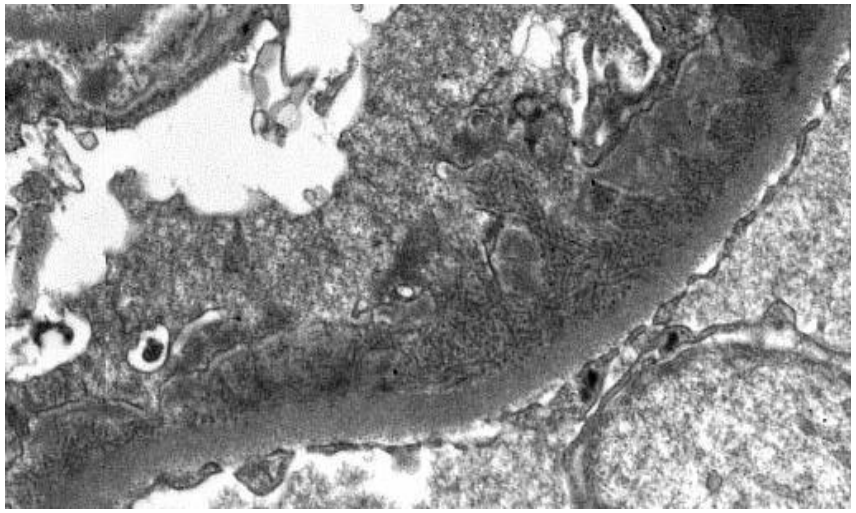
IgG2, lambda, PLA_2R positive staining (DNAJB9 can help to identify fibrillary GN)
screening for hematological malignancy











- **Membranous-like nephropathy with masked IgG-kappa**

Standard IF negative, only pronase digestion unmasked the deposits

Young patients with autoimmune phenomena

Secondary MGN

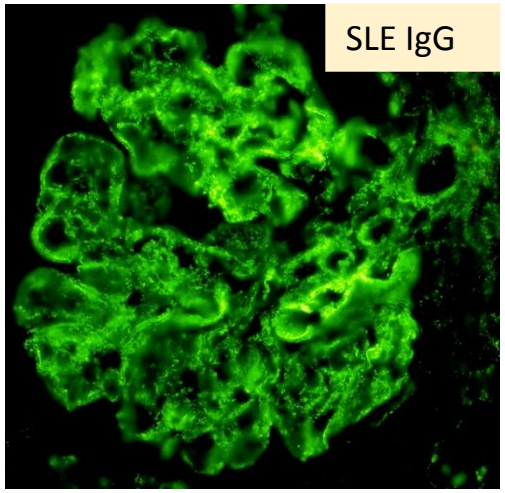
- Malignancy
- ***SLE:*** approximately 10-20 % of patients with lupus nephritis have MGN, called class V.

Some patients with class V present only with renal disease (no symptoms or serologic abnormalities of SLE).

Lupus should be suspected in any young woman with MGN.

PLA_2R antibodies are typically negative.

Exostin 1/2



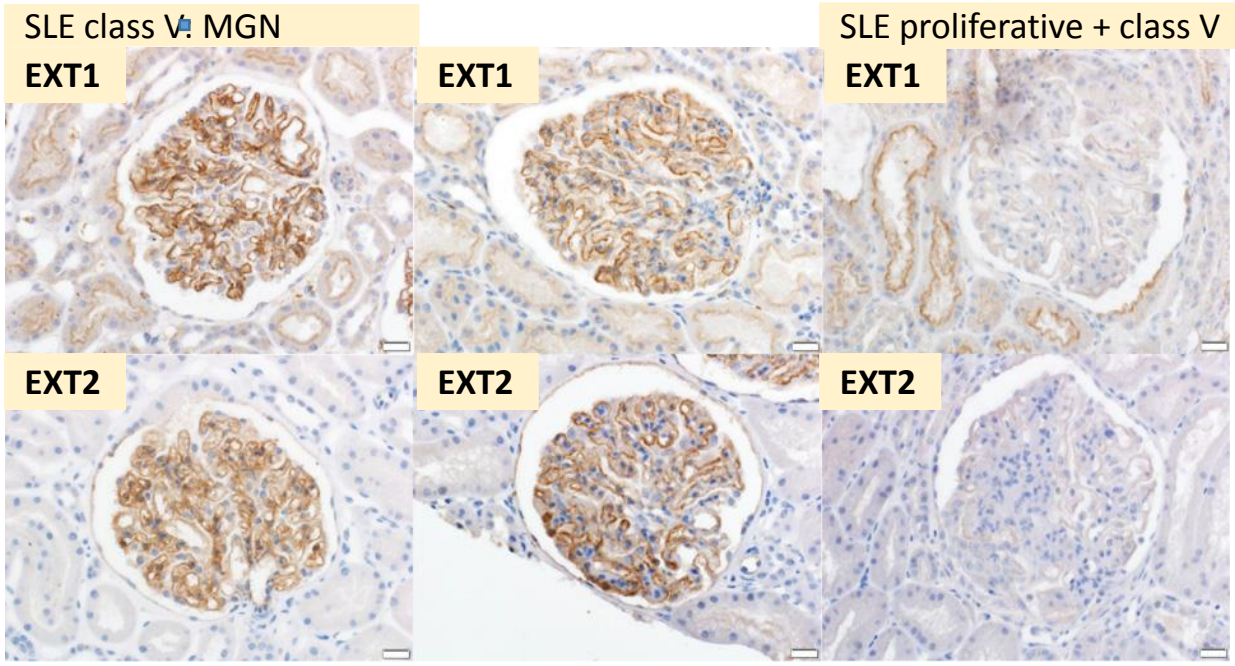
- Drugs (penicillamine 6%, NSAID)
- IgG4-related disease
- Infections (HBV, HCV, syphilis…)
- Sarcoidosis, GVHD…

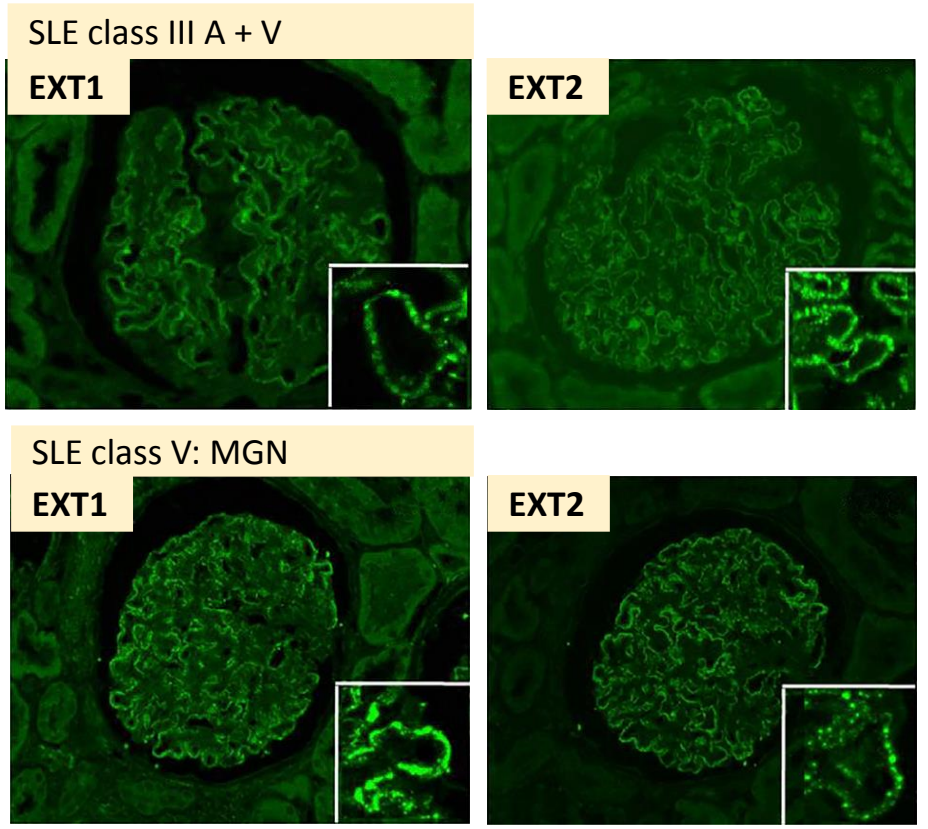
MGN Exostosin 1/Exostosin 2–Associated

- Using proteomics (mass spectrometry) and immunohistochemistry, the authors detected two proteins, exostosin 1 (EXT1) and exostosin 2 (EXT2), in the GBM of PLA2R-negative MGN. EXT1 and EXT2 were absent in all cases of PLA2R-associated MN and controls.
- Clinical and biopsy findings showed features of autoimmune disease, including membranous lupus nephritis, in 81% of the 26 EXT1/EXT2-associated MGN cases the authors identified.
- These findings suggest that EXT1/EXT2-associated MGN represents a ***distinct subtype of MN, most commonly associated with autoimmune diseases*** (secondary MGN).

Sethi S, RoncoP. Exostosin 1/Exostosin 2-Associated Membranous Nephropathy.

J Am Soc Nephrol. 2019 Jun;30(6):1123-1136.





Secondary MGN

MGN and malignancy

5-20% of adults with MGN over age of 65 years have been reported to have a malignancy (most commonly a carcinoma)

- tumor antigens can behave as ***planted antigen***
- the production of ***Abs formed against tumor antigens*** that also recognize similar or identical molecules present on podocytes (mechanism which connect some cases of ***thrombospondin*** associated MGN with malignancy)

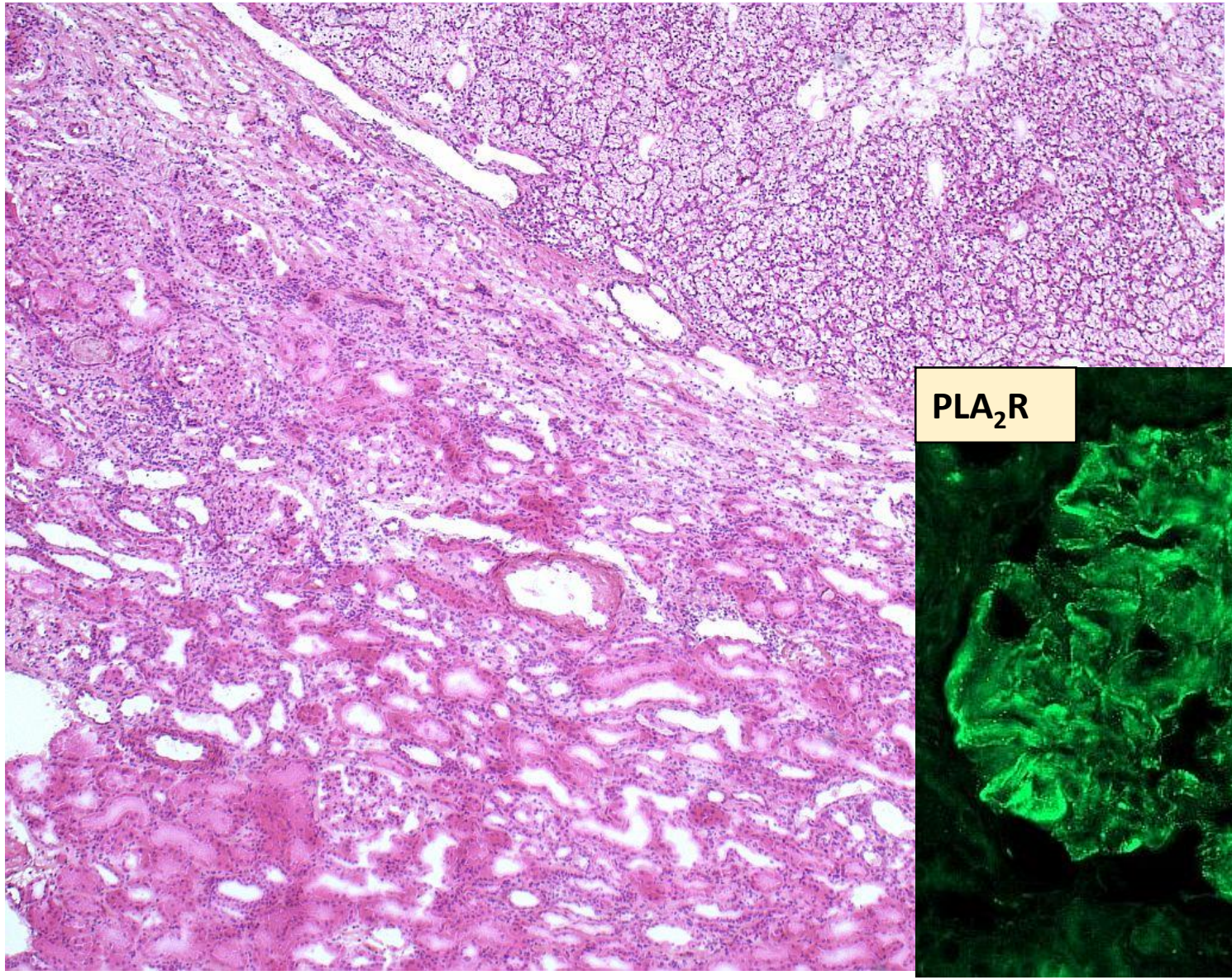
many cases of malignancy-associated MGN may also represent ***coincident disease processes***

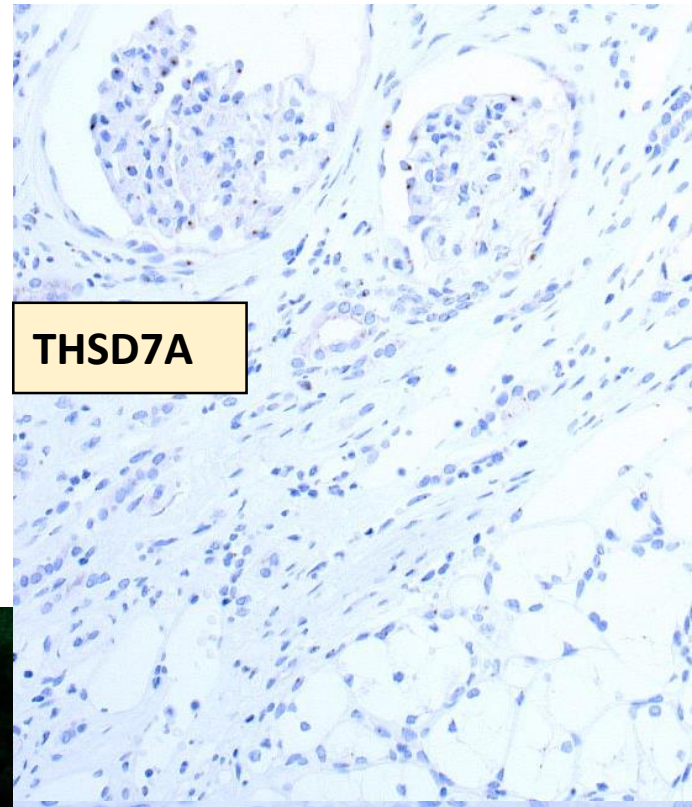
- the associated tumors are common in males over the age of 50 years, the same population with MGN.

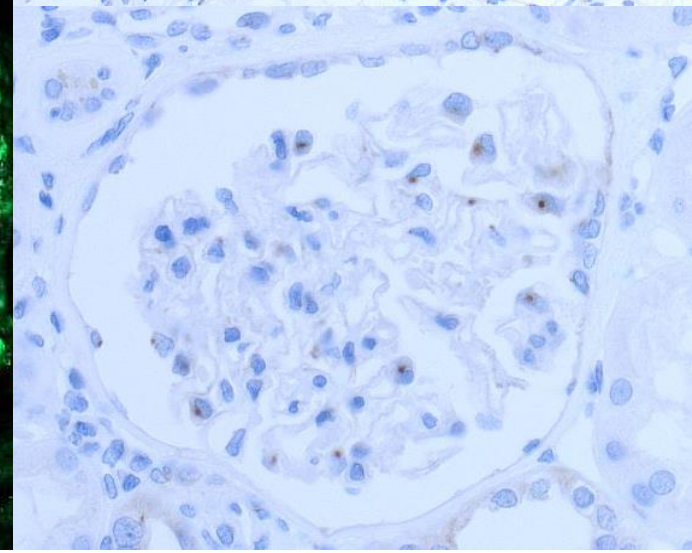
- remission of the NS with removal of the tumor does not necessarily imply a therapeutic response (a relatively high rate of spontaneous remission)

Patients with PLA_2R positive MGN should undergo age related screening for malignancy.

Clear cell ca & MGN







Secondary MGN

MGN and drugs

- Several groups of drugs are known to be associated with PU and development of MGN

NSAIDs, penicillamine, parenteral gold salts, alemtuzumab, mercurial salts, elemental mercury, and possibly anti-TNF agents

- The incidence of MN may be as high as 7 % in patients treated with penicillamine and 1-3 % in those treated with parenteral gold
- The mechanisms responsible for drug-induced MN are uncertain

Secondary MGN

MGN and Infections

- **syphilis** have been associated with MGN

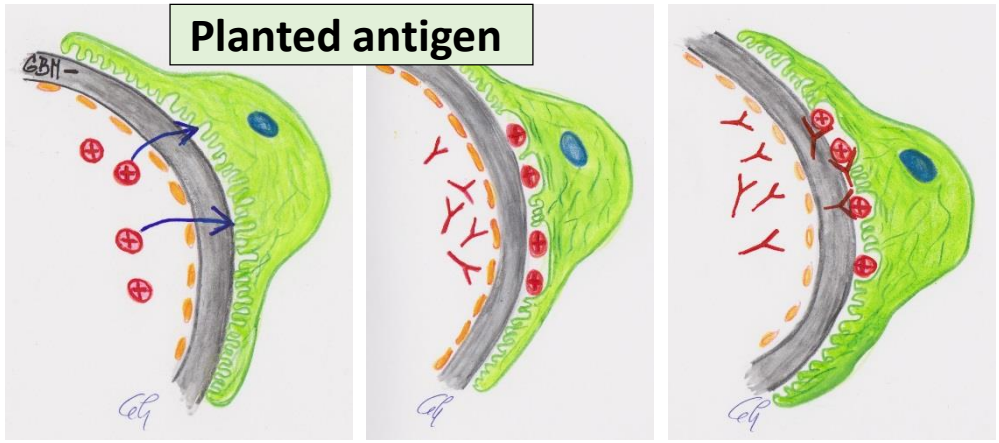
Treponemal antigens have been identified in the gli by IF

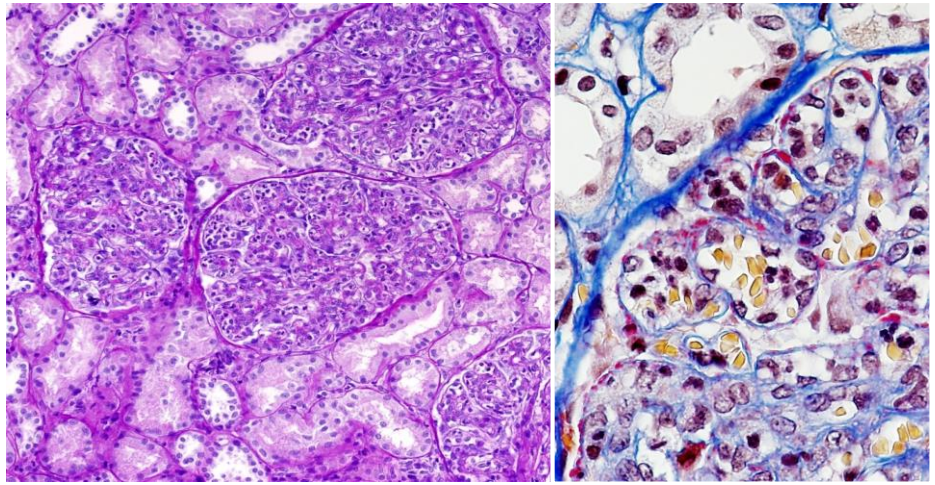
Eluates from glomerular deposits contain Abs specific for T. pallidum antigen

Treatment of syphilis can lead to resolution of the glomerular disease

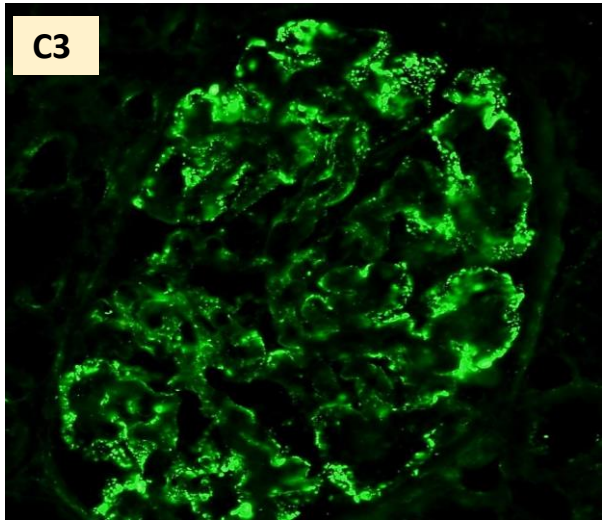
- **Hepatitis B virus** — MGN and HBV infection primarily occurs in children in endemic areas, asymptomatic carriers with no history of active hepatitis.
- ***e-antigen and cationic anti-e antibody*** are primarily deposited in the glomeruli
- Most studies have shown a low prevalence of anti-PLA_2R antibodies and/or PLA_2R staining of the immune deposits
- retrospective study of 39 cases of hepatitis B-associated MGN: 64% of cases with strong PLA_2R staining of deposits. Staining for the HBsAg colocalized with PLA_2R. Among the 6 cases for which serum was available, *all were positive for anti-PLA_2R antibodies*.
- This association was confirmed by Pierre Ronco group in patients in Africa.
- Xie Q, et al. Renal phospholipase A2 receptor in hepatitis B virus-associated membranous nephropathy. Am J Nephrol 2015; 41:345.

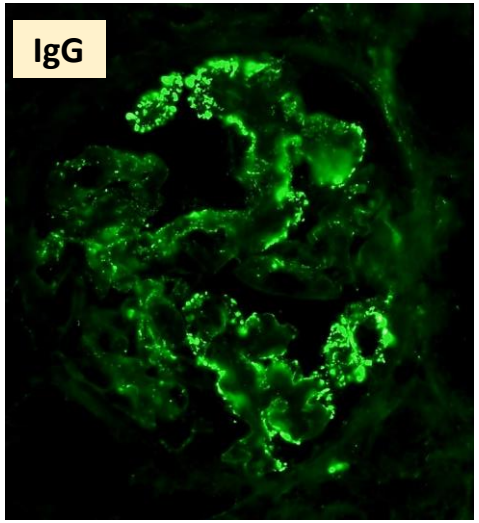
Pathogenesis and morphology of PIGN

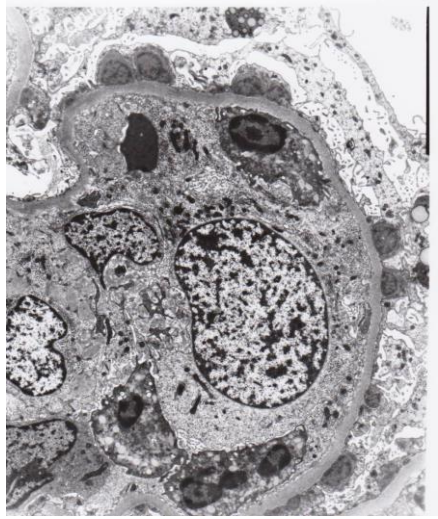




- PIGN represent immunologicaly mediated disease
- Agreement: streptococcal antigens enter the bloodstream and become localized in the glus and remain planted (for antigens it is advantageous to be cationic); Candidate: streptococcal cationic pyrogenic exotoxin B (SpeB)



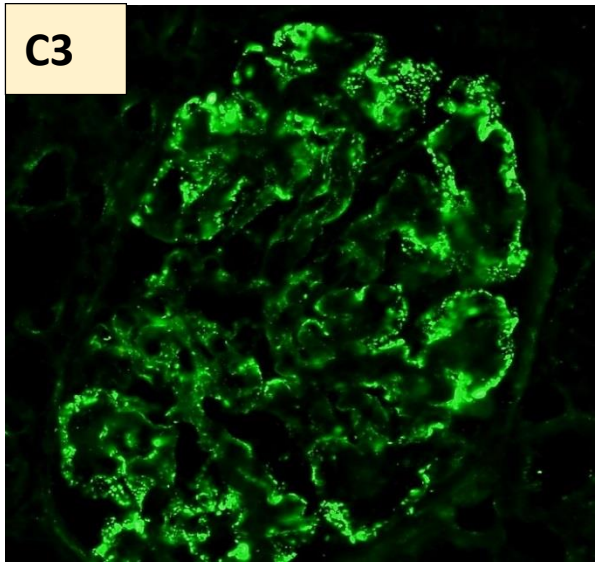


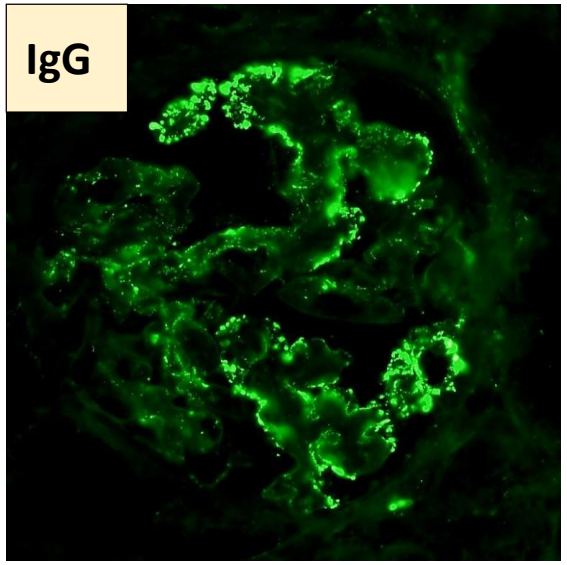


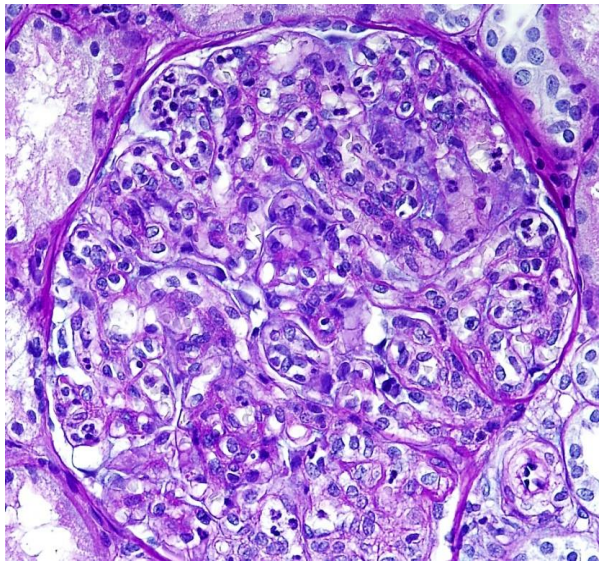
IgG and C3 positive staining in granular deposits along the capillary walls
EM: subepithelial electron-dense deposits, so-called **humps**
share location of deposits and IF with MGN
the classical form of PIGN is self-limiting similar to 30% of patients with MGN.

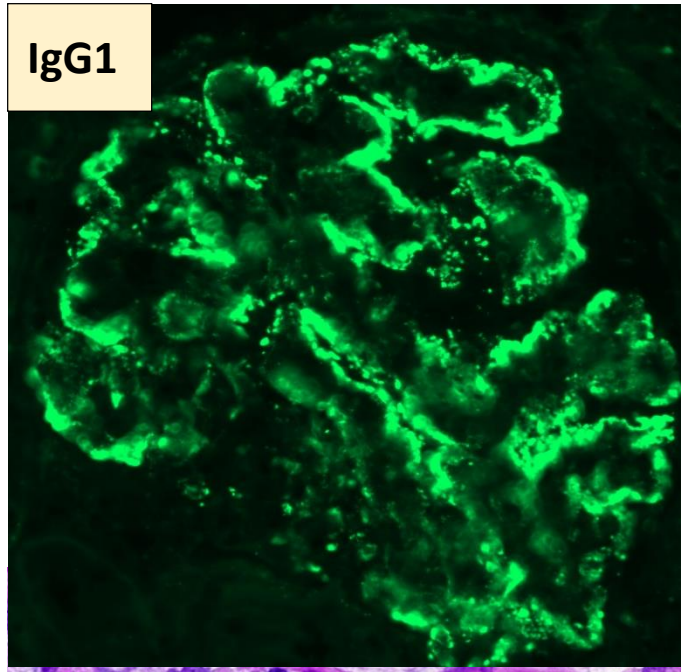
PIGN: immunologicaly mediated disease

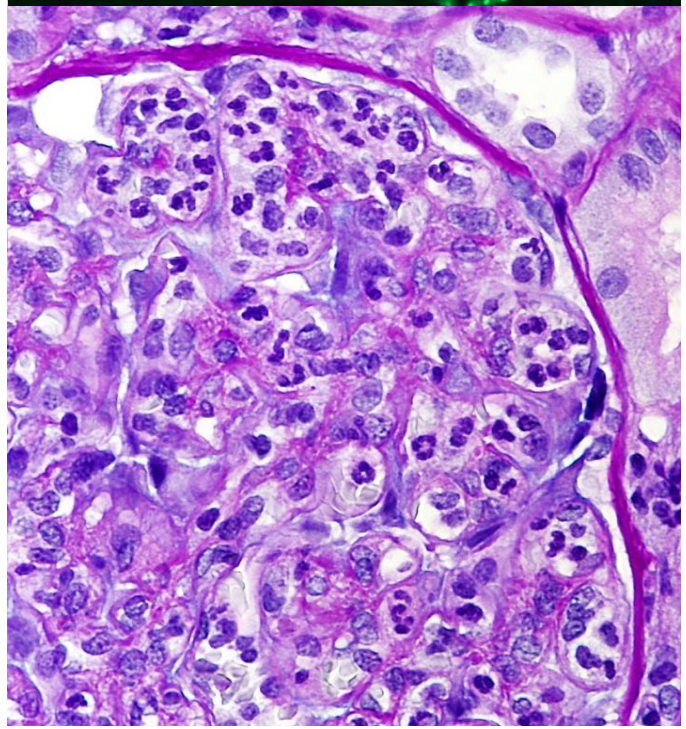
planted cationic antigens

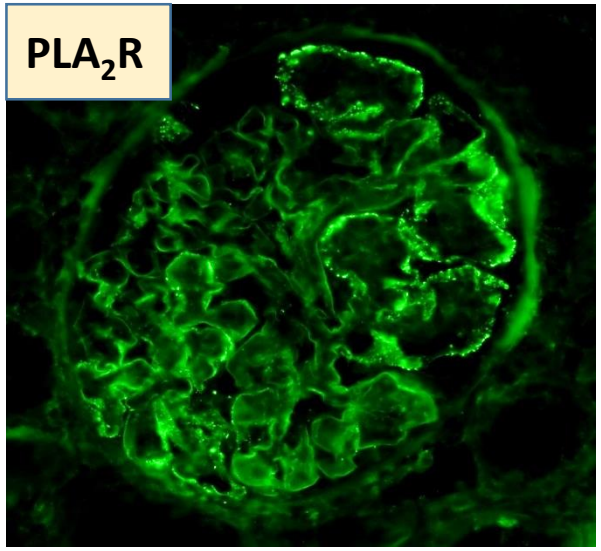


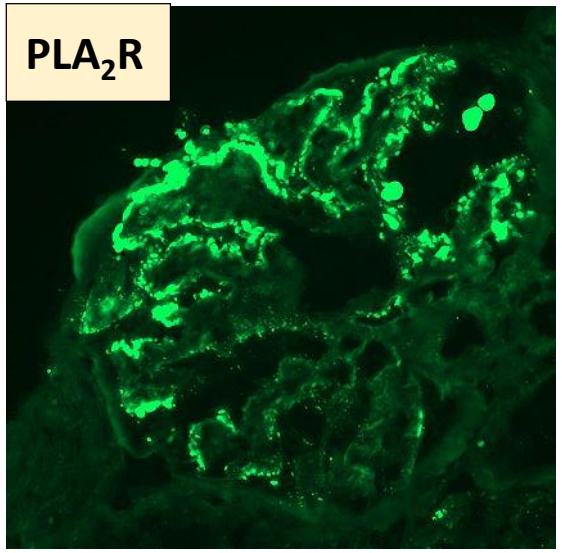


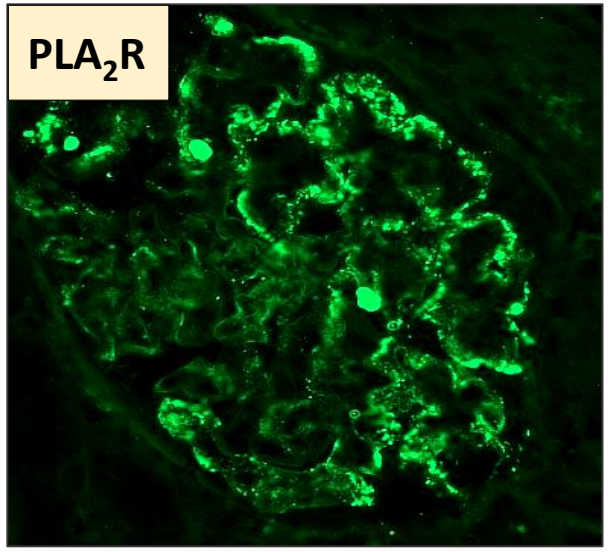






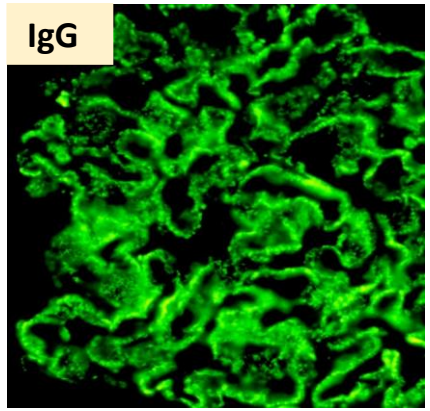


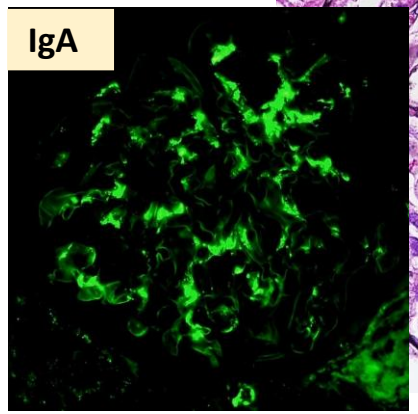


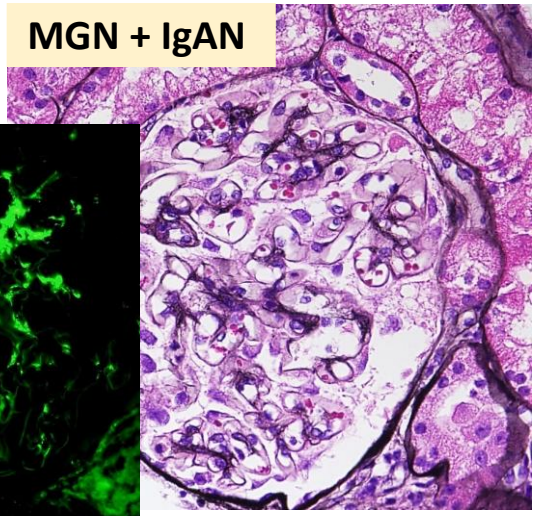


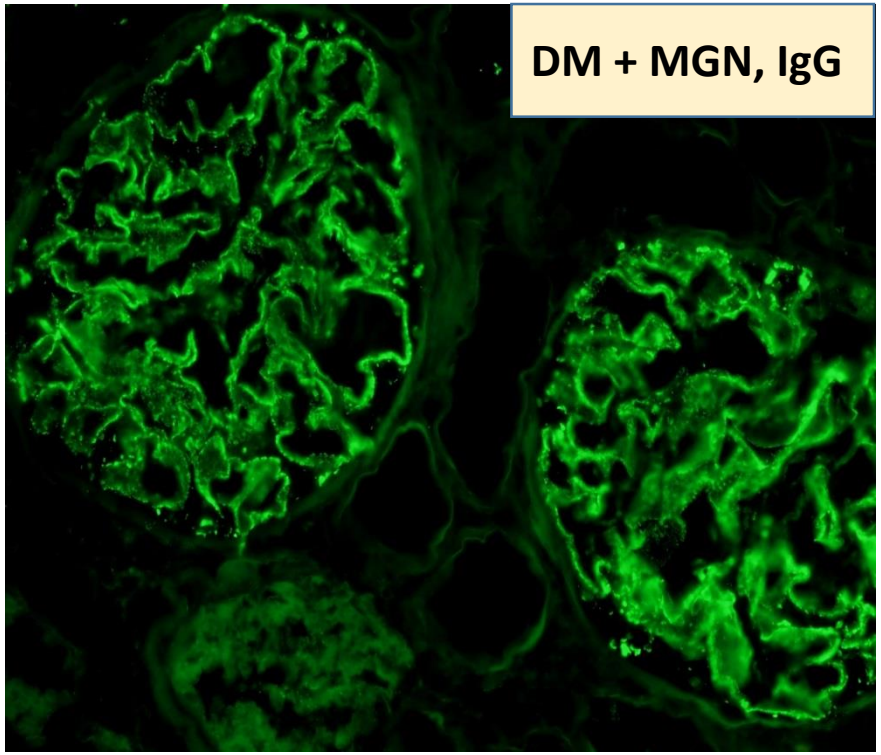
MGN with other glomerular disease

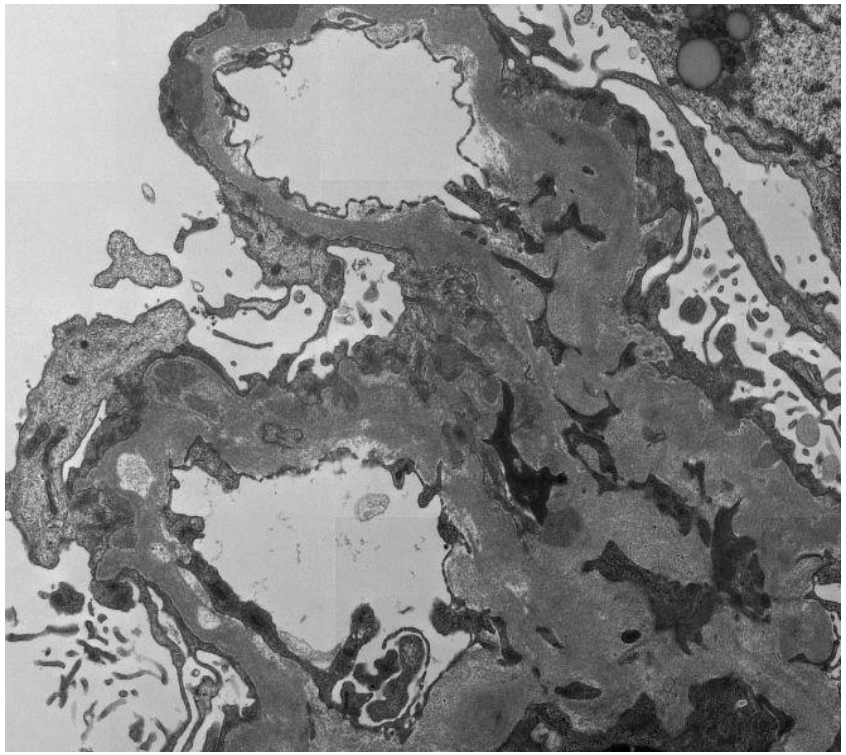
- DM
- ANCA (anti-GBM)
- IgA GN
- FSGS (part of podocyte injury)

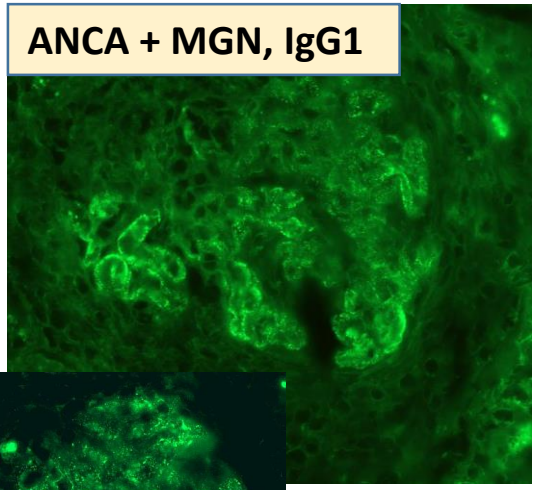


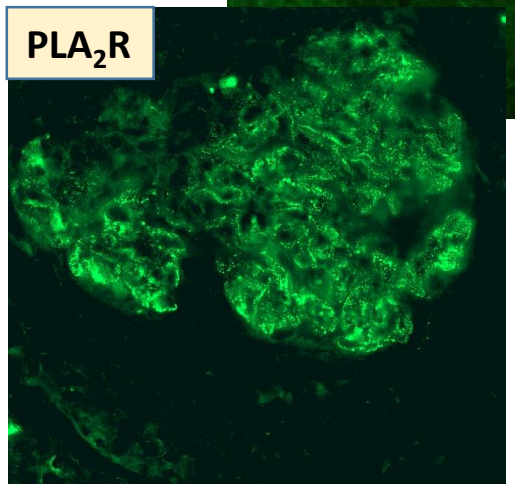








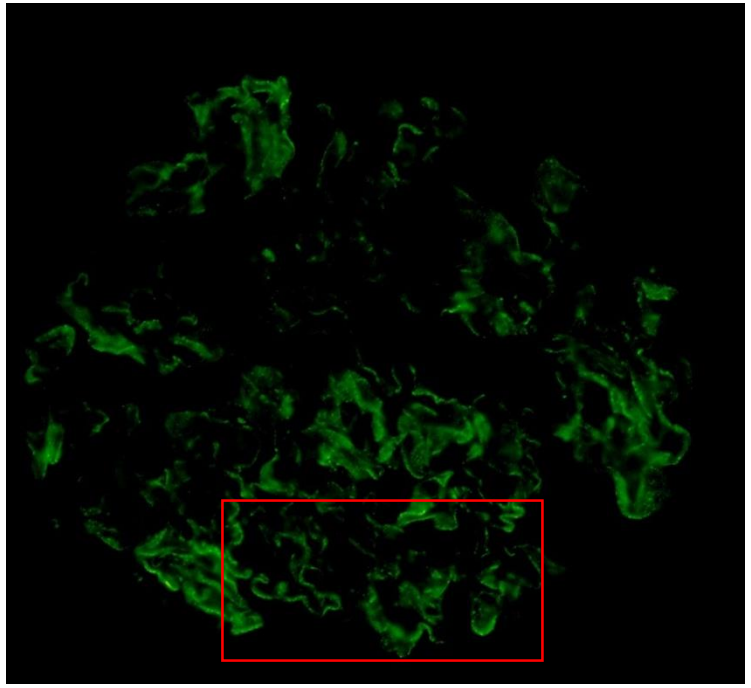


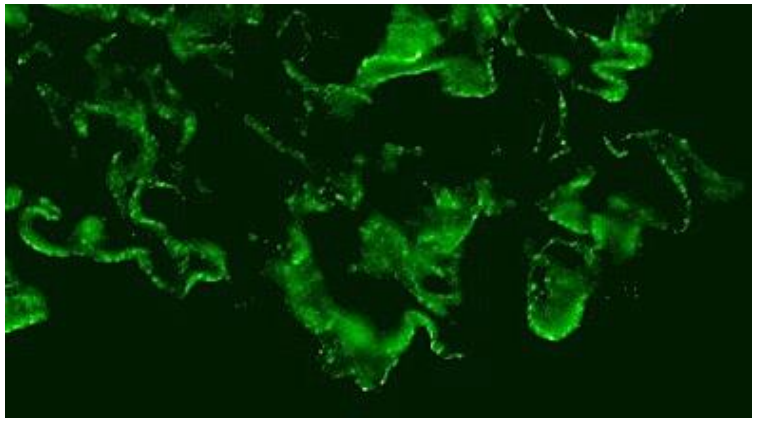


MGN and Transplantation

- MGN may occur in the transplanted kidney, either as recurrent or „de novo“

- **MGN recurs** in approximately 40-50% of patients, with 50% graft loss
- Patients with anti-PLA_2R Abs pre-transplantation: 60-70 % risk of recurrence (high anti-PLA_2R Abs titers: higher risk)
- Patients Abs negative lower risk (30 %)
- Histological recurrence occurs most often during the first year
- Second period around 5 years
- No information about the relevance of other Abs in disease recurrence
- rMGN is generally a progressive disease, treatment at the earliest stage, rather than after a period of observation



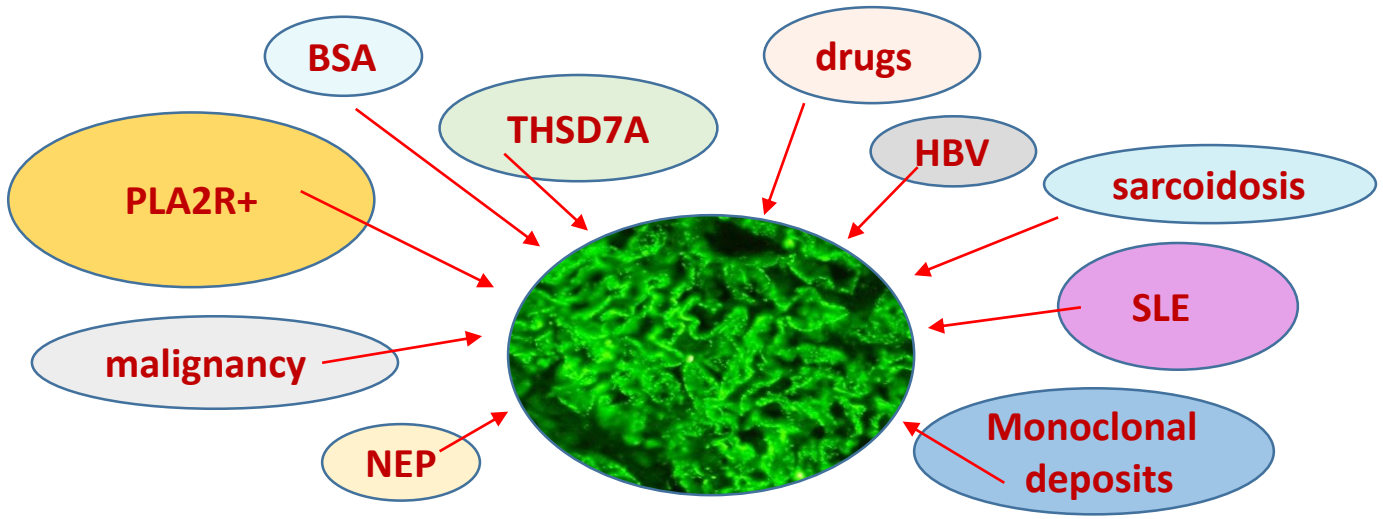


MGN and Transplantation

„De novo“ MGN

- 2% of adults and even more frequent in children
- Clinical presentation: variable (asymptomatic - NS)
- Later onset
- ***Morphology***: segmental distribution of deposits and mild to moderate mesangial proliferation
- ***Secondary MGN***: The patient management should include a careful search for underlying cause of MGN (infections, malignancy)
- De novo MGN: can be *associated with antibody-mediated rejection*.
- PLA_2R almost always negative, IgG1
- *The clinical impact of de novo MGN on the graft survival is still controversial and there is no formal contraindication to retransplantation*

Summary



- Identification of **PLA_2R** and **THSD7A** as autoantigens in primary MGN has fundamentally changed our approach to this disease

- **Anti PLA_2R antibodies can be detected in serum and can be used for diagnosis and monitoring of patients:**

a) Immunological remission of PLA_2R precedes clinical remission

b) High PLA_2R levels in the serum is associated with longer time to remission under immunosuppressive treatment.

- **One phenotype** can occur with *variable antigens* and utilizing *different mechanisms of complement activation* and *IgG subclasses*

Perspectives:

Only understanding the complex pathogenesis will offer opportunities for future therapeutic interventions

Many questions:

- Are IgG4 protective and IgG1 caused the damage?
- *What is the triggering factor?*
- Why the disease often starts later in life?
- What is the role of epitope spreading and how to block it early in the course of disease?
- What is the role of different subsets of plasma cells mainly *long-lived memory plasma cells* (CD20-)
- What is the role of genetic background?