Pathophysiology of kidneys – part I

Nephron and glomerular hemodynamics

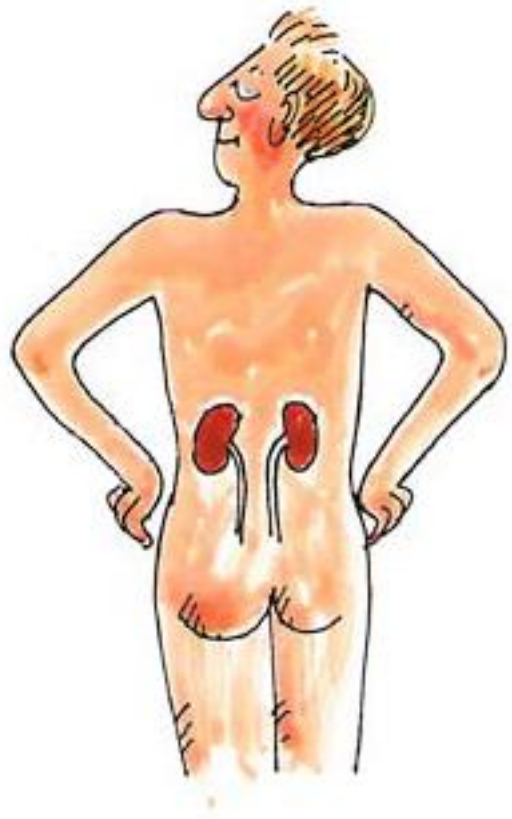
Glomerular filtration membrane and its pathologic changes

Proteinuria

Glomerular disease syndromes

- nephrotic vs. nephritic syndrome

Causes vs. histopathology of glomerular diseases



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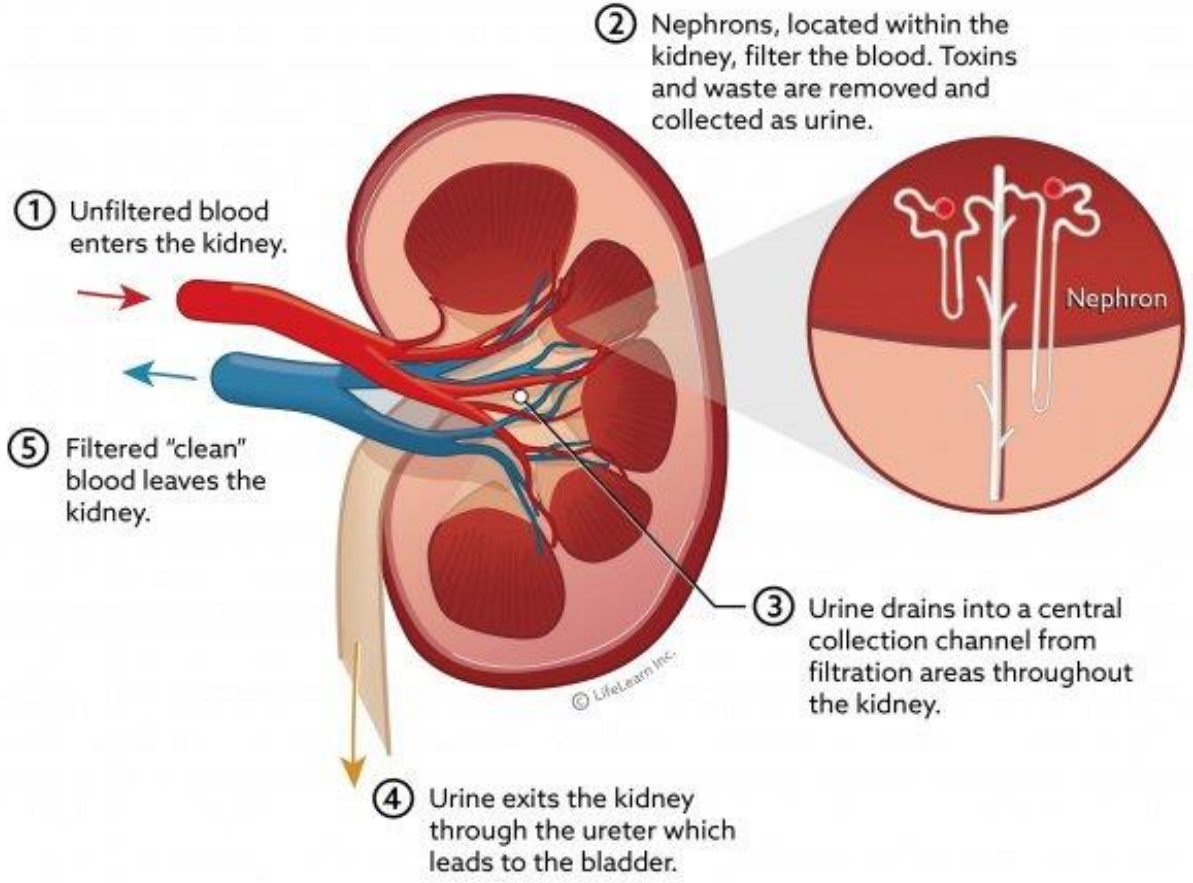
Functions of the kidney



A healthy kidney and the blood filtration process

- regulation of
  - — extracellular volume and blood pressure
  - — tonicity and osmolarity
  - — acid-base balance
  - — nitrogen metabolism
  - — calcium and phosphate homeostasis
  - — haematocrit
  - — excretion of waste products
  - — endocrine functions
  - — metabolic functions
    - gluconeogenesis
- in order to carry out all these functions/processes kidney has to have an excellent blood supply

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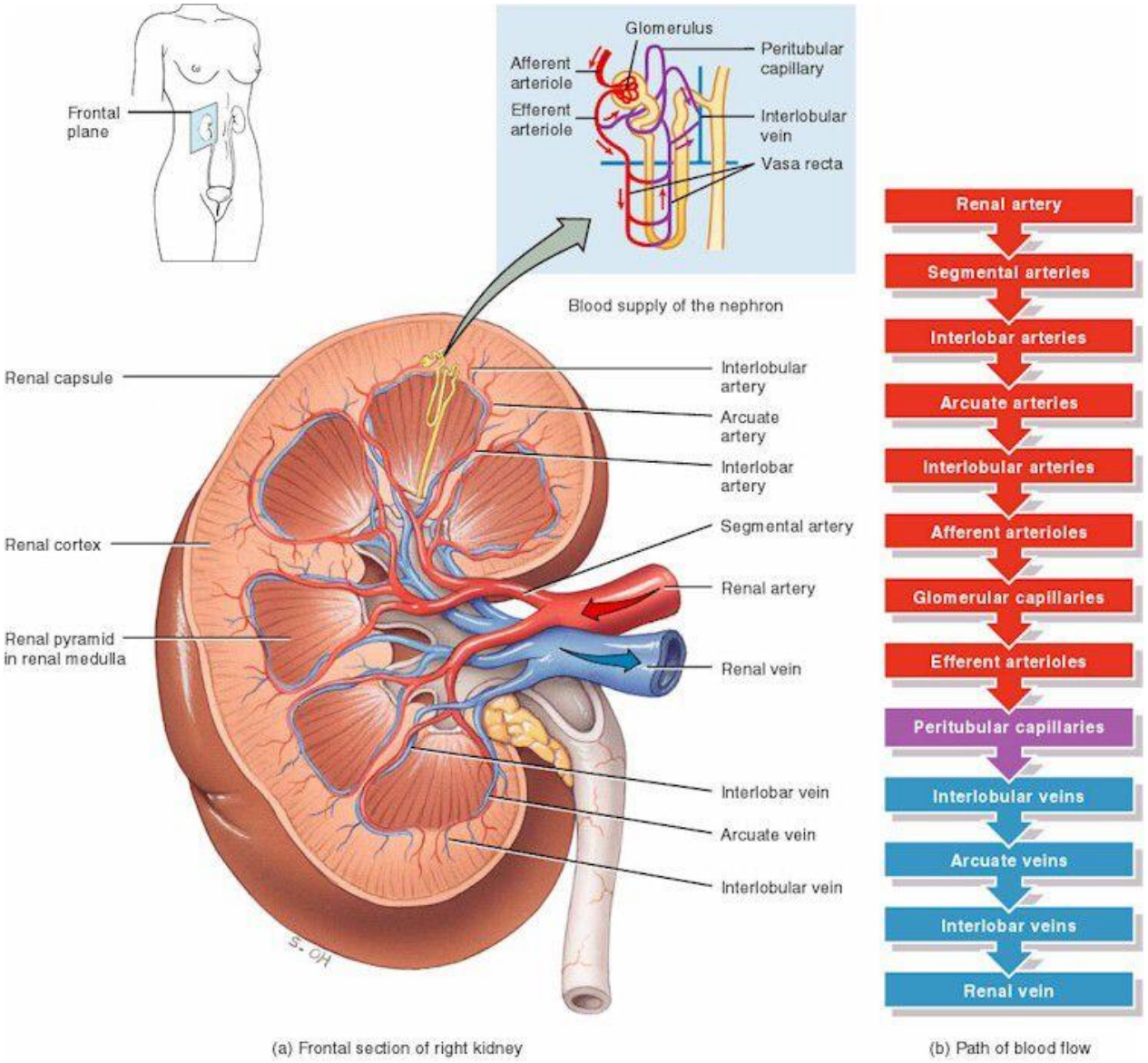
Kidney blood supply



- renal blood flow (**RBF**) **~1200 ml/min**, this represents ~**20-25% of cardiac output**
  - rather high considering the weight of kidneys (~350 g)
  - cortical flow >>> medullar flow
  - renal plasma flow (**RPF**) in haematocrit 0.45 **~600 - 700 ml/min**
- arterio-venous difference in oxygen saturation of haemoglobin is very low
  - given nearly 100% saturation of Hb with O_2 in the arterial blood, high Hb saturation in venous blood proves that great perfusion serves primarily to regulation purposes and not to nutrition !!!
    - how much O_2 left in venous blood?
      - heart ~35%, brain ~50%, kidney ~90%
- blood supply to kidney
  - via renal artery and its branches
    - **„portal“ circulation** = 2 capillary networks in series
      - 1. glomerular capillaries
      - 2. capillaries following efferent arterioles
        - peritubular capillary network (cortical nephrons)
        - vasa recta (juxtamedullar nephrons)

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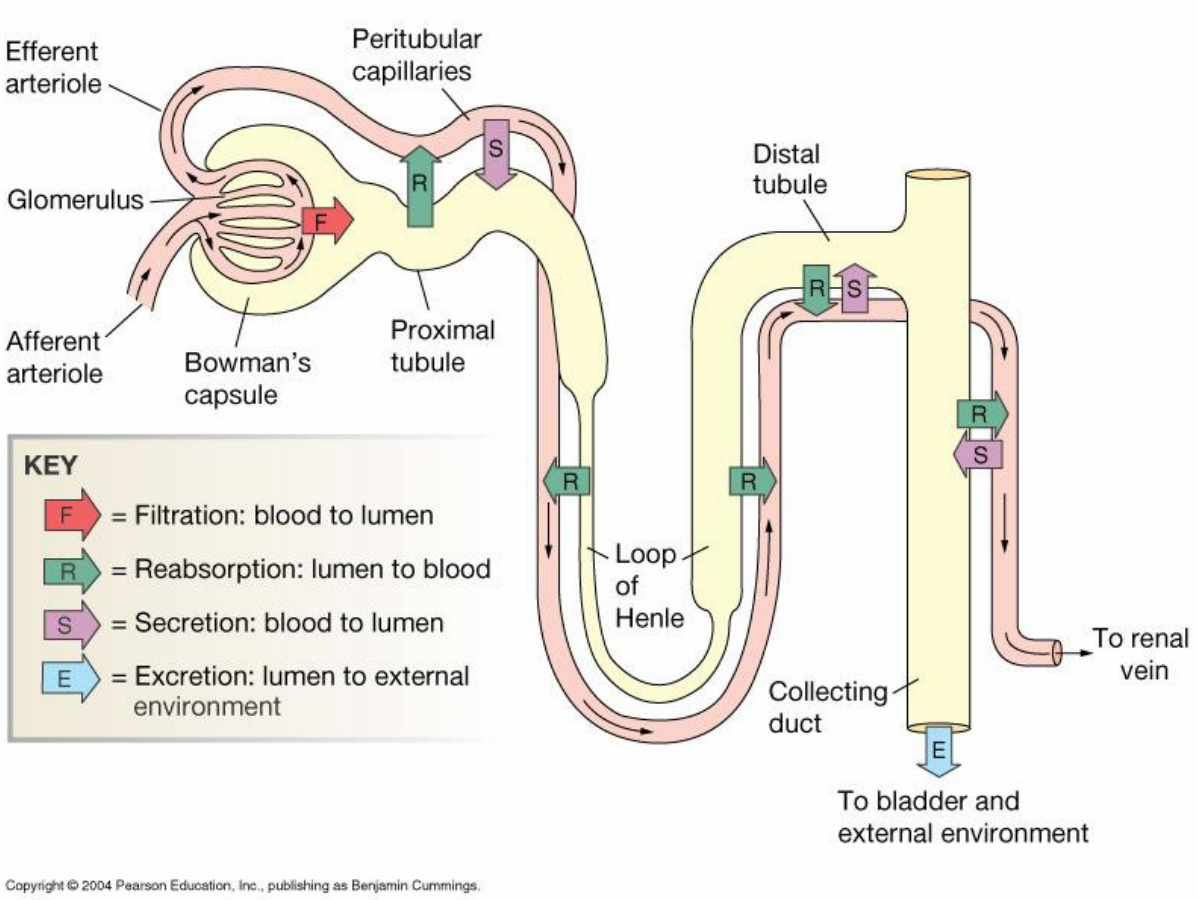
Kidney – processes involved in maintaining homeostasis



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- **glomerular filtration** – to maintain volume and BP
  - driven by hydrostatic and osmotic pressure gradients
    - though not typical Starling forces as in other capillaries
  - quantitatively
    - GFR ~20 - 25% RPF → **GFR ~120 – 140 ml/min** → ~180 l daily
      - ratio GFR/RPF = filtration fraction (120/600 = 0.2)
  - qualitatively
    - water and low molecular weight substances freely
    - others (cells and high molecular weight substances) limited by size (<65kDa) and charge (repulsion of negative)
- **tubular reabsorption** – to reclaim solutes, nutrients and other needed substances
  - daily filtered ~180 l, but 99% reabsorption (~178.6 L/day)) → 1.4–1.8 l of urine/day
  - typically symports
    - e.g. Na/Glc, Na/AAs, …
  - saturable capacity (transport maximum, Tm)
    - renal thresholds (e.g. Glc)
- **tubular secretion** – to get rid of waste products, hydrogen ions (ABB), drugs etc.
  - active (ATP)
  - secondary active
- **urine excretion**

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Nephron – structure & function correlation

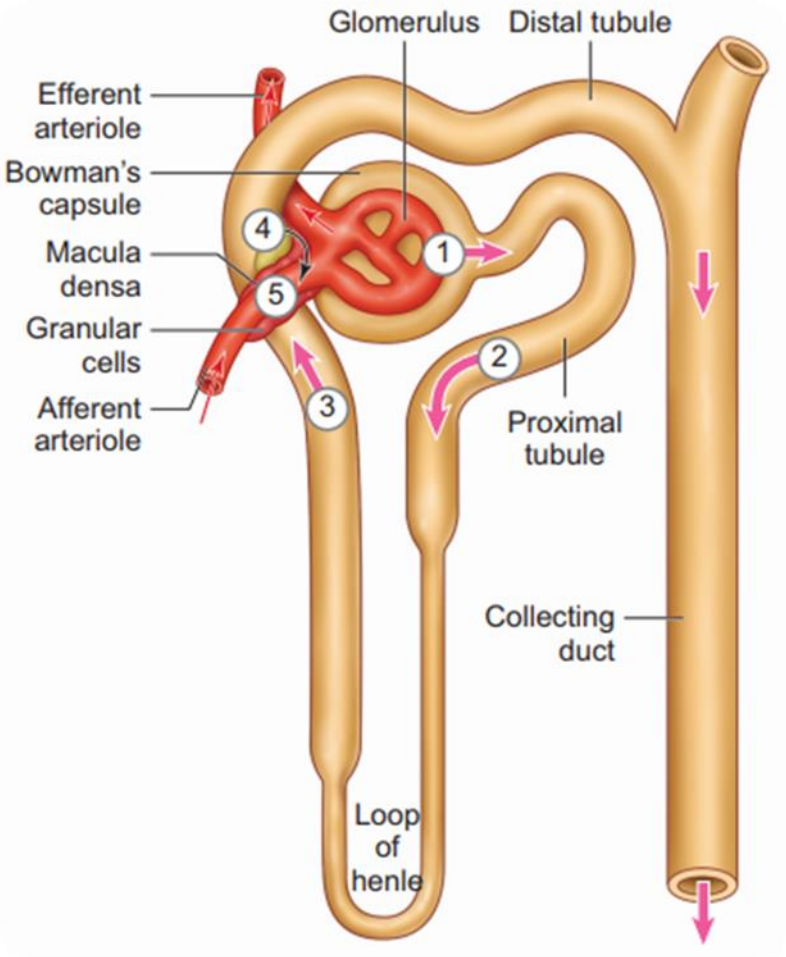

Figure 8.4 Structure of a Nephrons

- nephron has to meet all the requirements for a normal control of homeostatic parameters by kidney
  - **sufficient kidney perfusion** to keep sufficient volume of glomerular filtrate
    - multiple **autoregulation vs. systemic effects**
- ultrafiltration of the plasma (i.e. separation of blood components) is the first step in formation of urine
  - ultrafiltrate is free of cells and most proteins
  - concentration of low molecular weight substances is equal to plasma
- **GFR is a crucial parameter estimating the kidney function**
  - volume of glom. filtrate per minute
  - low range of normal interval ~100 mL/min/1.73m^2
  - natural age-related decline (>40 yrs) 0.4 - 1.2 mL/min per year
- normal function of tubular epithelia to reabsorb and event. secrete LMW substances and to regulate their concentration in plasma
  - **tubular reabsorption of >99% of glomerular filtrate**
- normal function of peritubular capillaries
  - in both cortical and juxtamedullar nephrons

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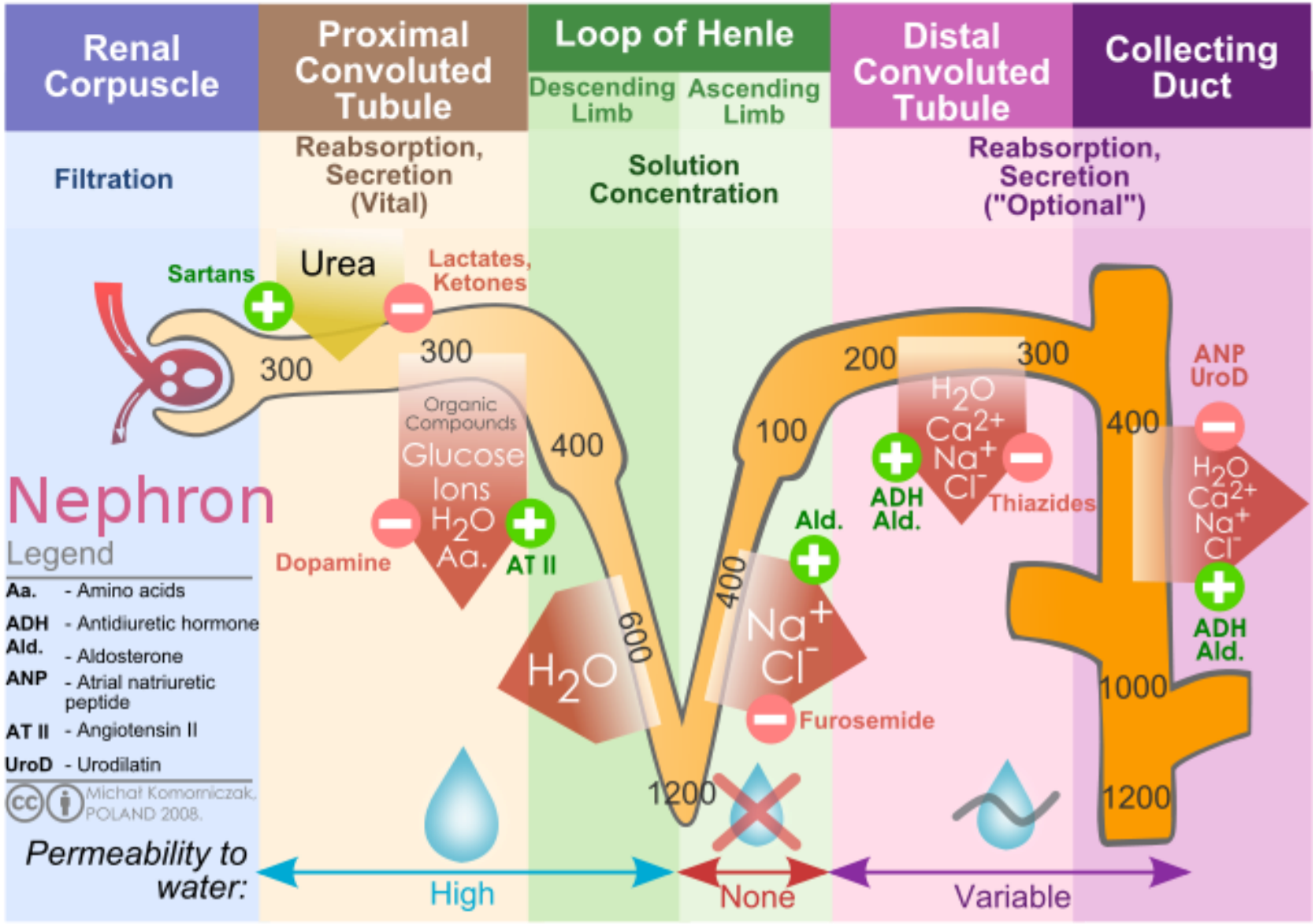
Reabsorption throughout the nephron



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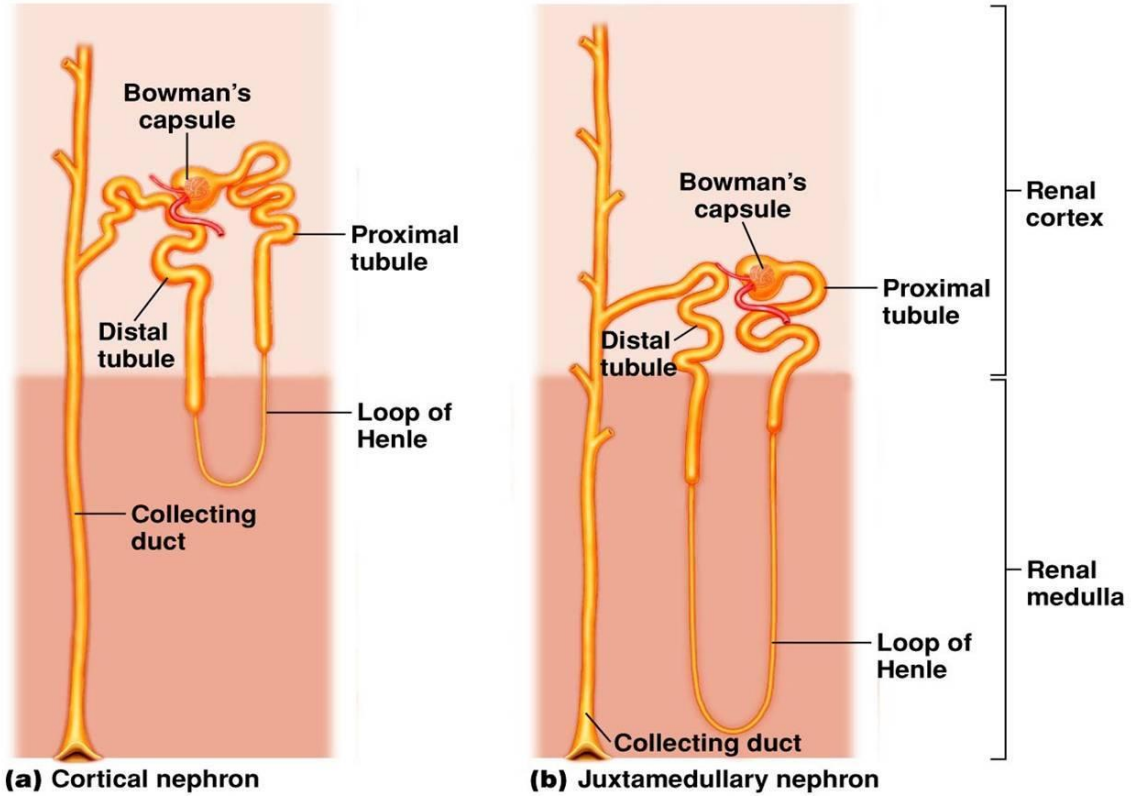
Two types of nephrons



- **(a) cortical nephrons (~70 – 80%)**
  - have a glomerulus located nearer to the outer 2/3 parts of the cortex
  - shorter loops of Henle (LH)
  - have peritubular capillaries which branch off the efferent arterioles
    - these provide nutrients to the epithelial cells
    - participates in reabsorption of water and solutes, but not in urine concentration
  - important for autoregulation
    - tubulo-glomerular feedback
- **(b) juxtamedullary nephrons (~20 – 30%)**
  - have a glomerulus near the junction of the cortex and medulla (inner 1/3 of cortex)
  - longer LH radiating deeply into the osmotically concentrated medulla
  - important for production of concentrated urine
    - capillaries - vasa recta (from efferent arteriole) together with LH form „counter-current" concentration multiplication system responsible for developing the osmotic gradients that are needed to concentrate urine
- various diseases can affect variably these two populations and thus have deferent effects on renal processes

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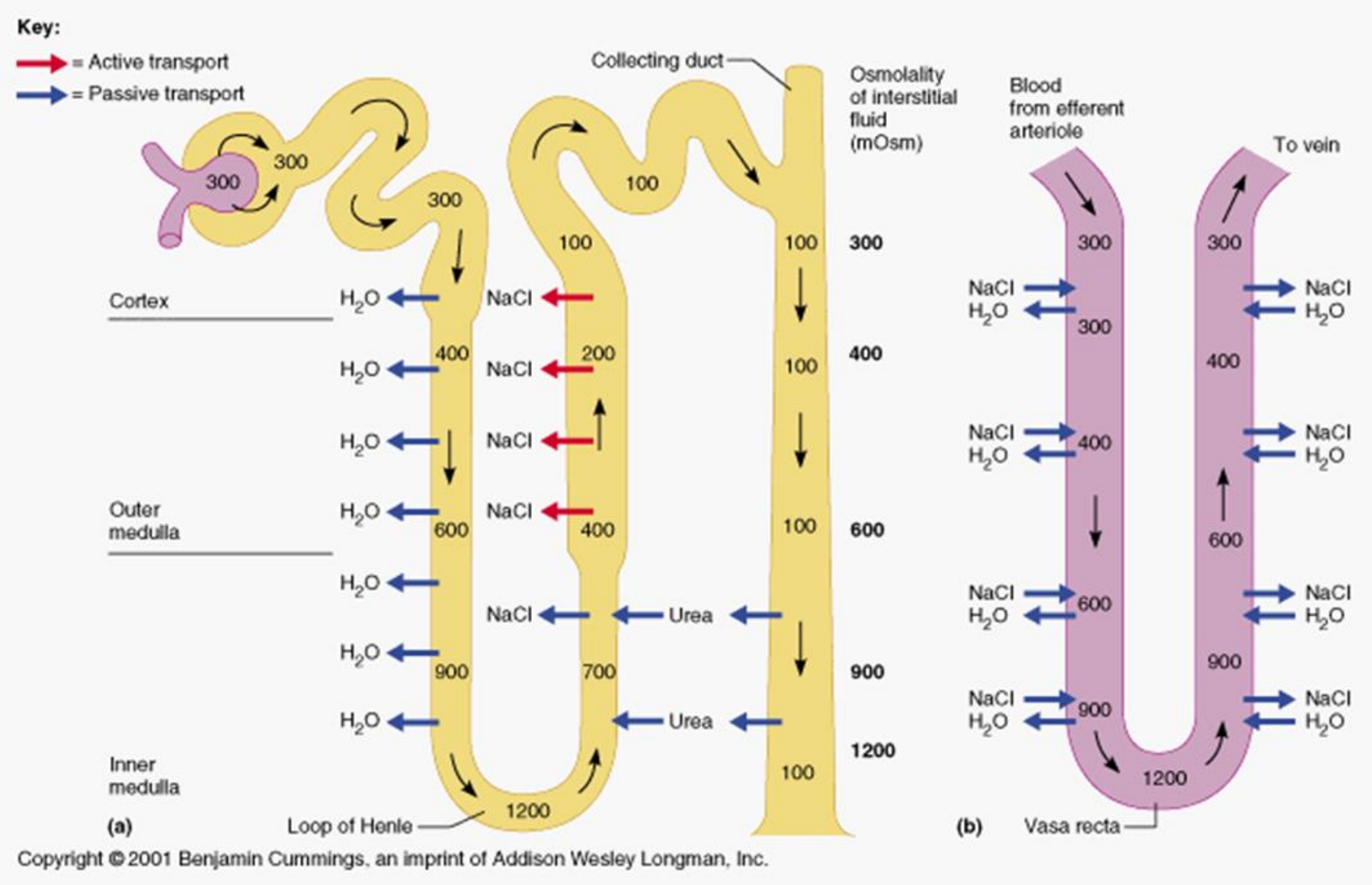
Counter-current system in medulla



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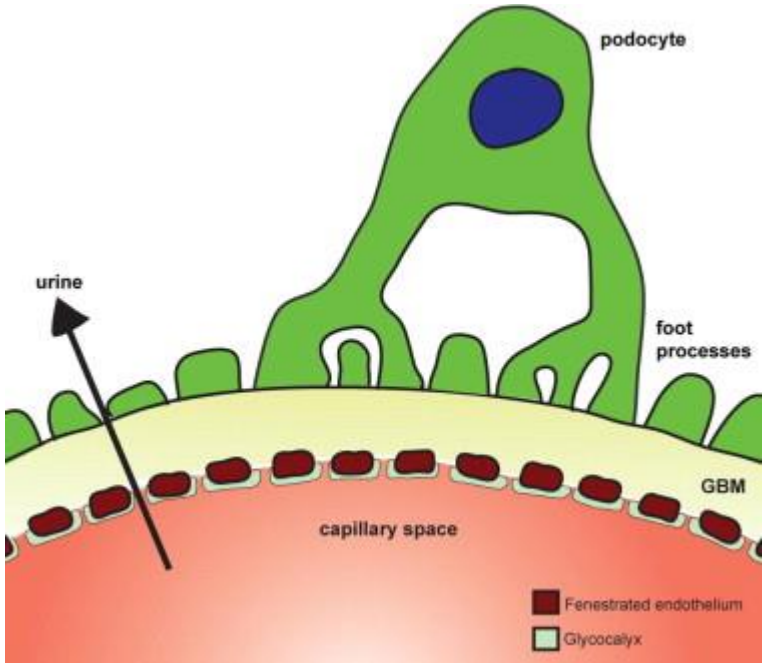

GLOMERULAR FILTRATION BARRIER AND ITS ABNORMALITIES/DAMAGE – PROTEINURIA

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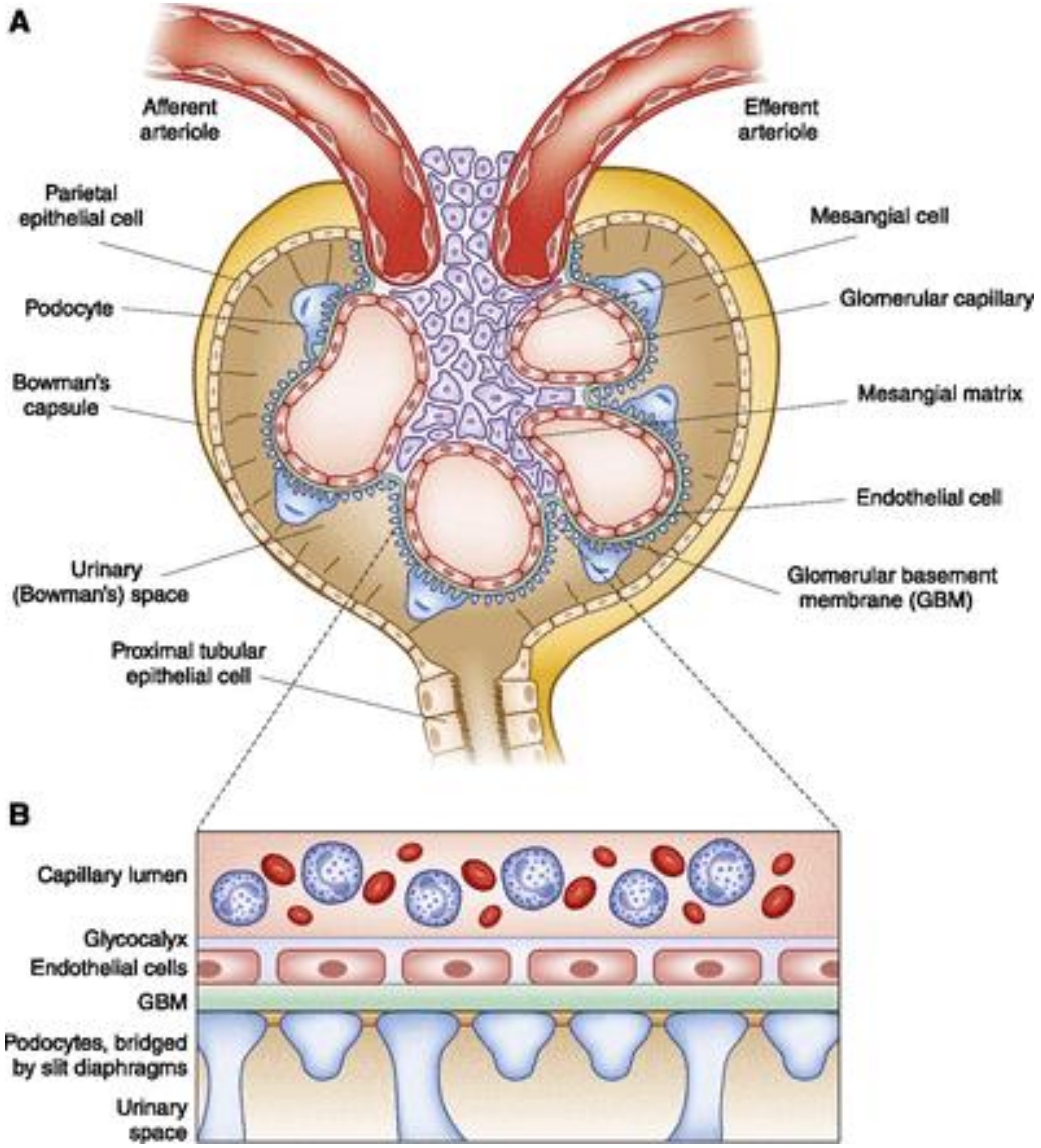
(A) Basic structure of the glomerulus and (B) the glom. filtration barrier



- (A) glomerulus
  - each glomerulus is composed of an **afferent arteriole**, which supplies the glomerular capillaries, and an **efferent arteriole**, into which they drain
  - **mesangial cells** and mesangial matrix provide structural support for the glomerular capillaries
  - **glomerular capillaries** are lined by specialized fenestrated endothelium, and then the **glomerular basement membrane**
  - on the urinary side of the glomerular basement membrane are **podocytes** with foot processes that wrap around the glomerular capillaries
  - the urinary space is lined by a cup-like layer of **parietal epithelial cells** which adhere to the basement membrane of Bowman's capsule
- (B) the glomerular filtration barrier
  - specialized molecular sieve, with properties that aid filtration of small solutes from the blood to the urine, while limiting the passage of macromolecules such as proteins and partly albumin

A. Richard Kitching, and Holly L. Hutton CJASN 2016;11:1664-1674

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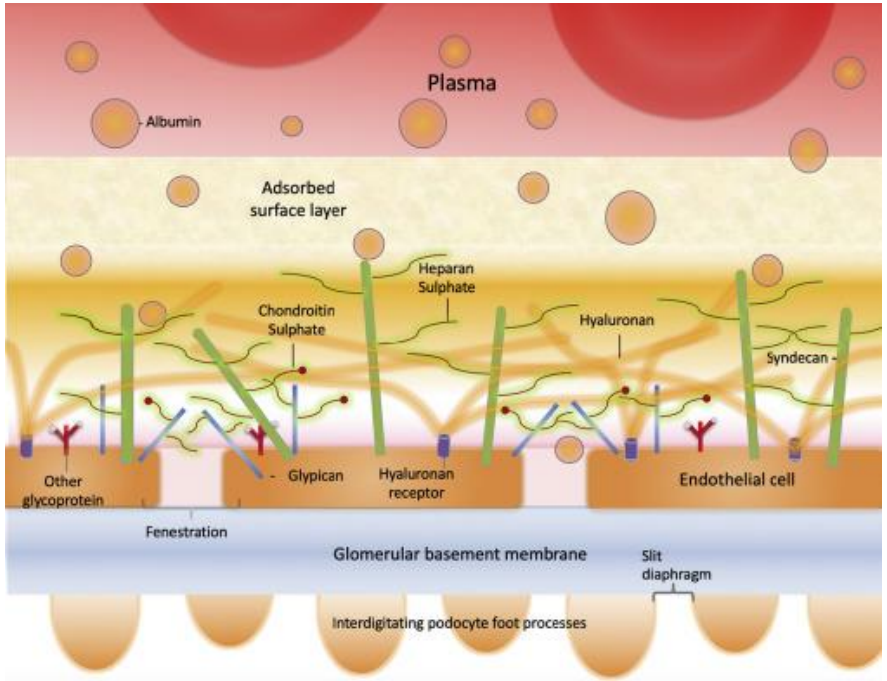
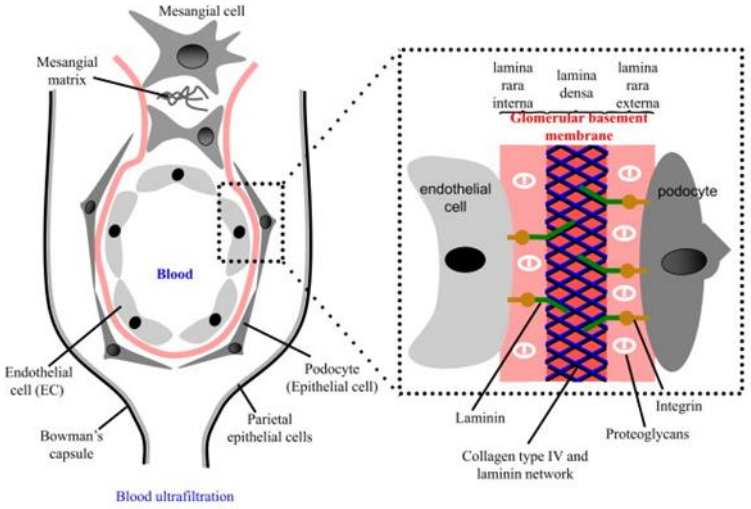
Structure of glomerular filtration membrane

- (1) endothelium
  - **fenestrae** – filter ~70-100nm ∅
    - separation of blood cells
  - **glycocalyx** – mesh of **anionic** biopolymers covering the luminal surface of endothelial cells
    - heparan sulphate is the dominant type, making up 50–90%
- (2) glom. basal membrane (**GBM**)
  - lamina rara interna – lamina densa – lamina rara externa
  - network of glycoproteins (collagen type IV, laminin, entactin, agrin, …) and mucopolysacharides
  - ~300nm thick with summary **negative charge**
    - size-selective separation of majority of plasma proteins >70kDa (~ 4nm ∅)
      - haemoglobin (~ 40kDa) yes
      - myoglobin (~ 17kDa) yes
      - β2-microglobulin (12kDa) yes, but reabsorbed
      - paraproteins (<70kDa) yes
    - neg. charge – heparansulphate, hyaluronic and sialic acid
      - albumin (~ 67kDa) mostly no/in limited extent yes
- (3) visceral epithelium of Bowmann capsule = **podocytes**
  - primary, secondary and tertiary foot processes (pedicles)
  - filtration slits ~20-30nm ∅
  - slit diaphragm (cell-cell junctions)
    - important contribution to size (as well as charge) separation of proteins
    - nephrin and other proteins
- (4) **mesangium**
  - indirectly affect filtration of proteins – mesangial cells contract and ↑ filtration pressure





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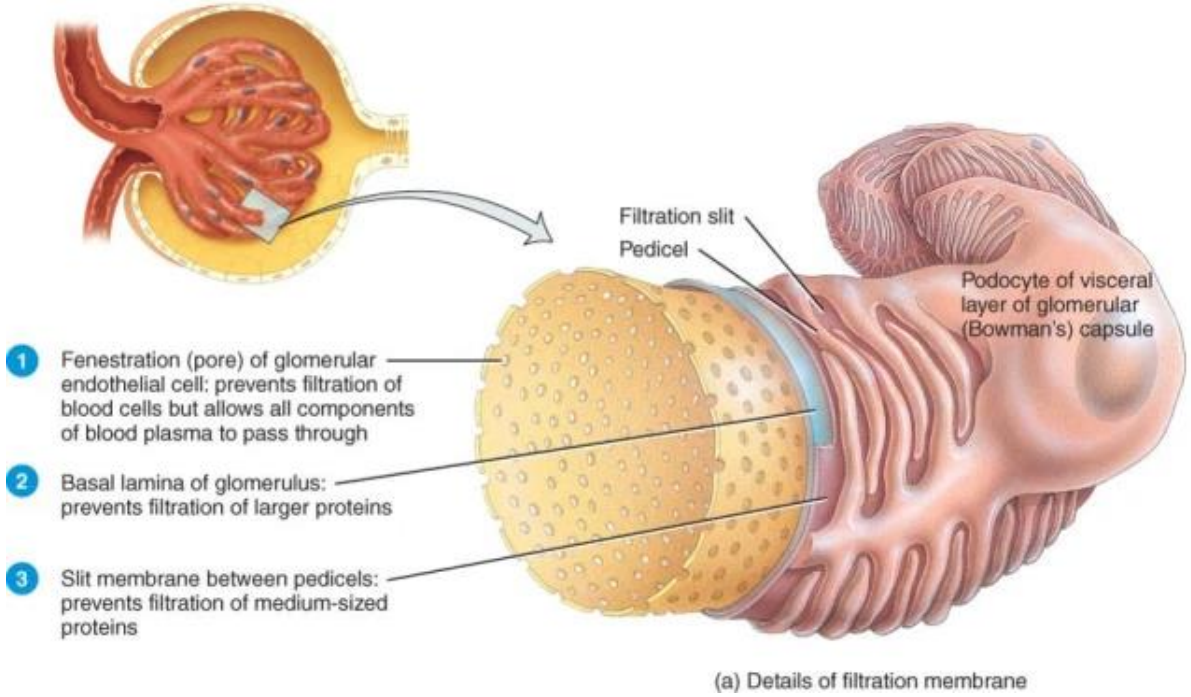
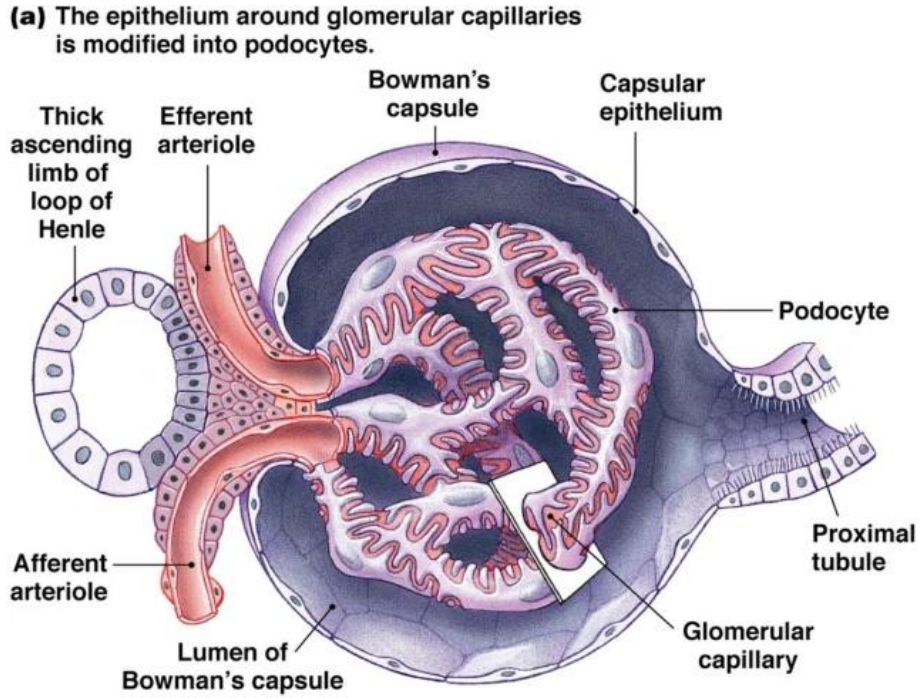
Properties of glomerular filtration membrane



(a) Details of filtration membrane



- size-selectivity, i.e. limit of mol. weight of filtered substances
  - <7kDa freely
  - 7-70kDa concentration dependent
  - >70kDa not filtered
- charge-selectivity
  - negative charge (also for proteins <70kDa)

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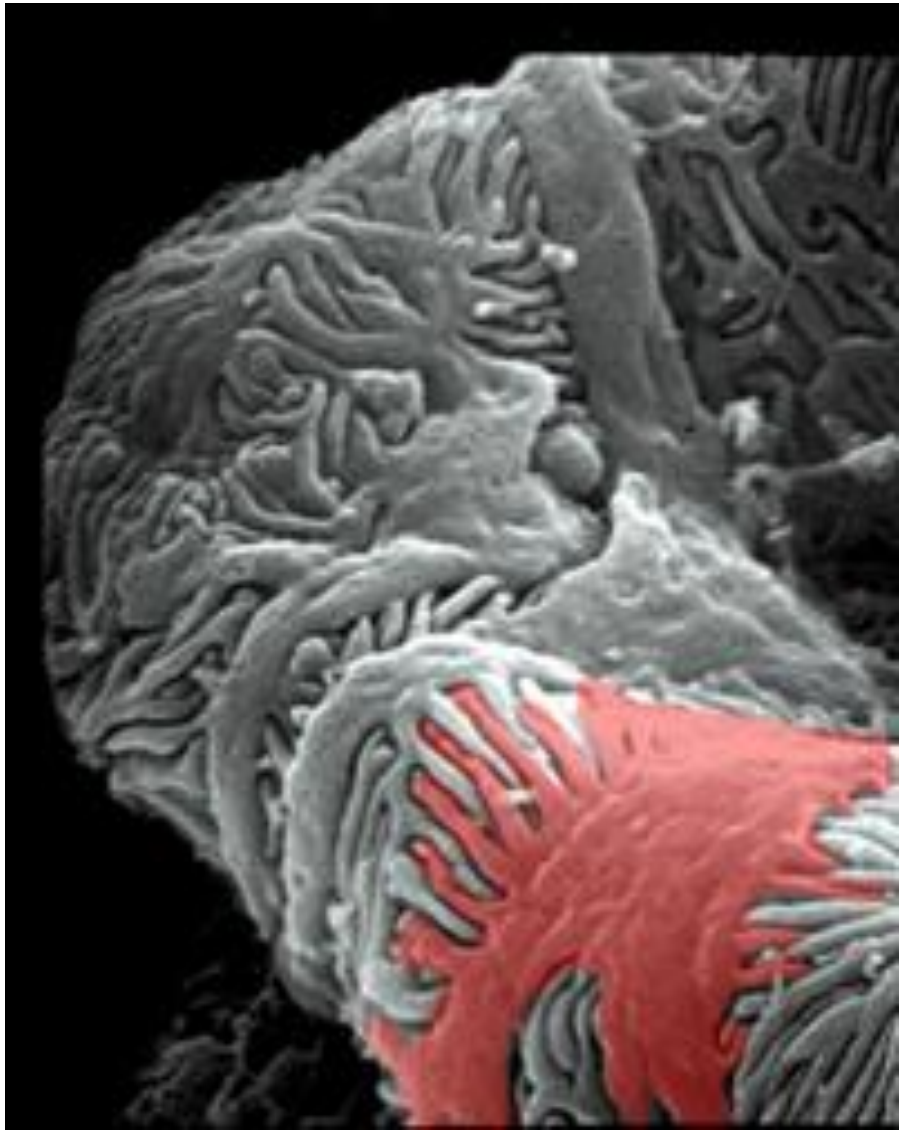
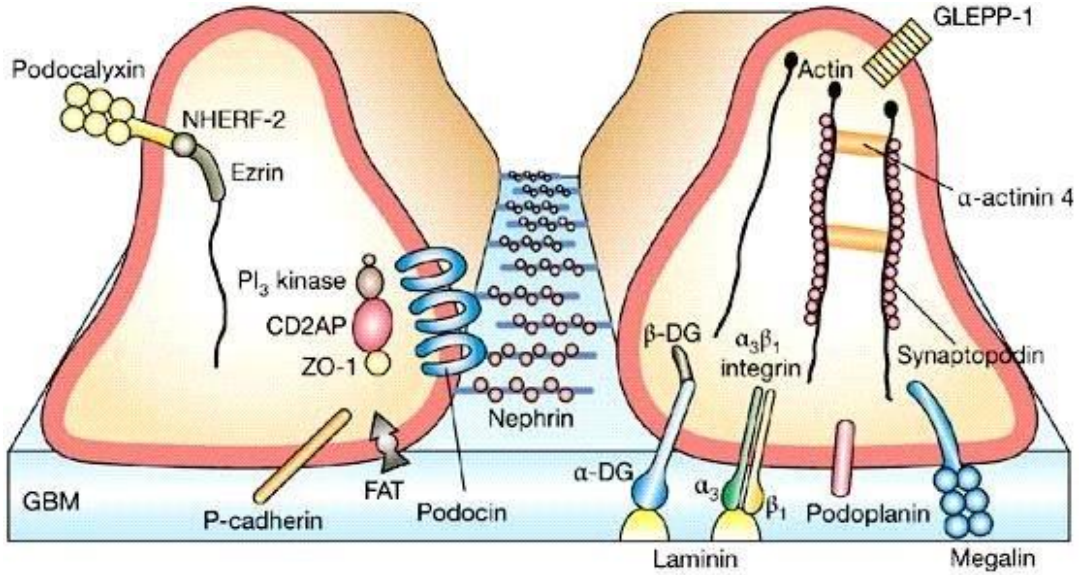
Podocytes – slit diaphragm





- (1) basal domain – anchoring to GBM
  - integrins, DG = dystroglycan
- (2) cytoskeleton - shape
  - actin, myosin, synaptopodin, actinin
- (3) junction domain – slit diaphragm
  - nephrin, Neph1, podocin, CD2AP = CD2-associated protein, ZO-1 = zona occludens-1 protein, densin, FAT = mammalian homolog of Drosophila fat protocadherin
- (4) apical domain – neg. charge
  - podocalyxin, podoplanin, podoendin, GLEPP-1 = glomerular epithelial protein-1, other proteins and receptors (NHERF-2 = Na+/H+ exchanger regulatory factor-2, …)

Johnstone DB and Holzman LB (2006) Clinical impact of research on the podocyte slit diaphragm. *Nat Clin Pract Neprol* **2:** 271–282 doi:10.1038/ncpneph0180

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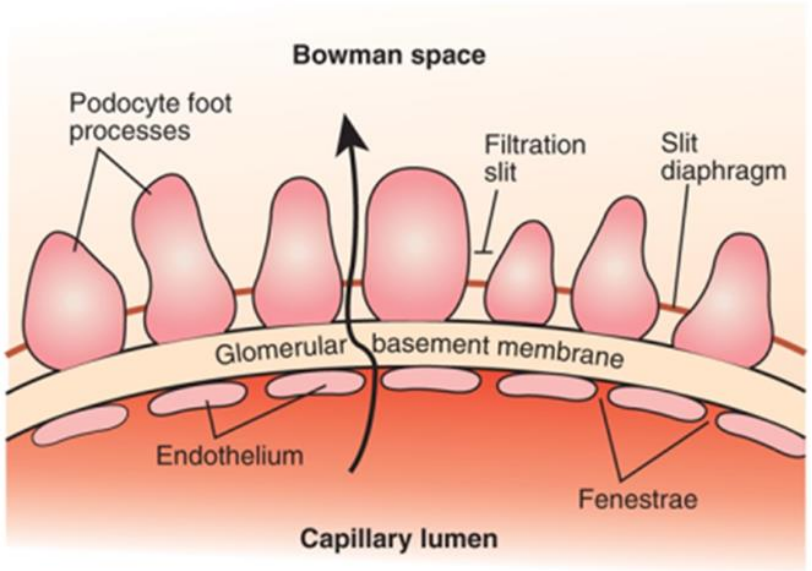
Proteins in urine (1) – physiologically

- physiological presence of proteins in urine
  - filtered in glomeruli but mostly reabsorbed and degraded in tubule
    - **albumin** (67kDa) - see further details
    - LMWP such as α1- a β2-microglobulins (33 resp. 12 kDa)
    - enzymes, apo-lipoproteins, binding proteins, peptide hormones, …
  - proteins produced by tubular cells
    - **Tamm-Horsfall protein** (= THP, uromodulin)
      - glycoprotein produced by cells of asc. arm of LH
      - unknown function (immunomodulation, protection against crystals or infection?)
      - main component of hyaline casts
    - uropontin
    - IgA immunoglobulin
    - nephrocalcin
- **physiological daily quantities:**
  - protein flow through renal arteries = 120 kg/d
  - protein filtered through glomerulus 1-2 g/d (<0.001%)
  - protein excreted by urine < 150 mg/d (<1% filtered)
  - composition 10-20% albumin, 60-80 % uromodulin
    - sensitivity of routine dg. methods ensures, that these proteins and albumin fragments are not detected
    - only clinically significant proteinuria (>0.5 g protein) gives positive results



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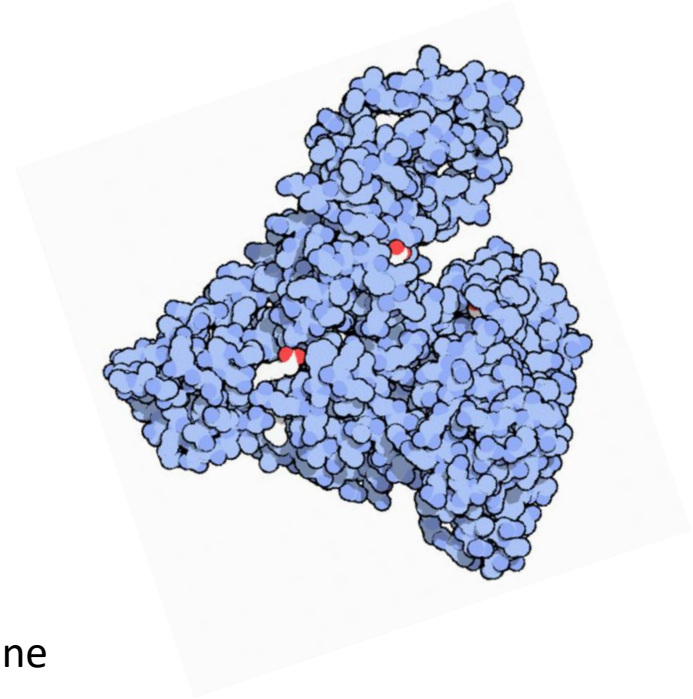
Human serum albumin (HSA) paradox

- HSA ~67kDa
- the molecule contains ~185 charged residues (Asp, Glu, Lys)
  - their surface distribution and overall charge is variable due to multiple functions of albumin:
    - transport (FFA, bilirubin, Ca, Mg, hormones, drugs, vitamins, …)
    - buffer / AB balance
    - enzyme activity (antioxidant, esterase)
    - oncotic pressure
    - AA pool
- handling of albumin by kidneys
  - (1) limited filtration
    - electrostatic repulsion of albumin was not always exp. proved
    - this concept was dominantly base on the absence of albumin in urine
      - but now using sensitive methods we can detect albumin and albumin residues in urine
  - (2) podocytes and tubular reabsorbtion
    - endocytosis = degradation ($\rightarrow$ AA and small fragments)
  - (3) tubular degradation



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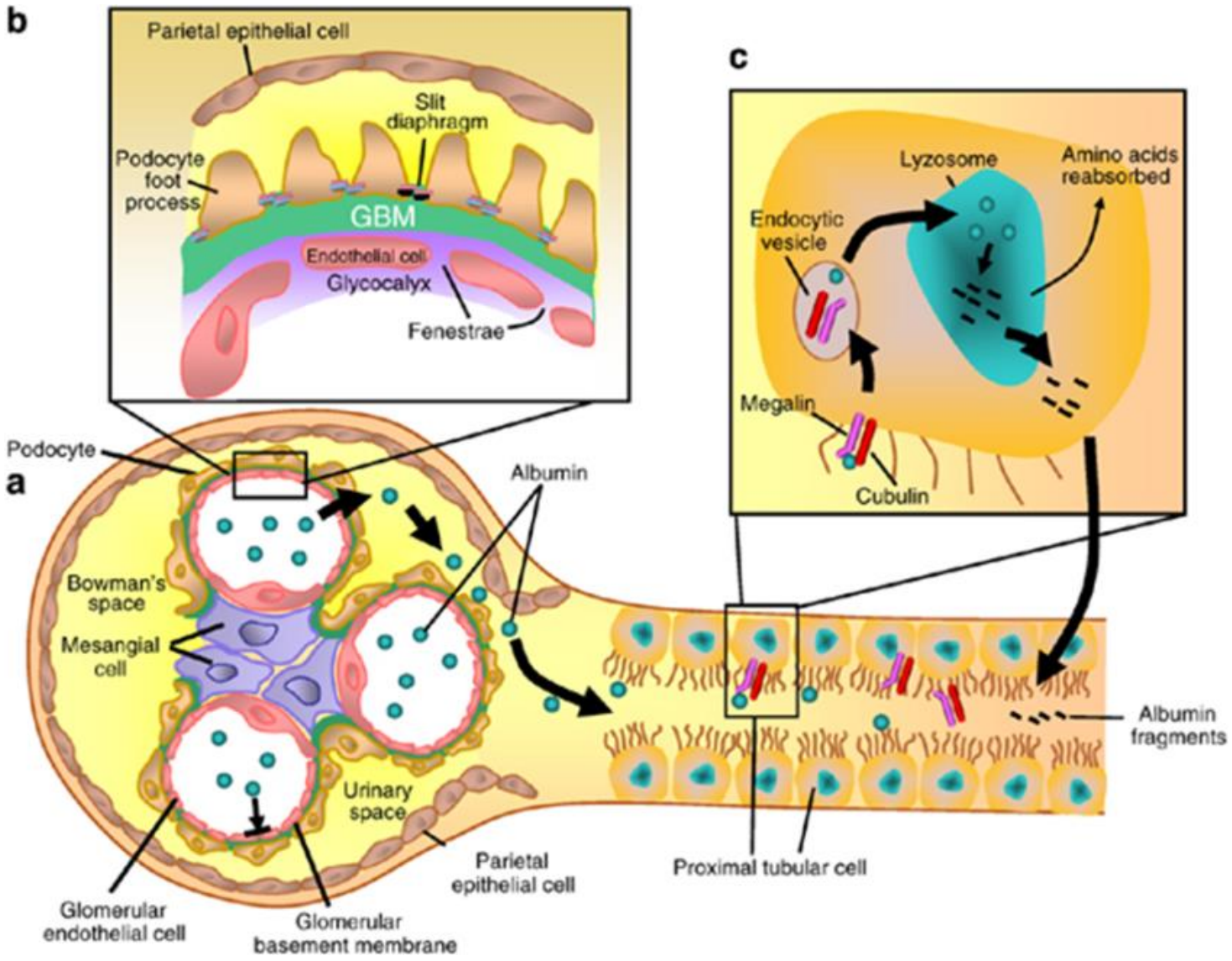
Normal renal handling of albumin



(a) Albumin (represented by green spheres) normally remains within the capillaries of the glomerular tuft, and does not escape into the urinary (Bowman's) space

(b) **Fenestrae** within specialized endothelial cells are covered by a negatively charged **glycocalyx**. Podocytes attach to the outermost aspect of the GBM by foot processes, between which are proteins comprising the size barrier **slit diaphragm**.

(c) The albumin that is physiologically filtered at the level of glomerulus into the urinary space is taken up by the megalin/cubulin receptor lining the brush border of **proximal tubular cells**. Albumin is internalized by vesicles, and upon lysozyme action, the resultant fragments are either reabsorbed or secreted back into the tubular lumen as albumin fragments

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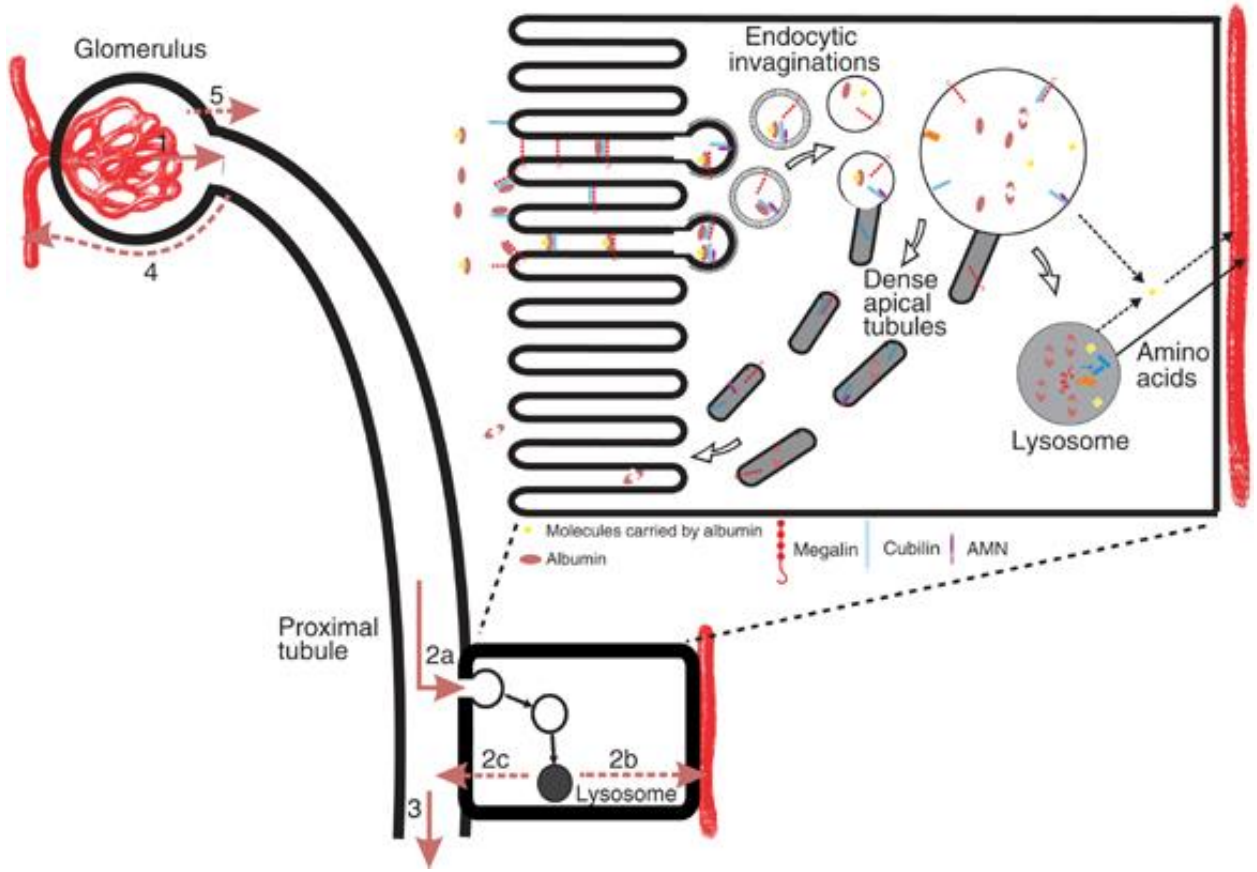
Mechanism of proximal tubular re-uptake of albumin

- receptor-mediated endocytosis
  - — high capacity/low affinity
    - the same mechanism is used elsewhere (e.g. absorption of complex of vit. B12/intrinsic factor in ileum)
  - — endocytic complex
    - megalin/cubilin – binding of albumin
      - — Imerslund-Graesbeck disease (mutation in cubilin gene) - **proteinuria**
      - — Fanconi syndrome (mutation in megalin gene) - **proteinuria**
    - NHE3 – necessary for acidification of endosome/lysosome
      - — NHE3 KO animals - **proteinuria**
    - ClC5 – interaction with cytoskeleton
      - — Dent's disease (mutation in ClC5 gene) - **proteinuria**
    - H-ATPase – necessary for acidification of endosome/lysosome



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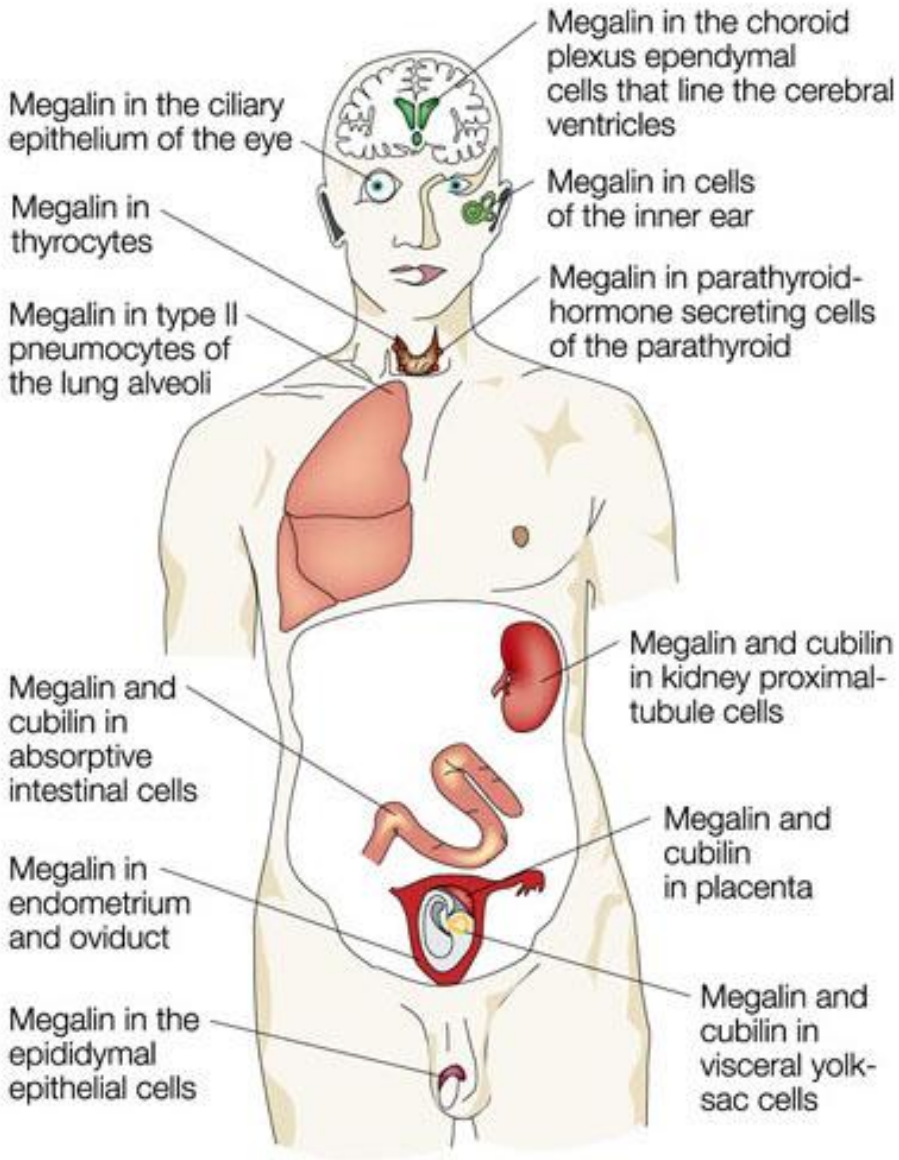
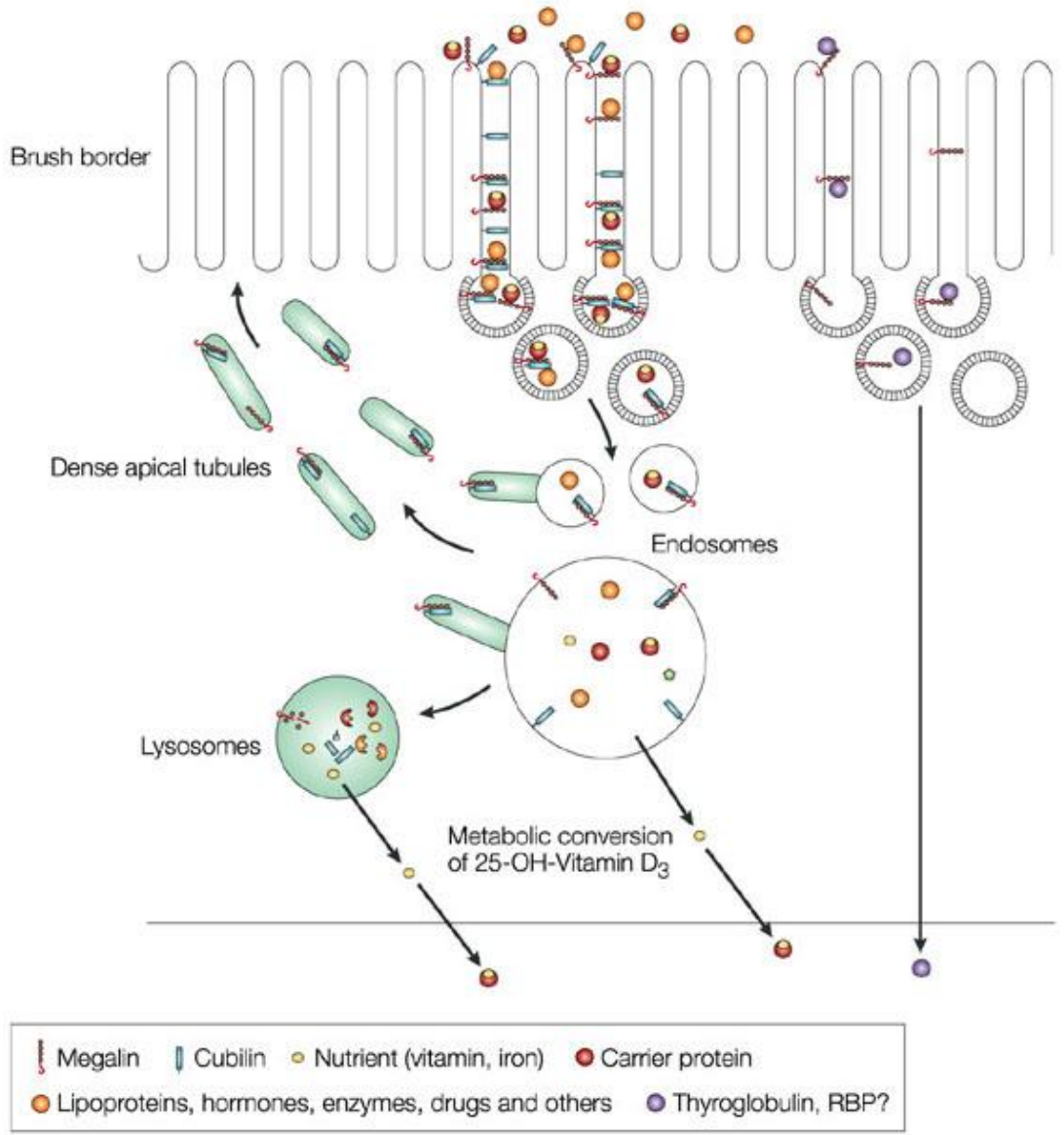
Megalin/cubilin





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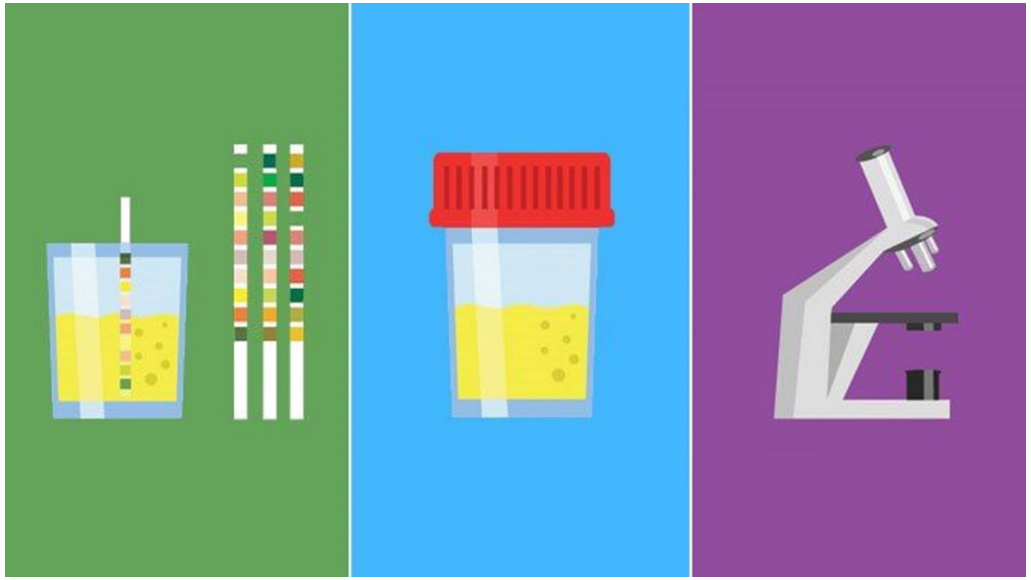
Proteins in urine (2) - proteinuria

- **(a) functional (benign) proteinuria**
  - appears occasionally as a result of altered glomerular hemodynamics (↑ hydrostat. pressure in capillaries), glomerular membrane intact
    - orthostatic, heavy exercise, fever, …
    - non-selective proteinuria
    - single occurrence of proteinuria does not constitute a pathological finding, test has to be repeated!!
- **(b) overflow proteinuria**
  - pathological increase of "small" proteins capable of passing to glomerular filtrate
    - e.g. haemolysis (α-β-dimers globin), rhabdomyolysis (myoglobin), paraproteins (light chains of Ig κ and λ (so called Bence-Jones protein)
- **(c) glomerular proteinuria** (often >1g/day)
  - **selective** - albuminuria, larger proteins retained, usually due to the loss of negative charge of glycocalyx, GBM or slit diaphragm or podocytes effacement
    - e.g. MCD
  - **non-selective without haematuria** – usually due to the podocytes loss or podocytes detachment
    - e.g. FSGS, membranous GN, diabetic nephropathy, amyloidosis
  - **non-selective with haematuria** – gross structural damage incl. rupture of GBM
    - e.g. IgA nephropathy, membranoproliferative GN, ANCA, …
- **(d) tubular proteinuria** (often <1g/day)
  - decreased reabsorption of small plasma proteins (mainly albumin and β2-microglobulin in urine)
    - congenital (Dent's disease (ClC5), Imerslund-Graesbeck disease (cubilin), Fanconi syndrome (megalin) etc.)
    - acquired (e.g. tubulointerstitial nephritis, hypertension)
    - microalbuminuria in early-stage of diabetic nephropathy



- test for urinary protein (= **urine analysis**)
  - **dip stick** – what is in the urine but not how much
  - **microscopy** – how the cells in the urine look like
  - **24hr urine collection** – quantitative
    - normal <150 mg/d
    - selective proteinuria 150-500 mg/d (microalbuminuria)
    - moderate proteinuria 1-3.5 g/d (nephritic syndrome)
    - severe proteinuria >3.5 g/d (nephrotic syndrome)
  - **calculated protein/creatinine ratio** - from spot urine sample

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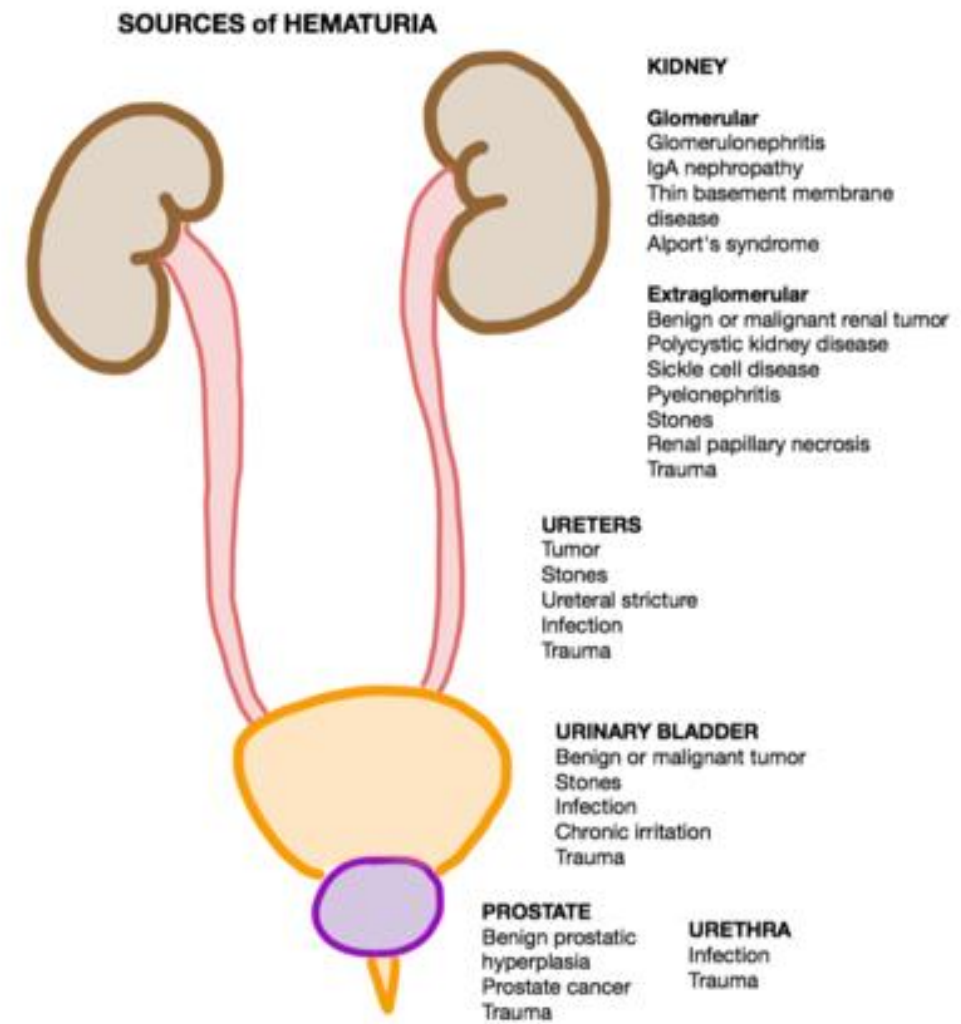
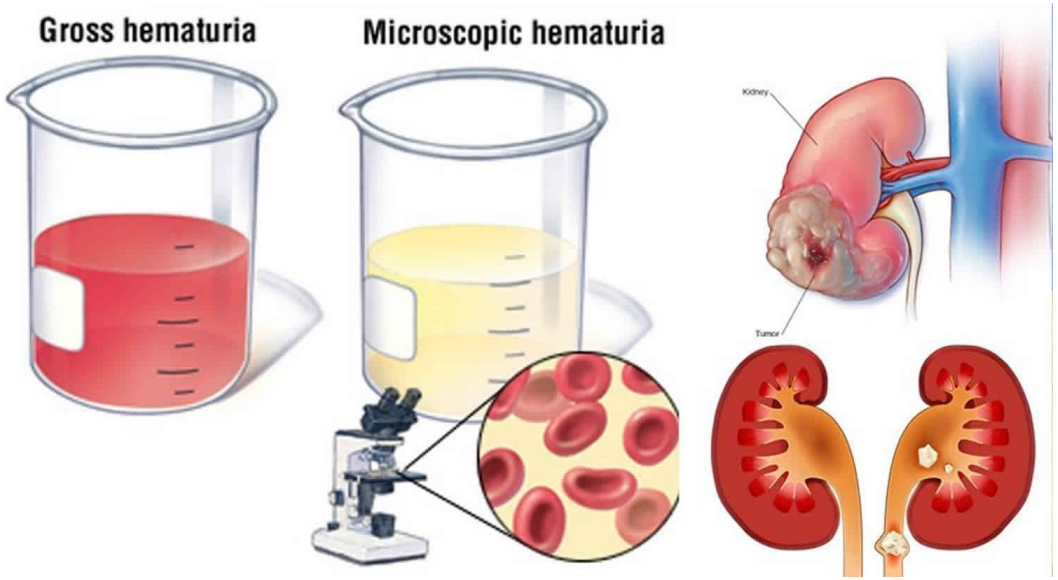
Isolated symptoms - haematuria





- microscopy
  - dysmorphic (+ RBC casts)
  - non-dysmorphic (no urine casts)

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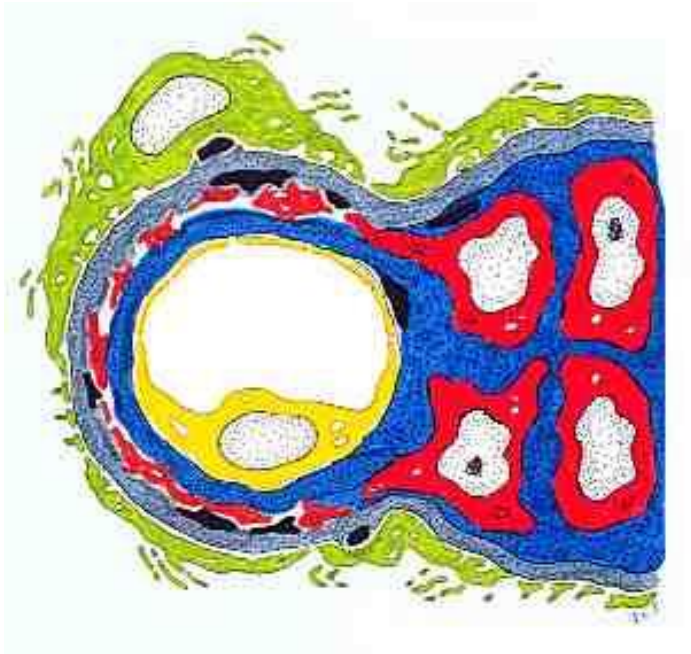

GLOMERULAR DISEASES (GLOMERULOPATHIES) – NEPHROTIC VS. NEPHRITIC SYNDROMES

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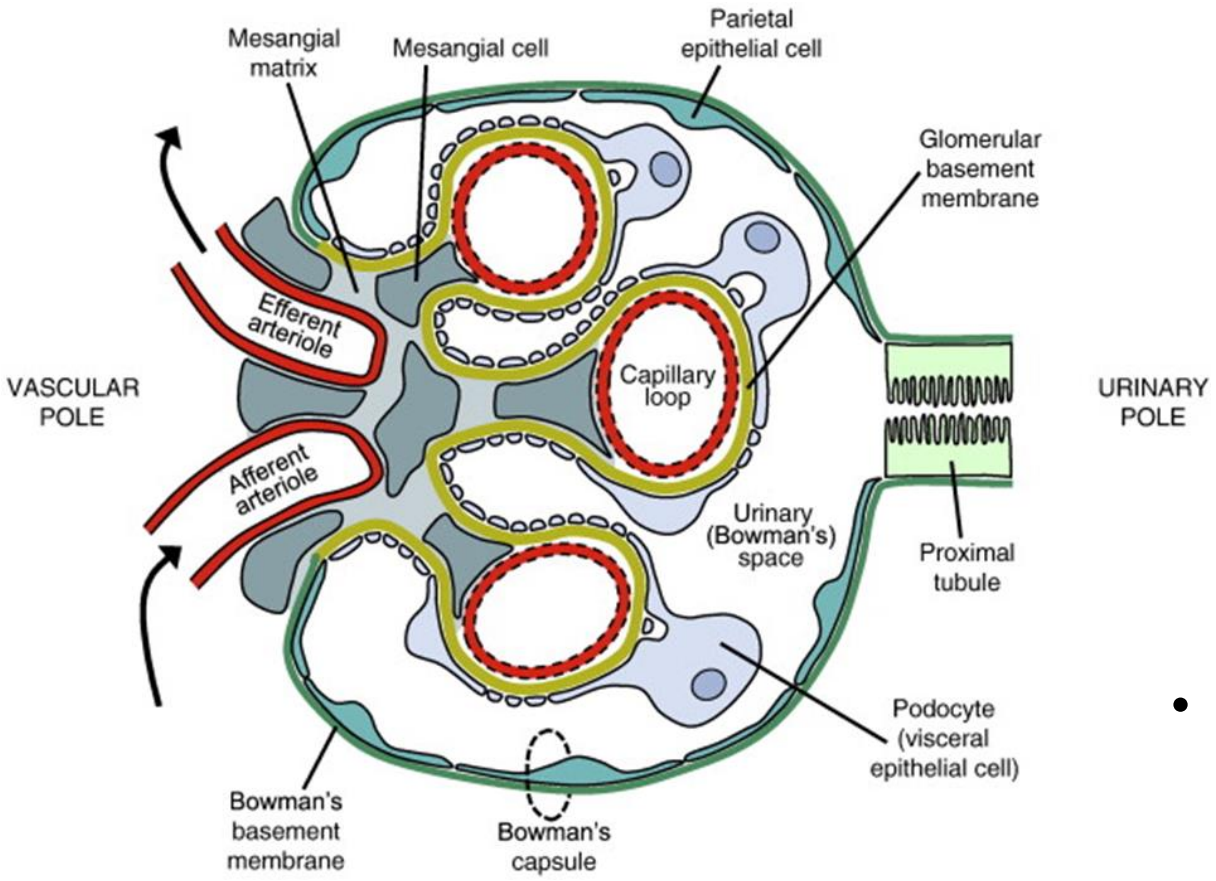
The players: cells Involved in glomerular diseases



- glomerulus has only limited spectrum of reactions to damage
  - **inflammation**
    - destruction or functional damage of some glomerular cells
      - e.g. podocytes
    - infiltration by other cells
      - e.g. neutrophils or macrophages
    - proteolytic destruction of filtration membrane (mainly by complement)
      - **hematuria and/or proteinuria**
  - **proliferation**
    - some glomerular cells – such as mesangial or parietal epithelial cells – can proliferate
    - others – such as podocytes – cannot (they only die)
  - **deposition of ECM**
    - fibrosis
    - scarring
    - sclerosis
      - **decrease of GFR**
- histologic appearance of glomerular disease depends on which particular part of glomerulus is predominantly affected
  - proliferative GN – mesangium
  - membranous GN – GBM thickening
  - sclerotizing GN – capillaries and synechia
  - membranoproliferative GN – parietal epithelial cells

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Classification of glomerular diseases is a nightmare

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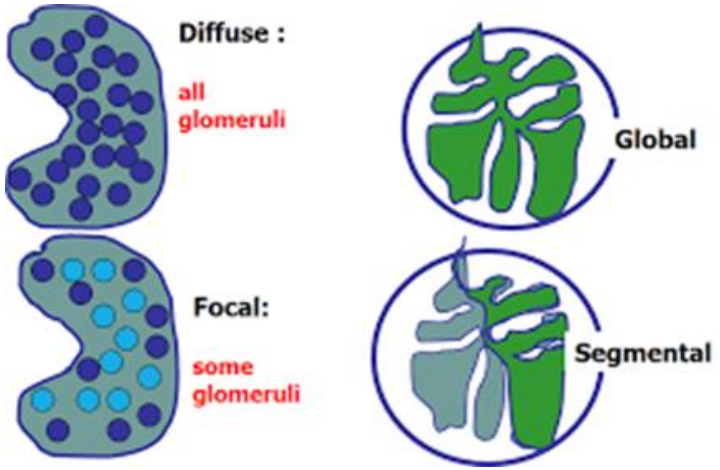
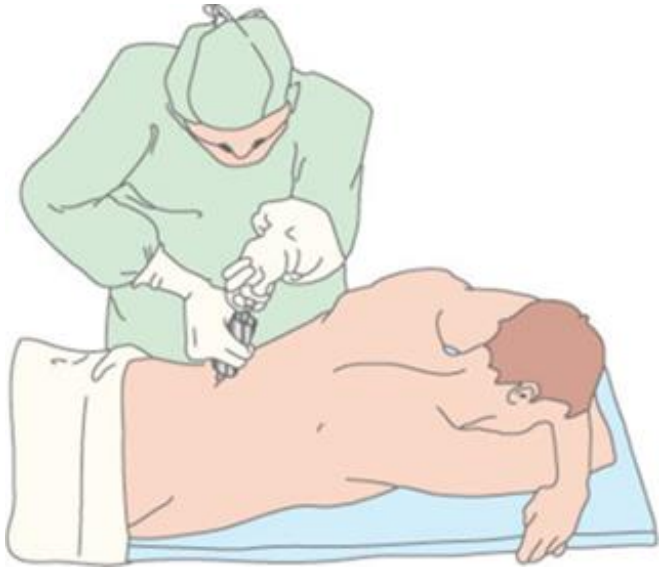
3D-classification of glomerular diseases

- **(1) clinically** according to symptoms and time course
  - **nephritic** syndrome × **nephrotic** syndrome × **isolated symptoms**
  - acute × chronic
- **(2) histo-pathologically** based on **kidney biopsy** (→ light microscopy, immunofluorescence, electron microscopy)
  - histologic classification of GN dominantly focuses on the number of affected glomeruli and predominantly affected cell type
    - **focal** (only some glomeruli affected) × **diffuse** (mote than 80% of glomeruli)
    - **segmental** (only certain structures) × **global** (all cell types affected)
  - and also degree of cellularity
    - non-proliferative × proliferative
- **(3) by cause (etiopathogenic)** if known! In fact, the exact cause of the majority of glomerular disease is unknown (idiopathic), therefore etiologic classification problematic
  - primary (often kidney-restricted)
    - IgA nephropathy
    - genetic
  - secondary (renal manifestation of a systemic disease)
    - systemic autoimmune diseases
    - post-infectious
    - vascular (vasculitis)
    - metabolic (diabetes, amyloidosis)
    - tumors (multiple myeloma)





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(1) Clinical manifestation of glomerular diseases - distinction between nephrotic vs. nephritic syndromes

Nephrotic syndrome

- massive loss of protein without concomitant finding of blood cells in urine
  - **glomerular proteinuria** typically >3.5 g/day and consequently
    - frothy urine
    - **hypoalbuminemia** / hyporoteinemia
      - oncotic pressure decrease → periorbital and pretibial pitting **edema**
        - low effective circulating volume leads to compensatory activation of RAAS
      - loss of Ig → susceptibility to infections
      - hypocalcemia, hypovitaminoses, …
  - protein loss becomes compensated by liver, the non-selectivity of this globally increased protein synthesis leads to
    - **dyslipidemia** (+ lipiduria)
      - ↑ lipoproteins, ↓ plasma LPL
    - **hypercoagulation** (risk of thrombosis – incl. renal vein)
      - ↑ clotting factors, ↓ AT III, ↑ hematocrit (heamoconcentration)
- etiopathogenetically a typical consequence of **podocytopathies** or **change of a negative chargé of glomerular structures**

Nephritic syndrome

- glomerular **haematuria**
  - macroscopic (visible by naked eye)
  - microscopic (urine analysis) - dysmorphic RBC - acanthocytes
  - plus **sediment** (RBC casts) plus sterile **pyuria** (leukocytes)
- **proteinuria** typically in a non- nephrotic range
  - if cells can go through so as can proteins
- gross damage to filtration barrier (involving endothelium and GBM) = **oliguria** and **AKI** (= azotemia)
- inflammation causes blockade of filtration and **hypertension** due to hypervolemia
- etiopathogenetically a typical consequence of **inflammation** leading to damage of **glomerular filtration barrier**

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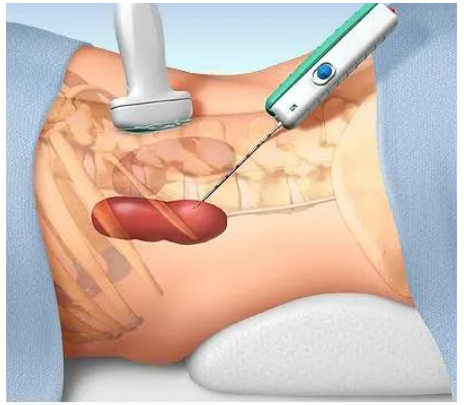
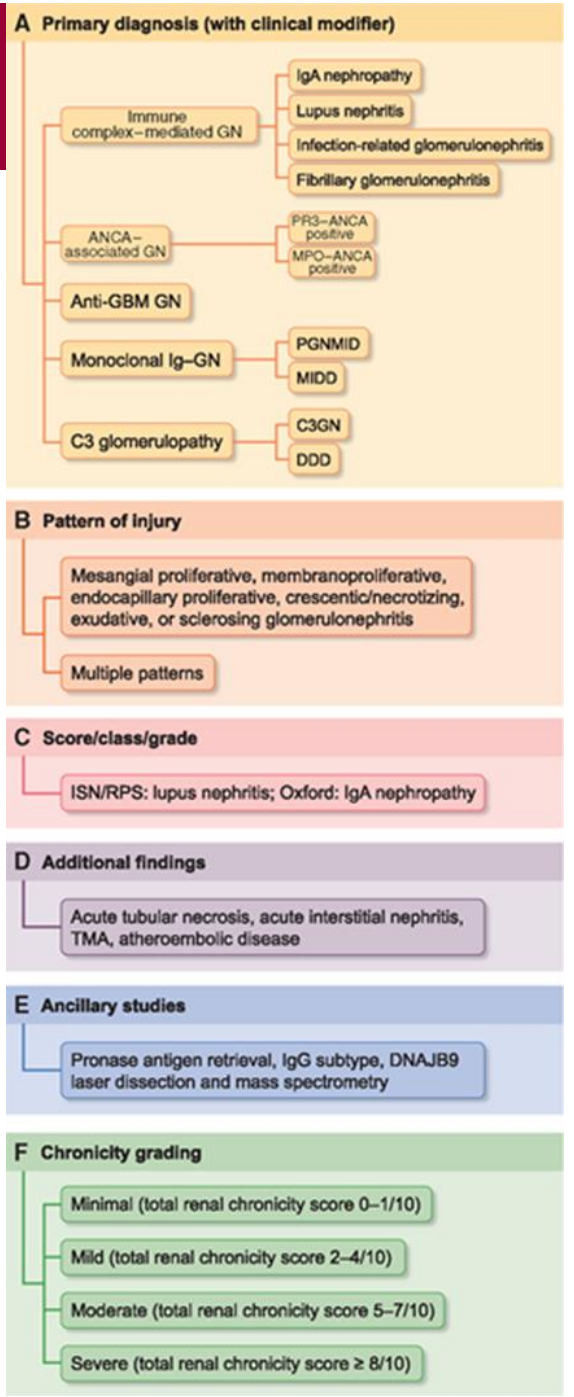
(2) What can kidney biopsy tell us in GN

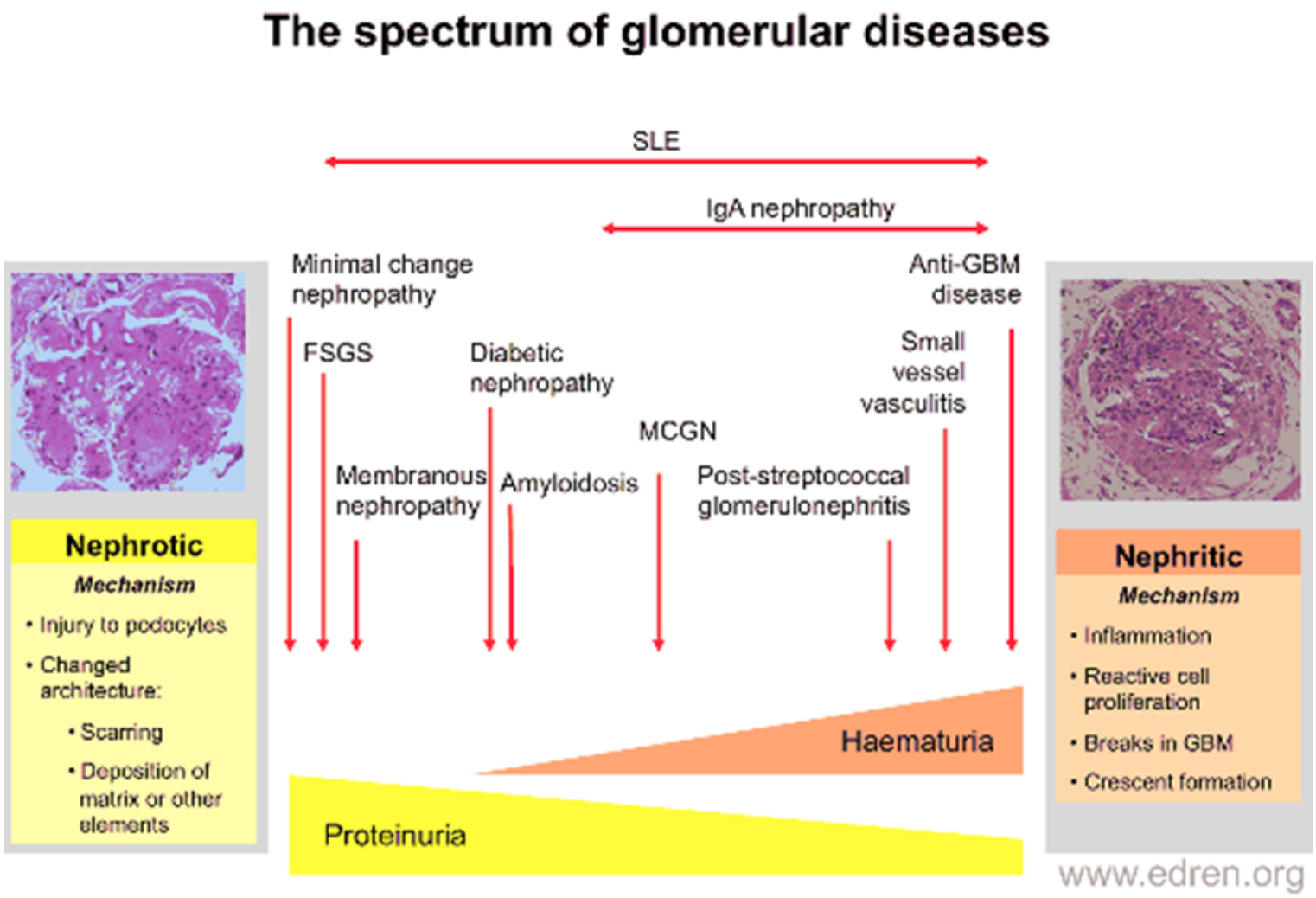
- A kidney biopsy is done to
  - **(1) determine the aetiology of the glomerulonephritis (GN)** - morphologically GN is classified into five groups (within each group there are specific disease entities):
    - **immune complex-mediated GN**
      - immune complex–mediated GN is most heterogeneous and contains many specific diseases such as lupus nephritis, IgA nephropathy, infection-related GN and fibrillary GN
    - **anti-neutrophil cytoplasmic antibody (ANCA)-associated GN**
    - **anti-glomerular basement membrane (GBM) GN**
      - Goodpasture syndrome
    - **C3 glomerulopathy**
      - ANCA GN, anti-GBM GN and C3 glomerulopathy are specific diseases in themselves
    - **monoclonal immunoglobulin-mediated GN**
      - includes proliferative GN with monoclonal Ig deposits and monoclonal Ig deposition disease (e.g. paraprotein)
  - **(2) severity of the lesion - revealed by the pattern of injury**
    - such as crescentic, necrotizing, diffuse proliferative, exudative, membranoproliferative, mesangial proliferative or a sclerotising GN
  - (3) identify whether other lesions, related to or not related to the GN, are present on the kidney biopsy
  - (4) and finally to ascertain the **extent of chronicity of the GN**
    - by evaluating the extent of glomerulosclerosis, tubular atrophy and interstitial fibrosis and vascular sclerosis present on the biopsy
- Kidney biopsy specimen is processed for
  - light microscopy (LM)
    - glomeruli, tubules and interstitium, vessels
  - immunofluorescence (IF)
    - type of positivity (linear, granular, negative)
    - location in glomerulus
    - type of Ig and complement
  - electron microscopy (EM)





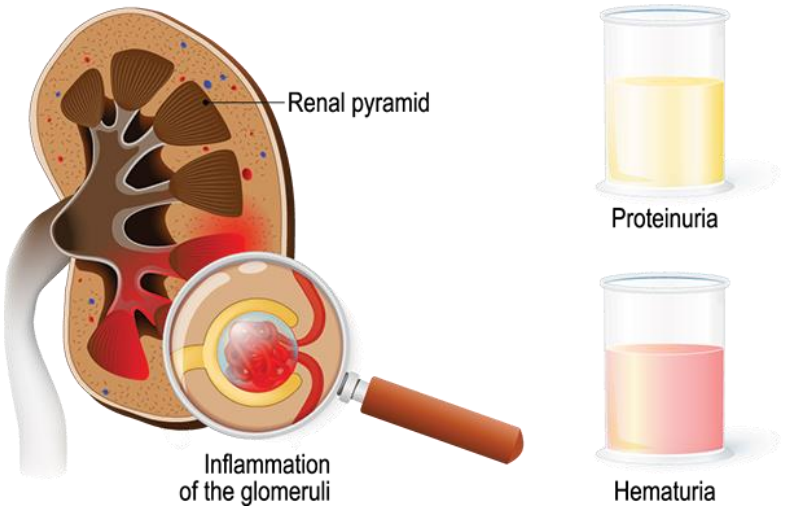
Overview of standardized classification and reporting of GN

Clinical syndrome vs. histopathology????



(3) Etiopathogenic types of GN

- primary
  - anti GBM antibody (Goodpasture's syndrome) + hemoptysis
    - typically manifests by rapidly progressing crescentic GN
    - IF: **linear deposits** of IgG and C3 **in the GBM**
    - the antigen common to GBM and alveoli is the NC1 domain of a peptide (α3) within the type IV collagen and the peroxidase peroxidasin
    - the trigger for the formation of these antibodies is unclear
    - treatment is plasmapheresis to remove the IgG
  - IgA nephropathy (Berger's disease)
    - deposits in mesangium and glomerular capillaries
    - post-infection but very soon after e.g. respiratory infection (1-3 days)
  - **C3 glomerulonephritis**
    - membranoproliferative GN based on kidney biopsy
    - immune complexes (activation of complement classical pathway) or complement-mediated (alternative pathway) injury
- secondary
  - ANCA (anti-neutrophil cytoplasmic antibody) associated vasculitis/GN
    - isolated or sign of Wegener's granulomatosis
  - post-SC GN
    - post-infection but delayed after e.g. respiratory infection (1 - 3 weeks)
  - lupus nephritis
  - hepatitis C
- **immunological assessment is an extremely important clue**



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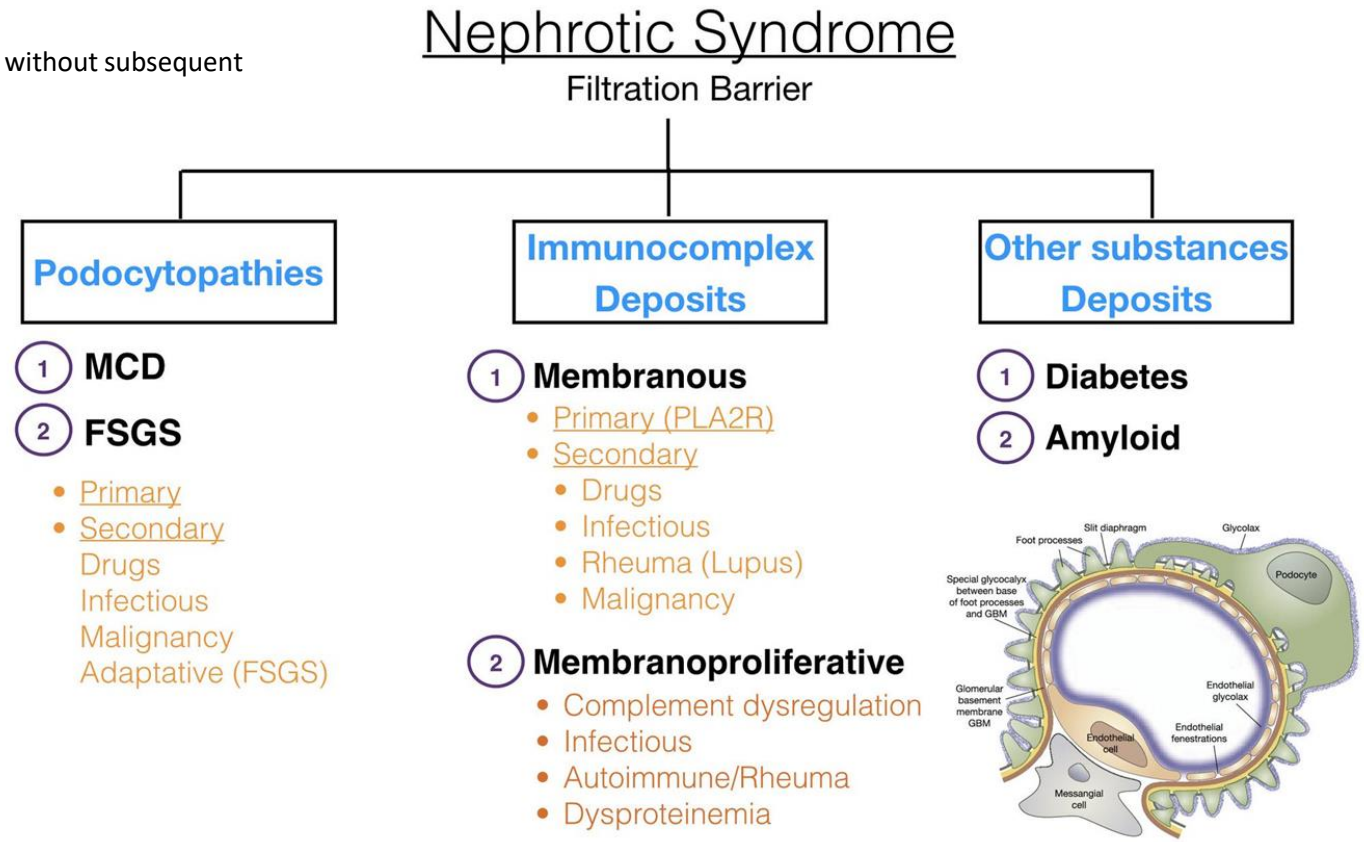
(1) Glomerular diseases manifesting as nephrotic sy

- damage to filtration barrier involving
  - mainly podocytes
  - some immune mechanisms
    - immune complex deposition and concomitant complement activation but without subsequent attraction of other immune cells
  - metabolic or hemodynamic factors
  - genetic abnormalities
- etiopathogenesis
  - primary
    - MCD (minimal change disease)
    - membranous GN
    - FSGS (focal segmental glomerular sclerosis)
  - secondary
    - SLE
    - membranoproliferative GN (type I, II and II)
    - diabetic nephropathy
    - amyloidosis
    - infections (hepatitis B, C, HIV)
  - hereditary = congenital
    - disorders of GBM
      - Alport syndrome (mutation in collagen gene)
        - however proteinuria can be in non-nephrotic range and hematuria is always present
      - Fabry disease
      - disorders of podocytes (slit diaphragm)
        - mutations in genes for nephrin, podocin, …



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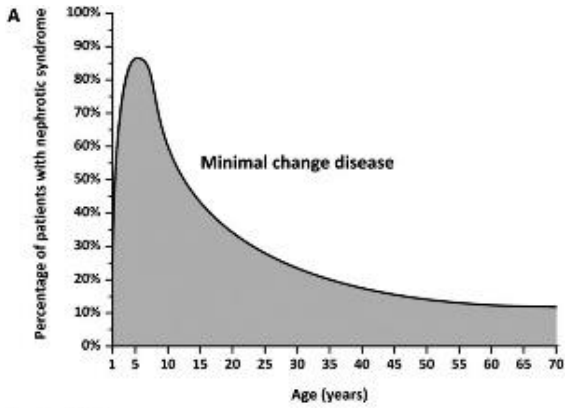
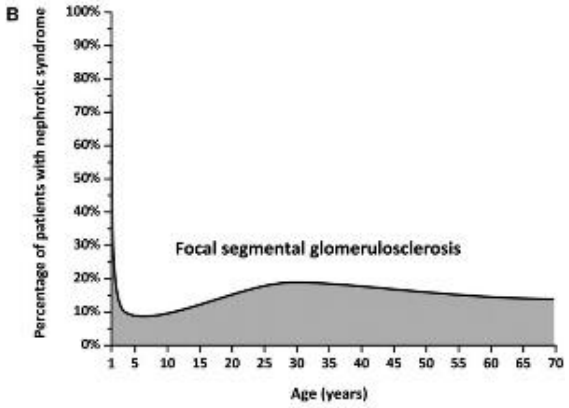
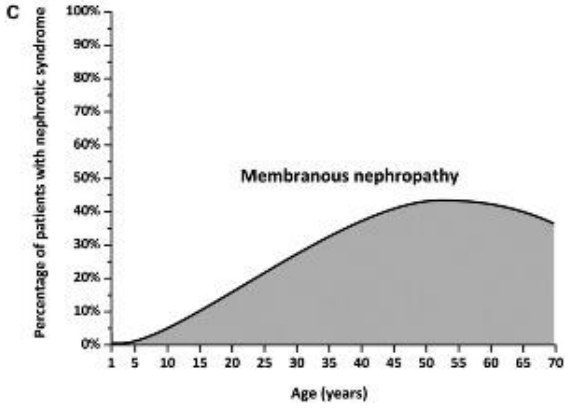
Primary types of nephrotic syndrome







(A) MCD (minimal change disease)

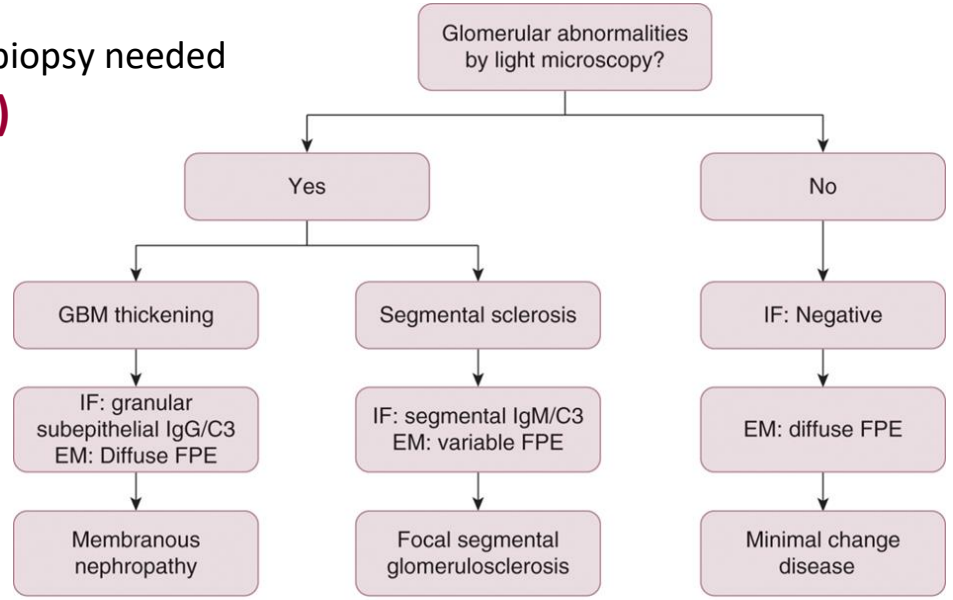
— typically kids
— selective proteinuria (albuminuria)
— kidney biopsy
  - LM: no or minimal
  - IF: typically negative or low-intensity mesangial IgM
  - EM: podocytes foot process effacement
— responsive to steroids (remission) – in that case no biopsy needed

(B) FSGS (focal segmental glomerular sclerosis)

— typically adults
— non-selective proteinuria
— kidney biopsy
  - LM: focal segmental glomerular sclerosis
  - IF: typically negative
  - EM: GBM thickening non-responsive to steroids

(C) membranous GN

— typically adults
— non-selective proteinuria
— kidney biopsy
  - LM: mesangial expansion and capillary wall thickening
  - IF: sub-epithelial deposits of IgG and C3 complement protein
  - EM: GBM thickening and „spikes“ (deposits of IgG and C3)



Abbreviations: **IF,** immunofluorescence microscopy; **EM,** electron microscopy; **FPE,** foot process effacement

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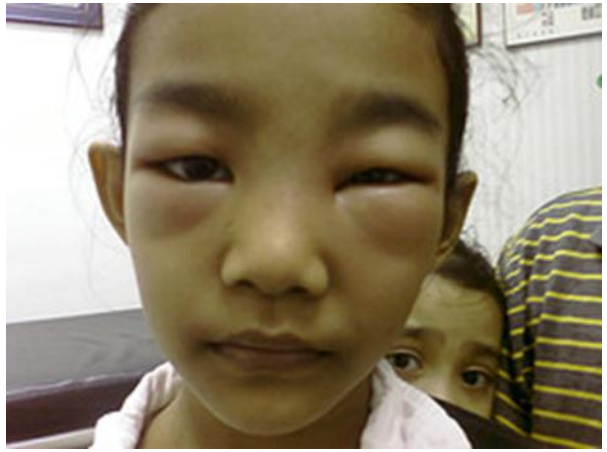
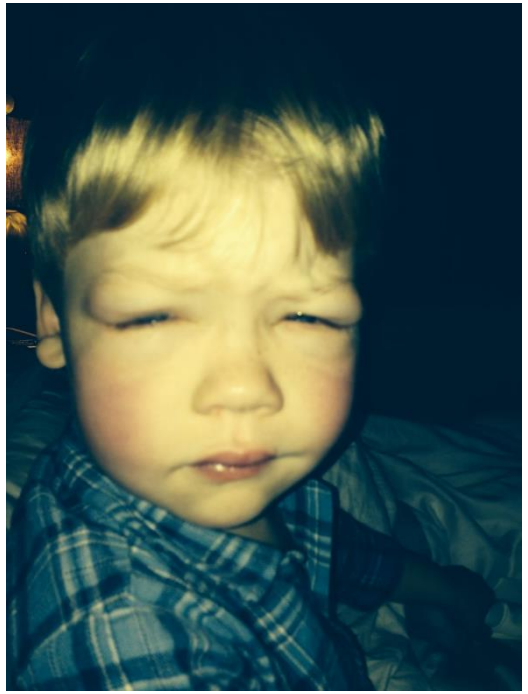
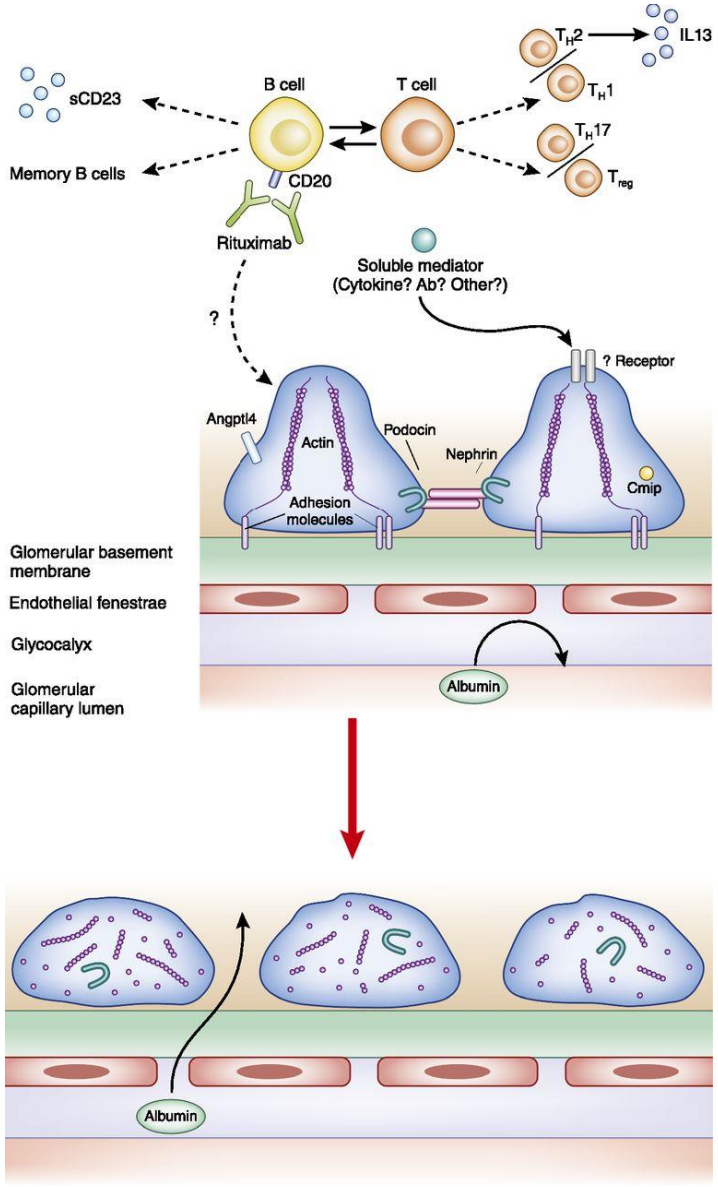
GN example (1a): Minimal change disease (lipoid nephrosis)







- 10-50/100 000 children
- various time course depending to steroid responsiveness but rarely progresses to renal failure
- pathogenesis of primary MCD unclear but indicates imbalance in T cell subpopulations during the active phase of the disease with a prevalence of circulating CD81T suppressor cells and type 2T helper cell (Th2; IL4, IL5, IL9, IL10, and IL13) cytokine profile in patients
- In the last few years, clinical evidence of the effectiveness of B cell depletion via rituximab, an anti-CD20 monoclonal antibody, in different forms of nephrotic syndrome has suggested a role for B cells as drivers of disease

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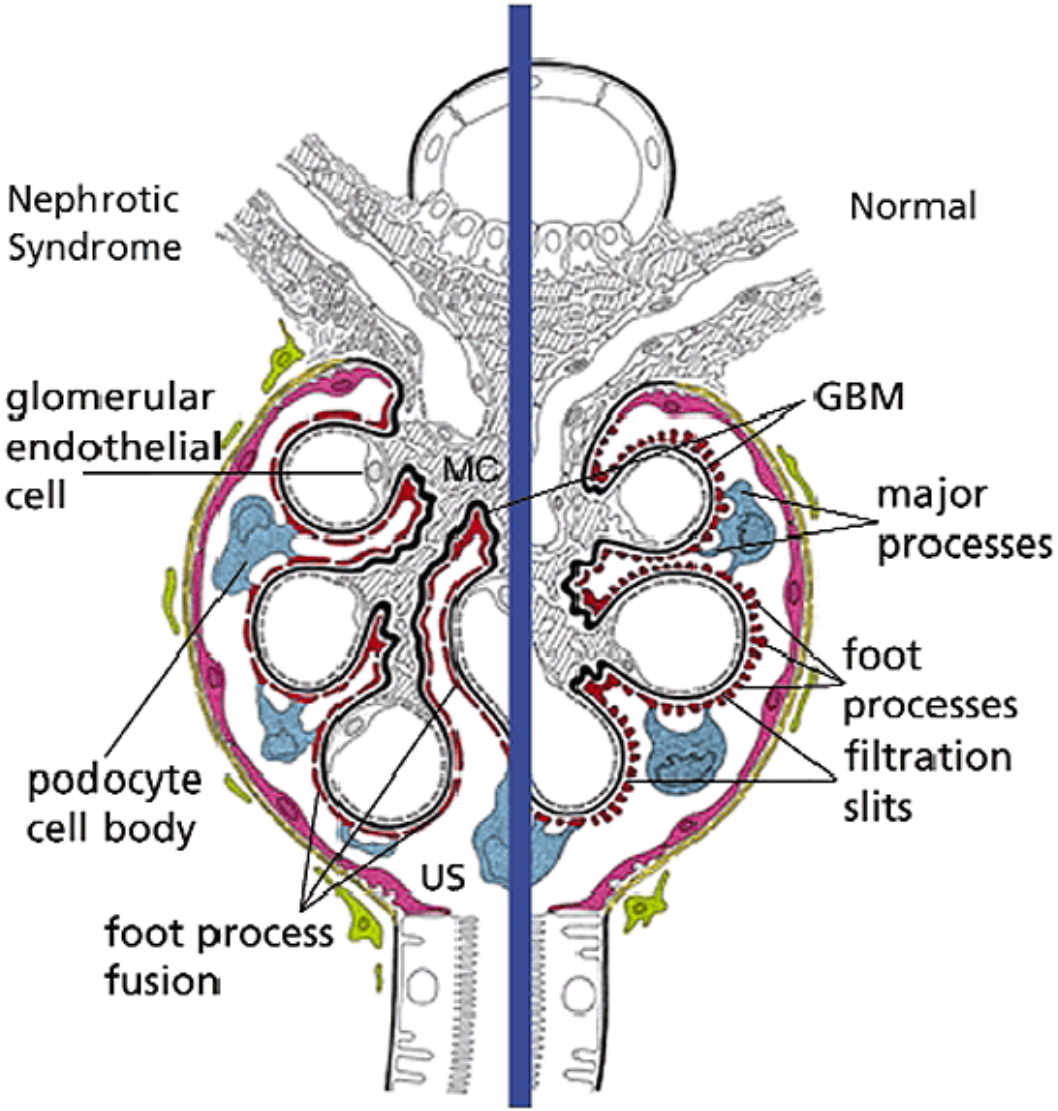
Podocytes - foot-process effacement



- = "smoothening" of podocytes - universal sign of damage to podocytes
  - secondary forms of MCD
- correlate with degree of proteinuria ("chicken or egg"?)
- variable etiology of podocyte damage
  - ROS (→ DNA damage, apoptosis, peroxidation of lipids)
  - AT II (→ apoptosis, hypertrophy, ↑ TGF-b, ↓ nephrin)
  - MMPs (→ ↓ GBM, ↓ nephrin-Neph complex)
  - mechanical stress (→ apoptosis, hypertrophy)
  - growth factors (→ ↑ MMPs, GBM, …)
  - hyperglycemia (→ ↓ neg. charged apical protein)
- **loss of podocytes ⇒ proteinuria ⇒ glomerulosclerosis**
  - synechia between naked GBM and parietal epithelium of Bowmann capsule → sclerotisation (FSGS)
  - **podocytes do not regenerate**

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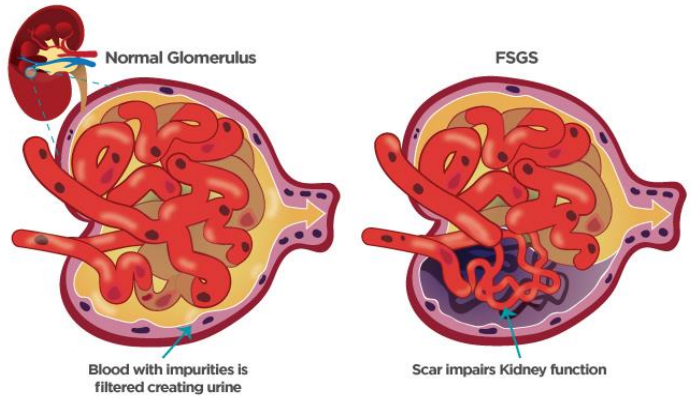
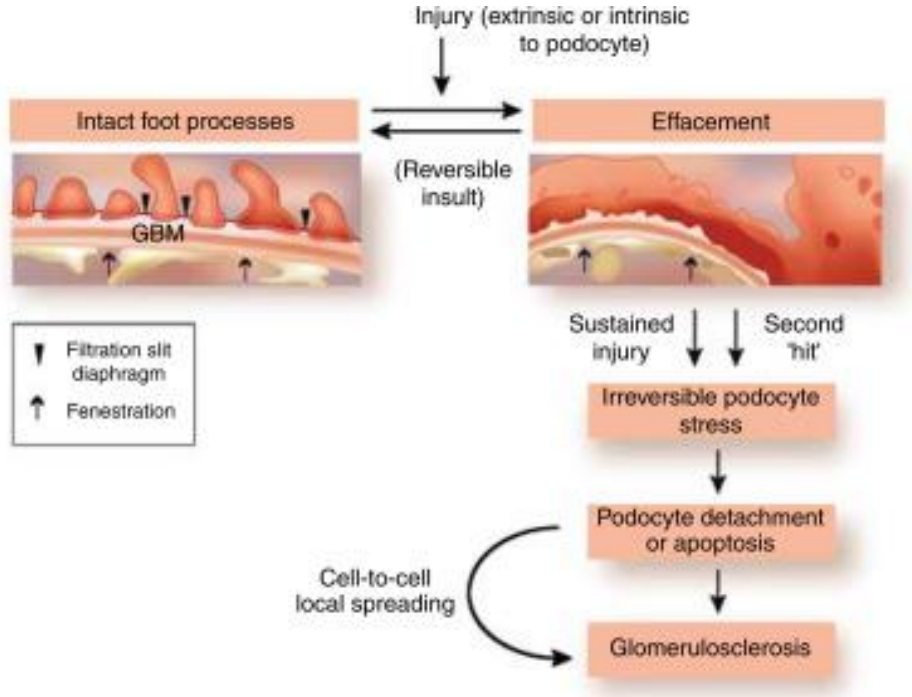
GN example (1b): Focal Segmental Glomerulosclerosis

- podocyte damage (effacement) and loss is a dominant feature of FSGS pathogenesis
  - does circulating factor affecting podocyte shape and function exist?
- exposure of GBM simulates the synechia with parietal epithelia
  - scarring and sclerotizing of capillaries
- idiopathic forms has a strong genetic component
  - see further
- secondary forms are common though
  - vesicoureteral reflex, obesity, medication, infections, HIV, heroin, glomerular hyperfiltration, …
- some forms are sensitive to corticosteroids, others not
  - successful treatment of steroid-dependant FSGS with rituximab suggests a potential role for B lymphocytes





Difference Between Primary and Secondary FSGS

Primary FSGS	Secondary FSGS
Usually abrupt onset of nephrotic syndrome	Less proteinuria; slow onset
Normal-sized glomeruli, less parenchymal atrophy	Glomerular hypertrophy in unaffected glomeruli Focal interstitial fibrosis/ tubular atrophy and global glomerulosclerosis
Diffuse global podocyte foot process effacement	Less prominent and segmental podocyte foot process
No IC, TRI, or other causes	Evidence of a secondary cause (IC, crescents, TRI, DM, Fabry, Alport, HTN) effacement

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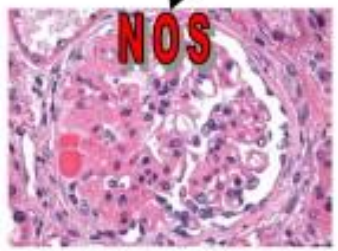
Histologic Variants of Focal Segmental Glomerulosclerosis (FSGS).



Defining Features: The usual generic form of FSGS. FSGS Foot-process effacement is variable.

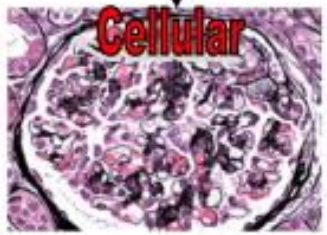
Associations: Primary or secondary Cross-sectional studies suggest this is the most common subtype.

Clinical Features: May present with the nephrotic syndrome or sub nephrotic proteinuria.

Defining Features: Perihilar hyalinosis and sclerosis involve the most of the glomeruli with segmental lesions. Perihilar lesions are located at the glomerular vascular pole. In adaptive FSGS, glomerular hypertrophy occurs (glomerulomegaly). Foot-process effacement is relatively mild

Associations: Common in adaptive FSGS associated with obesity, elevated body mass, reflux nephropathy, hypertensive nephrosclerosis, sickle cell anemia, and renal agenesis.

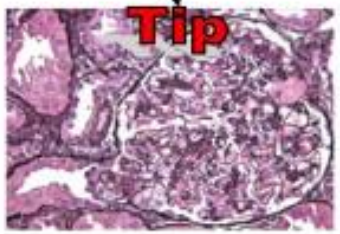
Clinical Features: In adaptive FSGS, patients are more likely to present with subnephrotic proteinuria and normal serum albumin levels.



Defining Features: Expansile segmental lesion with endocapillary hypercellularity, often include foam cells and infiltrating leukocytes, with variable glomerular epithelialcell hyperplasia.

Associations:U sually primary, but also seen in a variety of secondary forms. Found in earlystage in the evolution of sclerotic lesions.

Clinical Features: Usually presents with the nephrotic syndrome.



Defining Features: Segmental lesion involving the tubular pole, with either adhesion to tubular outlet or confluence of podocytes and tubular epithelial cells.

Associations: Usually primary. Probably mediated by physical stresses on the paratubular segment owing to the convergence of protein-rich filtrate on the tubular pole, causing shear stress and possible prolapse

Clinical Features: Usually presents with abrupt onset of the nephrotic syndrome. More common in white race

Defining Features: Implosive glomerular-tuft collapse with hypertrophy and hyperplasia of the overlying visceral epithelial cells. Hyperplastic glomerular epithelial cells may fill the urinary space, resembling crescents.

Associations: Primary or secondary to Viruses: HIV-1, parvovirus 19, SV40, EBV, CMV, hemophagocytic syndrome

Clinical Features: Most aggressive variant of primary FSGS with black race with severe nephrotic syndrome

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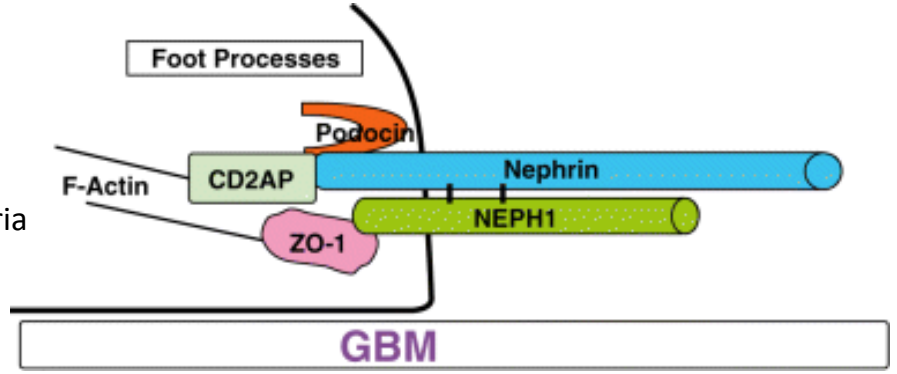
Importance of podocyte slit diaphragm – lessons from genetics

- (1) study of familiar forms of nephrotic syndrome (**leading to FSGS**) led to the identification of majority proteins of slit diaphragm of podocytes
  - nephrin (Finnish-type congenital nephrotic syndrome, NPHS1)
    - congenital. defect of development of pedicles and slit diaphragm
    - massive and potentially lethal proteinuria starting during the fetal period
    - parenteral nutrition and peritoneal dialysis necessary till the transplantation
      - in comparison Alport syndrome (mutations in collagen IV) leads to only moderate proteinuria
      - deletion of heparansulphate in mouse models do not lead to any proteinuria
  - podocin (familiar steroid-resistant nephrotic syndrome, NPHS2)
    - early postnatal proteinuria
  - other syndromes with significant proteinuria (CD2AP, NEPH1, FAT, TRPC6, …)
- (2) it is possible to induce nephrotic syndrome experimentally by polyclonal antibodies against slit diaphragm or by monoclonal ab. against nephrin, podocin, …
- (3) clinical importance
  - glomerulopathies are dominant cause of proteinuria
    - current classification of glomerulopathies based histopathologic appearance (= non-specific)
  - future (?) molecular-biologic classification
    - diagnostics, prognosis, treatment (steroids y/n), benefit of transplantation (family member/donor), …



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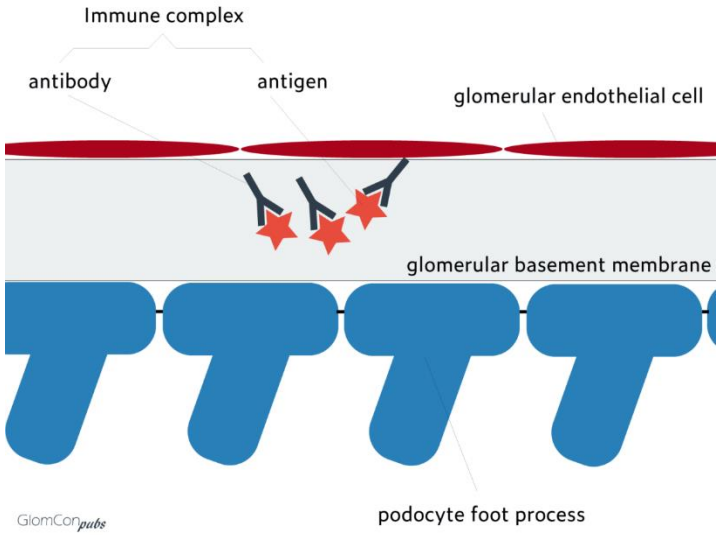
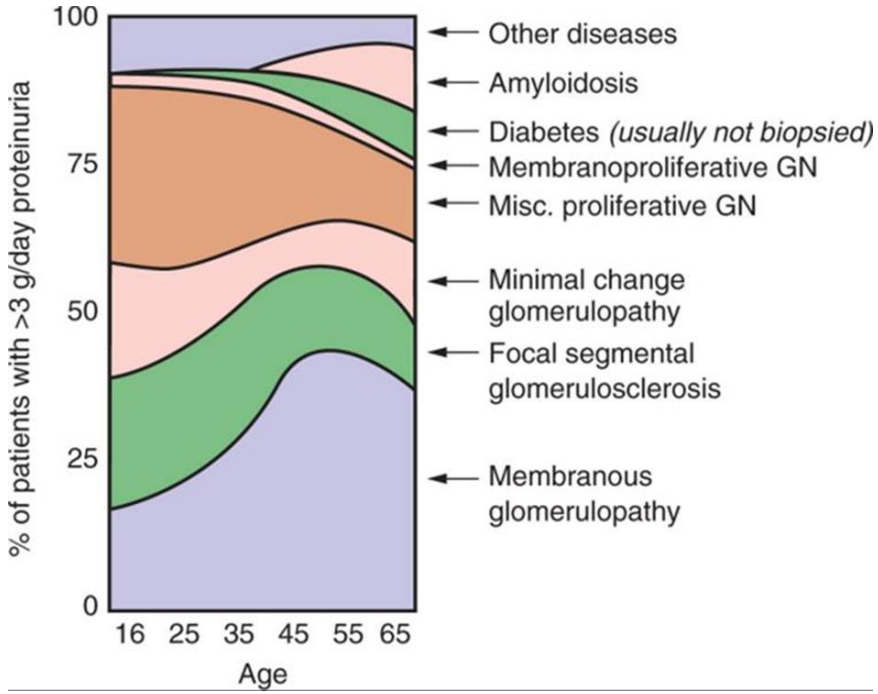
GN example (1c): Membranous GN/nephropathy





- MN is one of the most common causes of nephrotic syndrome in adult patients
- The characteristic pathological findings
  - intra-membranous immune complex deposits at GBM or
  - subepithelial deposition of circulating IC is also a cause
    - in primary MN, antigen-antibody complexes develop also in situ by targeting of podocyte antigens rather than trapping of circulating immune complexes
      - most commonly against M-type phospholipase A2 receptor (anti-PLA2R)
      - neural endopeptidase

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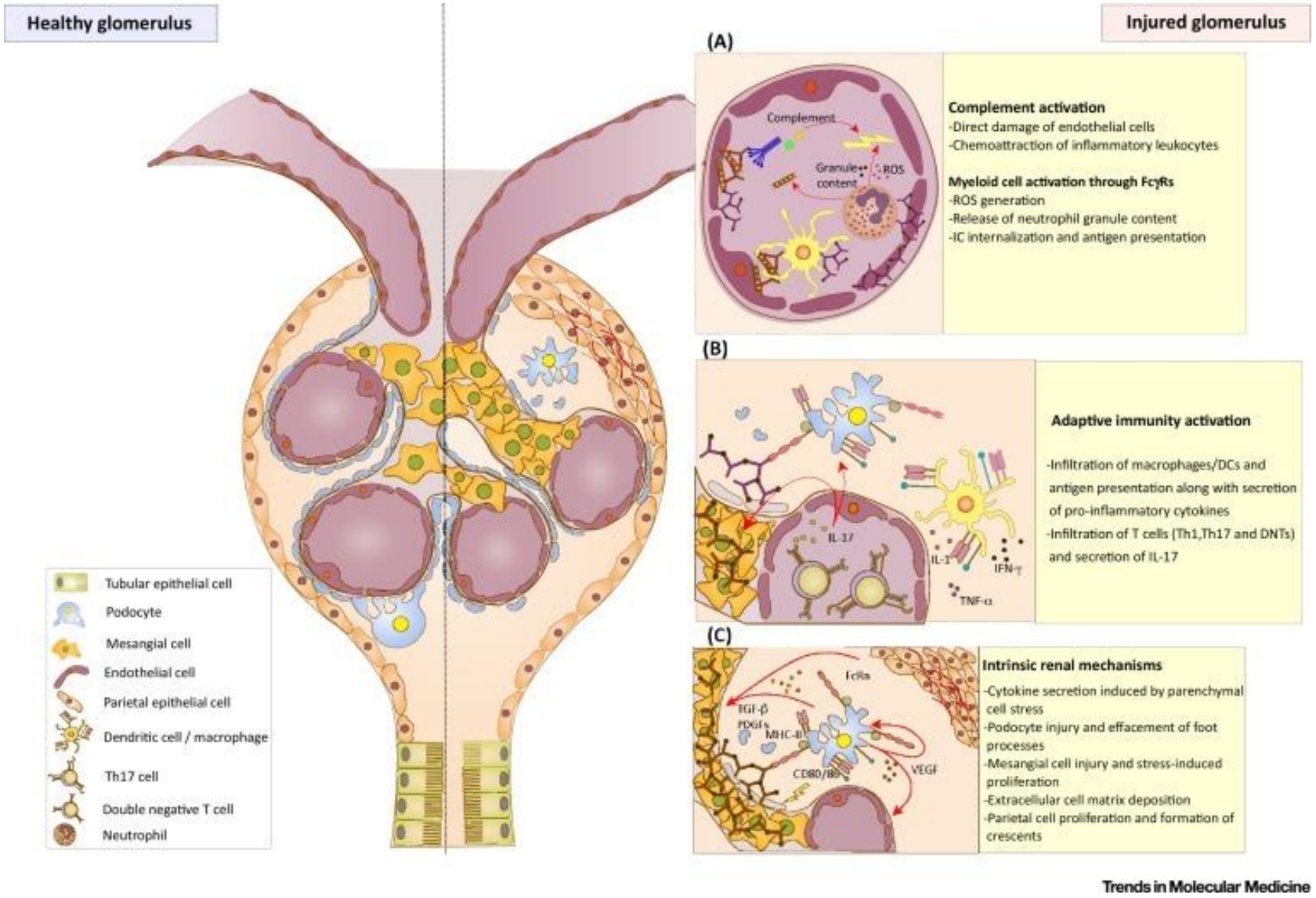
GN example (1d): Lupus nephritis secondary to SLE: as an example of very heterogeneous response to IC deposition



- SLE as a disease that develops in two phases
  - (1) the establishment of a chronic autoimmune response that follows tolerance failure and manifests by the presence of autoantibodies and activated T cells
    - particularly against intra-nuclear antigens such as chromatin and ribonucleoproteins
    - this response underlies the hallmark laboratory feature of SLE: antinuclear antibodies
  - (2) the development of immune-mediated tissue inflammation that directly results in clinical manifestations and disease morbidity
- complex interplay between genetic susceptibility factors and environmental elements shape the disease phenotype incl. glomerular damage
- SLE presents as a remitting-relapsing, heterogeneous disease that can affect different organs with varying severity
  - skin, kidneys, and joints, as well as the hematopoietic, nervous and cardiovascular systems
- most organ involvement in SLE results from inflammation initiated by deposited immune complexes that activate complement and attract effector cells (e.g., neutrophils)
  - although virtually all patients with SLE exhibit **immune-complex (IC)** deposition in the **glomerular mesangium**, only about 40–60% develop glomerulonephritis
- IC-mediated glomerulonephritis attributes a passive role to the kidney, however, recent work in humans and mice with lupus has revealed that the reaction of resident kidney cells to local inflammation determines the magnitude of the inflammatory response and ultimately the outcome of the immune-mediated insult

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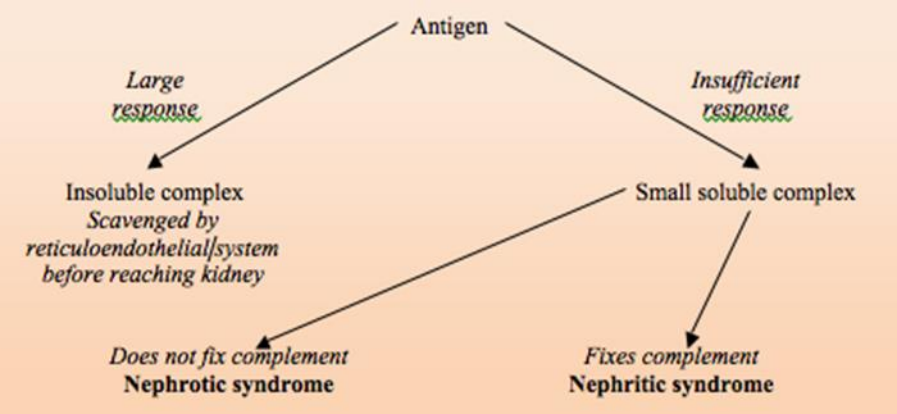
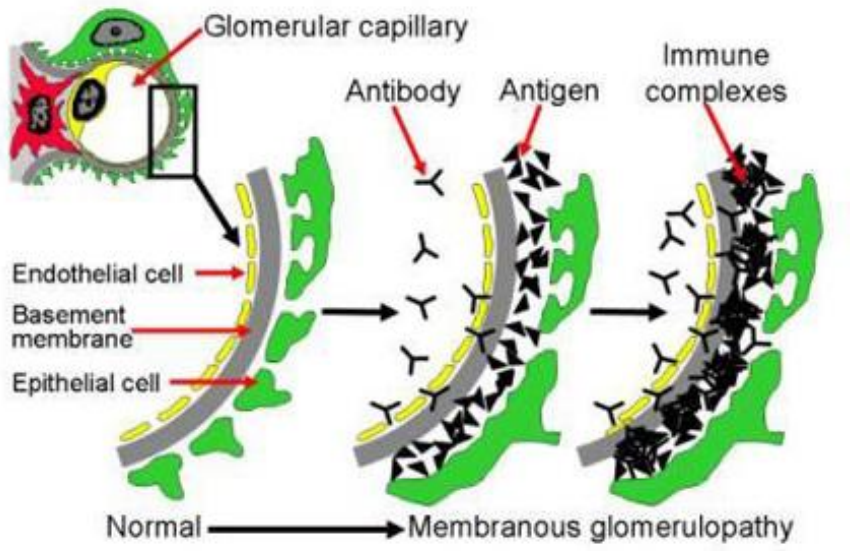
Immune complexes: Nephritic vs. nephrotic syndrome?





- Both syndromes can be caused by the formation of soluble complexes of antigens after an insufficient clearing from the immune system, however their location matters!!!
  - **sub-epithelial** IC deposition as found in **membranous nephropathy** leads to a non-inflammatory complement-mediated damage because the anaphylotoxins produced during the local activation do not reach circulating leucocytes
    - hence the **nephrotic syndrome**
  - **sub-endothelial** IC deposition is associated with a brisk inflammatory response because the produced anaphylotoxins easily come into contact with circulating cells
    - hence the **nephritic syndrome**
  - given the broad variety of IC formation in **SLE**, the variety of its renal phenotype spectrum is not surprising ranging from nephrotic to nephritic or combination of both

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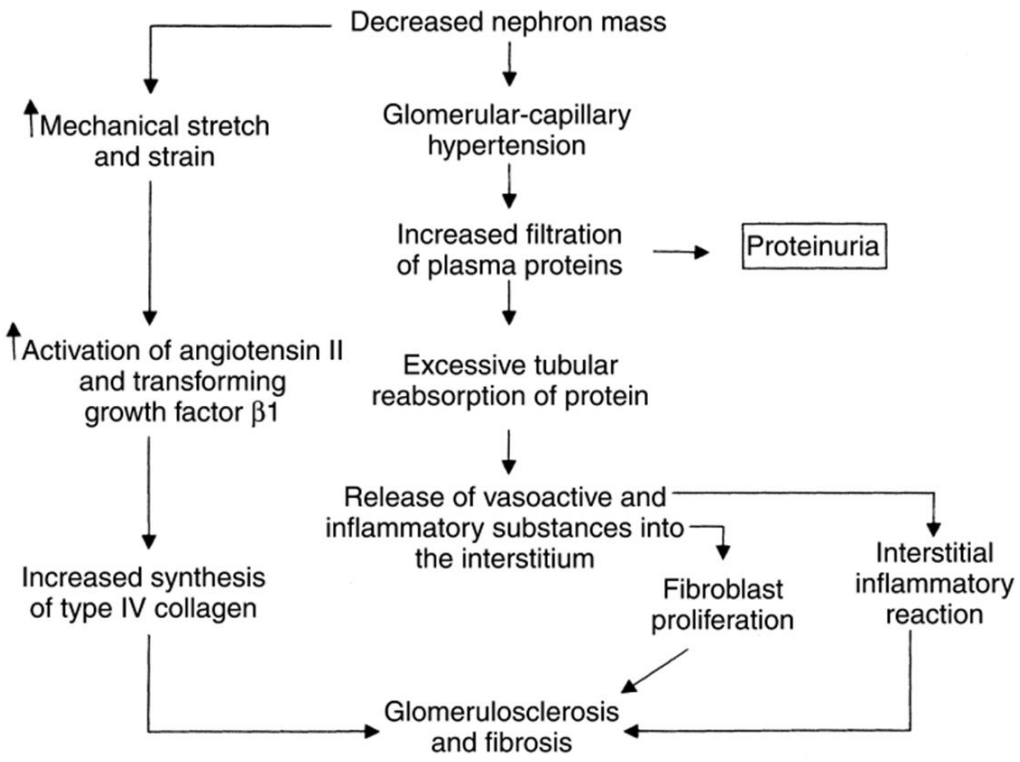
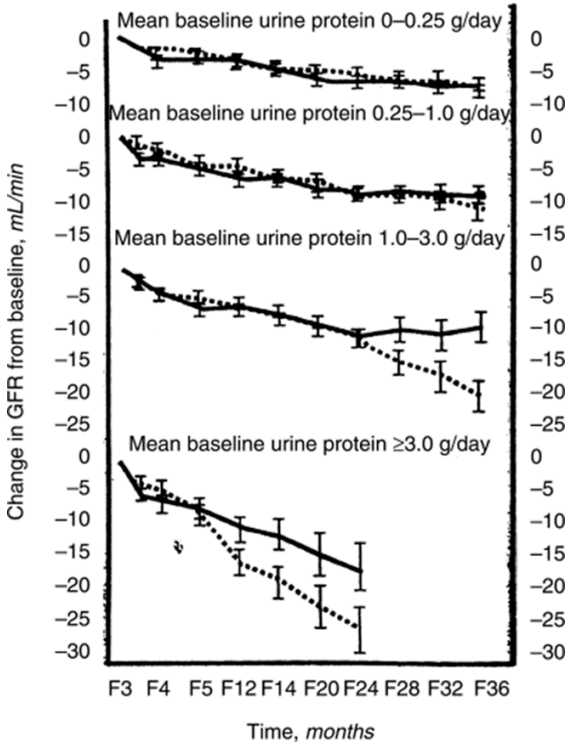
Consequences of nephrotic syndrome: proteinuria results in the development of glomerulosclerosis, interstitial fibrosis, ↓ GFR and ESRD



Progressive renal and cardiovascular disease: Optimal treatment strategies
Matthew R Weir

- albumin in small concentration necessary survival factor for tubular cells
- however, larger quantities of proteins in tubules lead to inflammation and interstitial sclerotisation
  - perpetuation of renal damage!!!
- mean decline in GFR (mL/min) over a 36-month period in groups with four different mean baseline 24-hour urine protein levels in non-diabetic patients with chronic renal failure in the MDRD study
  - compared in each of these four groups are the
    - normal blood pressure group (dashed line; 140/90 mm Hg; 102-107 mm Hg MAP)
    - intensive control group (solid line; 125/75 mm Hg; 92 mm Hg MAP)



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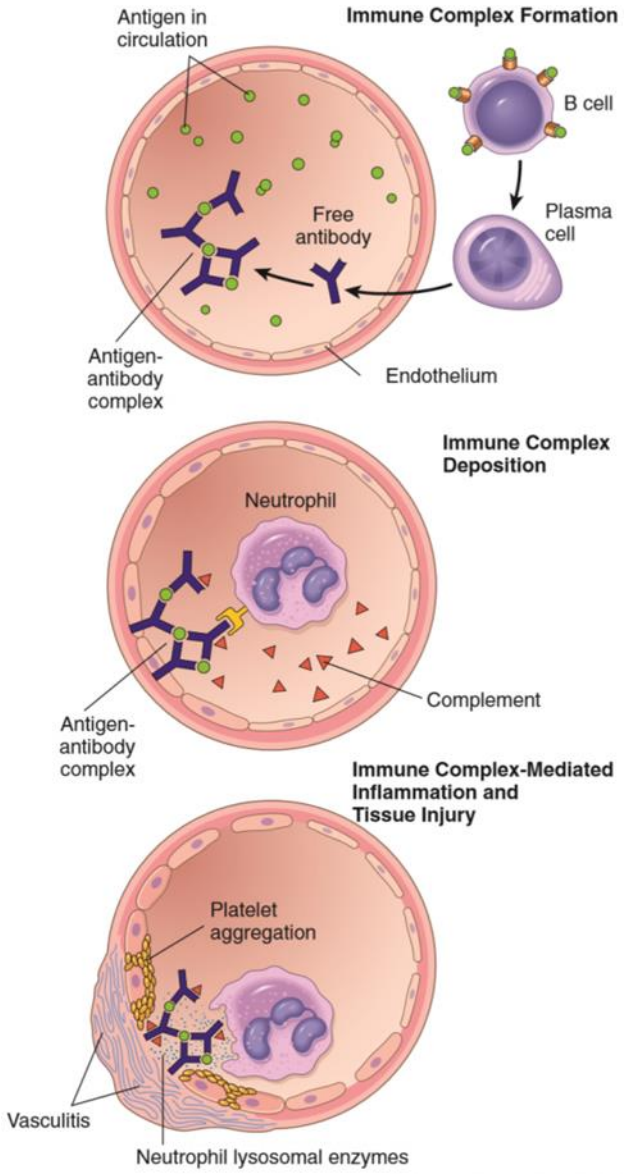
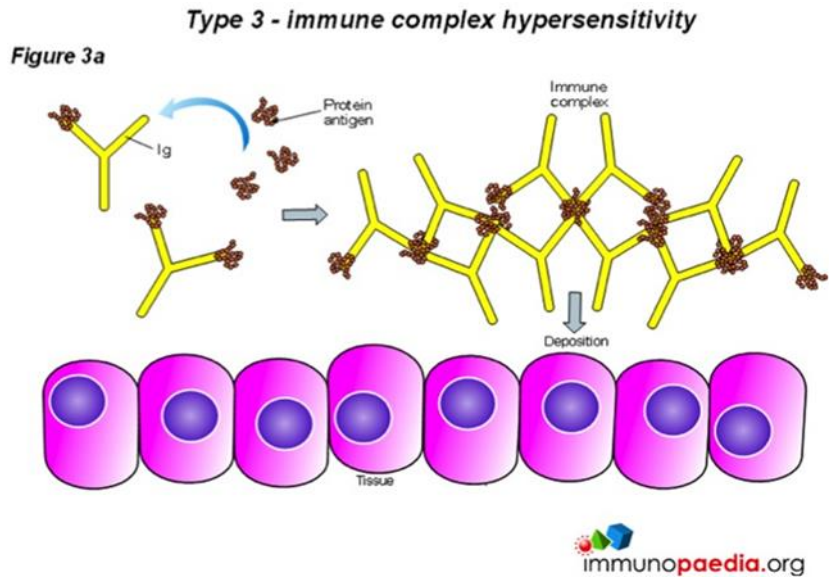
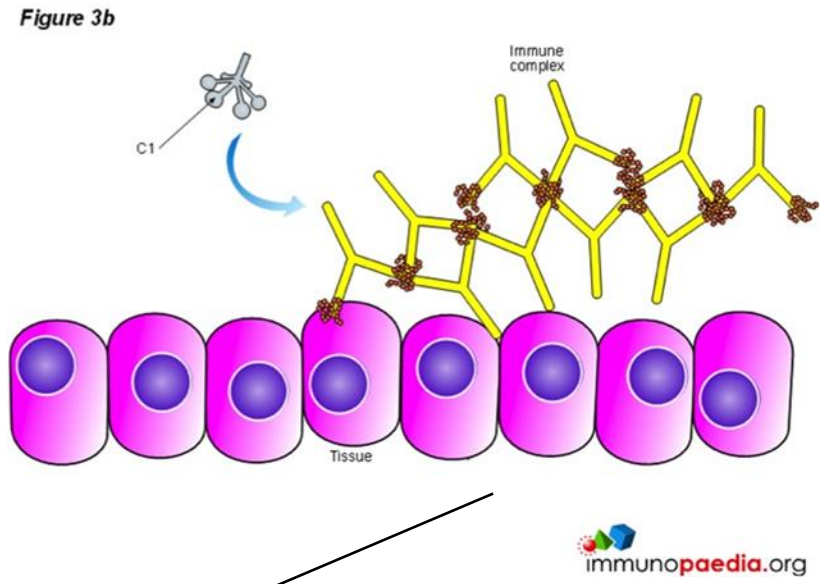
(2) Glomerular diseases manifesting as nephritic sy

- damage to filtration barrier due to inflammation = **glomerulonephritis**
- often acute, presenting with dramatic clinical picture, requiring **kidney biopsy**
- immune mechanisms pathogenically responsible
  - **(1) immune complexes (IC) mediated (90 %)**
    - antigens: circulating, in situ (kidney tissue specific Ag) or planted in kidney
      - bacteria (β-hemolyt. Strepto-, Staphylo-, Pneumococci), parasites, viruses, endotoxin, cell organelles (in SLE), drugs, …
  - **(2) antibody-mediated (10%)**
    - against GBM, anti-neutrophil or against glomerular cells
- GN can be kidney-restricted or accompanied by systemic disease

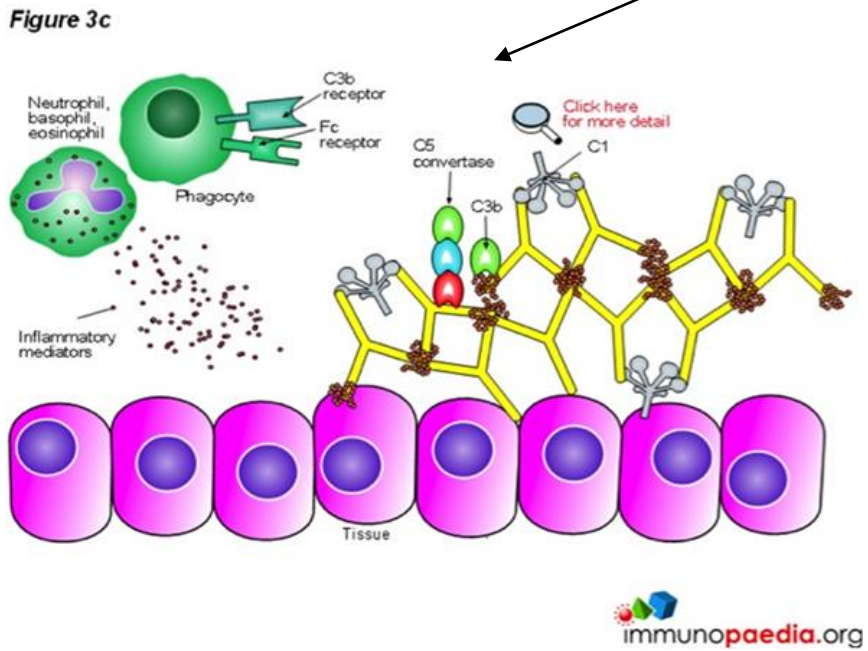


General pathogenesis of acute immune-complex mediated GN

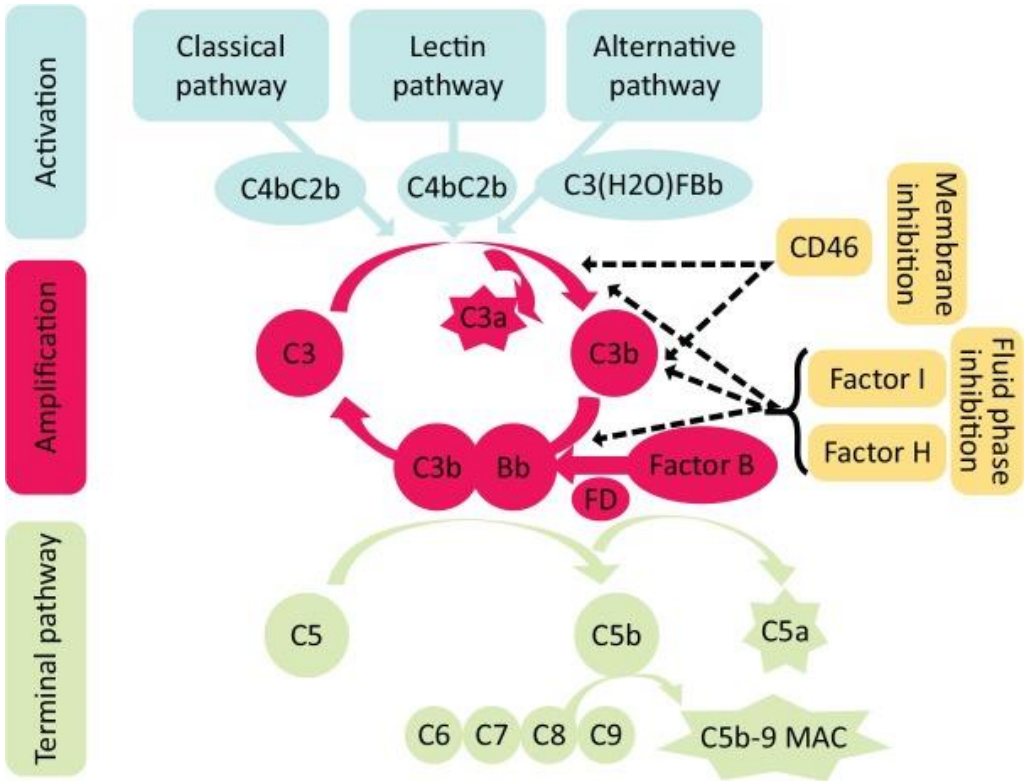




- activation of complement by classical pathway (low C3 in lab test)
  - formation of opsonins, chemotaxis
  - inflammatory infiltration by neutrophils and macrophages
  - glomerular cell lysis
- protease degradation of cells and ECM
- healing
  - growth factors – cell proliferation
  - connective tissue (mesangium) – fibrosis and sclerosis



The role of complement in renal disease



- Complement is a critical early component of the innate immune response consisting of serum and cell surface proteins that work in a cascade culminating in the production of a cell membrane attack complex (MAC) that destroys and removes pathogens
  - ~30 plasma- and membrane-bound proteins representing ~10% of plasma protein
- There are 3 major pathways for complement activation converging
  - classical
    - IgM or IgG, complexed with antigen, bind complement protein C1q
  - lectin
    - Mannose-Binding Lectin (MBL) bind to carbohydrate residues found on pathogen cell surfaces
  - alternative
    - constantly active at a low level, allowing rapid amplification of complement activation in response to pathogens
- The complement system involves numerous regulatory molecules that protect the host from uncontrolled tissue destruction and activation by the complement system
  - kidney is particularly susceptible to damage by complement, therefore loss of complement control is etiopathogenically important as exemplified by the prototypical diseases:
    - atypical haemolytic uraemic syndrome (aHUS)
    - C3 glomerulopathy sub-type of membranoproliferative glomerulonephritis (MPGN)
- Measurement of serum C3 and C4 levels can provide important diagnostic information in the assessment of a patient with kidney disease
  - low levels indicating over-activation and consumption
- Therapeutic inhibition of complement

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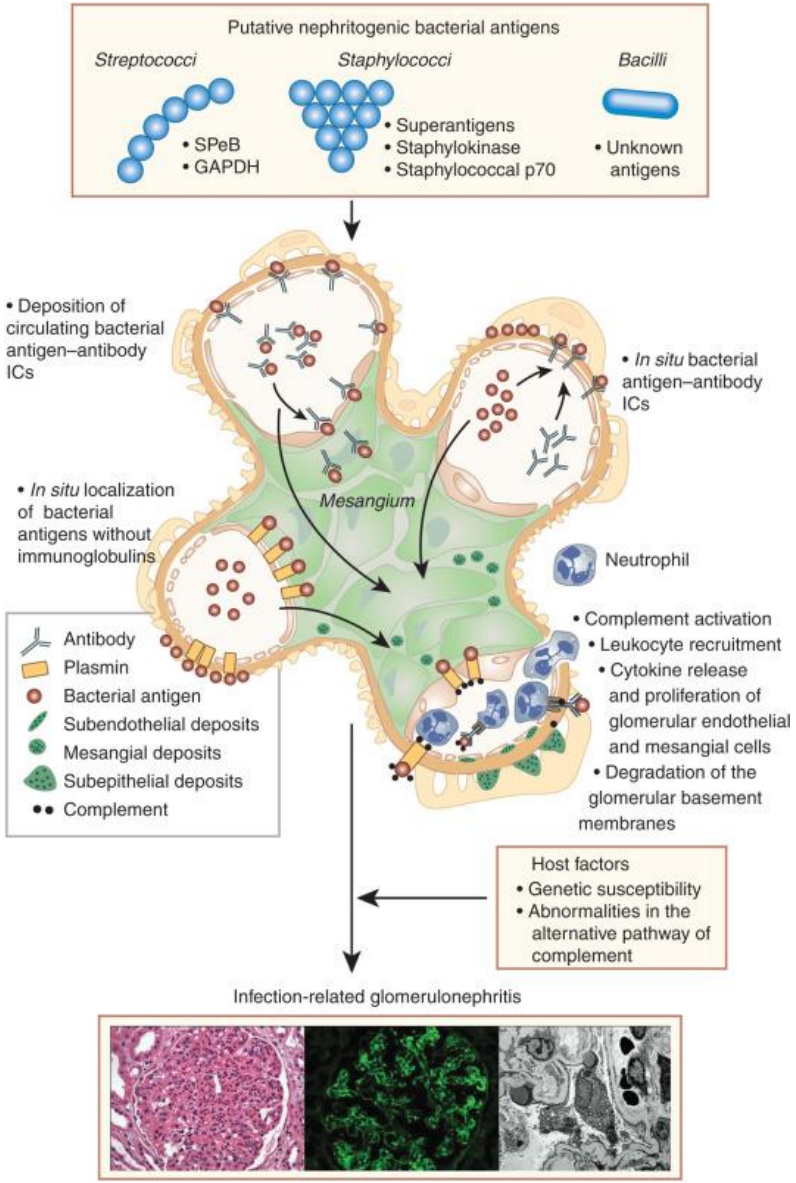
GN example (2a): Post-streptococcal (infection-related)

Bacterial infection-associated glomerulonephritis

Acute post-streptococcal (or post-infectious) glomerulonephritis (APSGN)
- Occurs following resolution of infection and an infection-free latent period of 1–8 weeks
- Abrupt onset of glomerulonephritis
- Usually involves paediatric patients

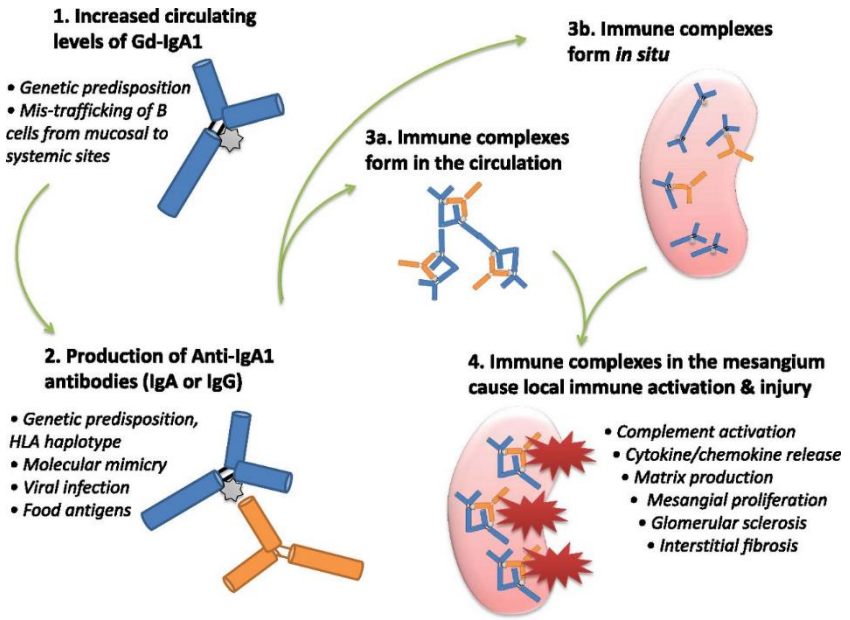
***Staphylococcus* infection-associated glomerulonephritis (SAGN)**
- Associated with *S. aureus* and *S. epidermidis* infections
- Infection is usually active when glomerulonephritis develops
- Aggressive immunosuppressive therapy is contraindicated, especially when active infection is evident

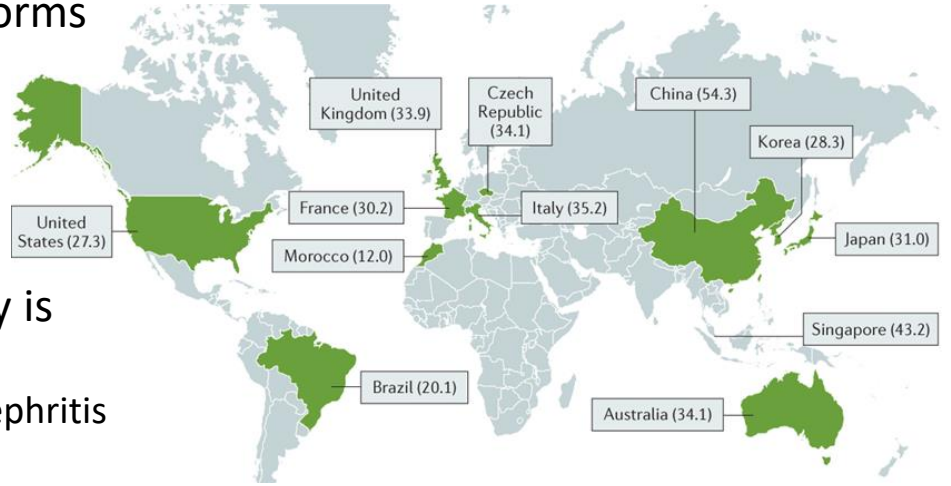
Glomerulonephritis of other infections
- Subacute bacterial endocarditis
- Shunt nephritis
- Indwelling central venous catheter infections
- Deep-seated visceral abscesses



GN example (2b): IgA nephropathy (Berger's disease)

- IgAN is the most common primary GN worldwide among patients undergoing renal biopsy
  - ~30% of patients with IgAN develop end-stage kidney disease 20 years after renal biopsy
- IgAN is characterized by dysregulation of the immune system, which causes an abnormal synthesis of deglycosylated IgA1
  - increase of **circulating galactose-deficient IgA1** elicits autoimmune response
  - gd-IgA1 can form complexes with specific autoantibodies that become deposited in glomeruli and induce the development of mesangial proliferation and matrix expansion by complex mechanisms
- Mesangial IgA deposition (together with C3) is the hallmark of IgA nephropathy
  - hence **mesangioproliferative GN**
- The spectrum of clinical manifestations in IgAN is wide, from incidental asymptomatic microscopic haematuria, to a rapidly progressive forms leading to kidney failure
  - the most common presentation in clinical settings is haematuria and moderate proteinuria with relatively preserved kidney function
  - mesangial-derived mediators released following mesangial deposition of IgA1 lead to podocyte and tubulointerstitial injury via humoral crosstalk
- Furthermore, global distribution of patients with IgA nephropathy is different between various regions of the world
  - prevalence is shown as percentage of biopsy-proven primary glomerulonephritis





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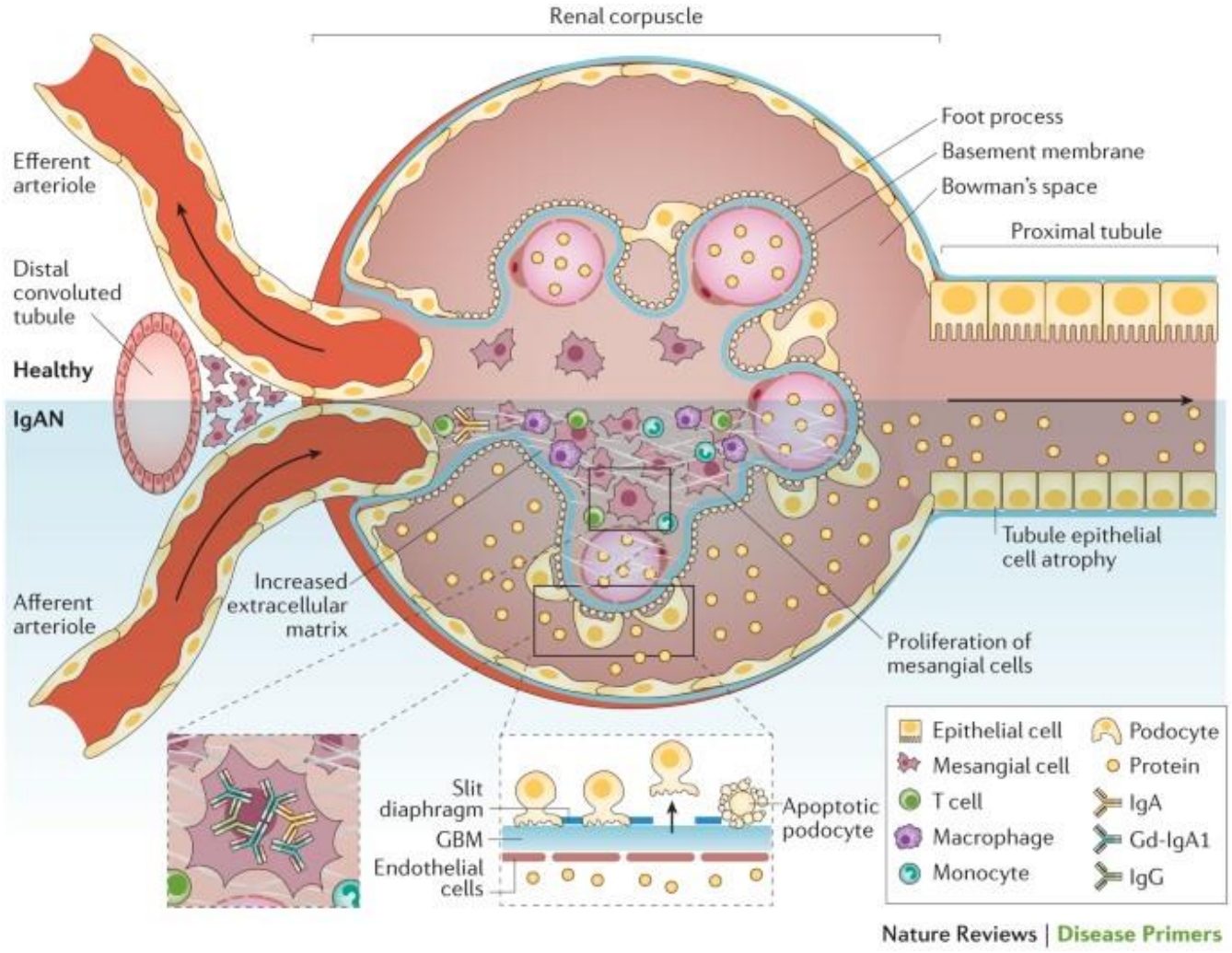
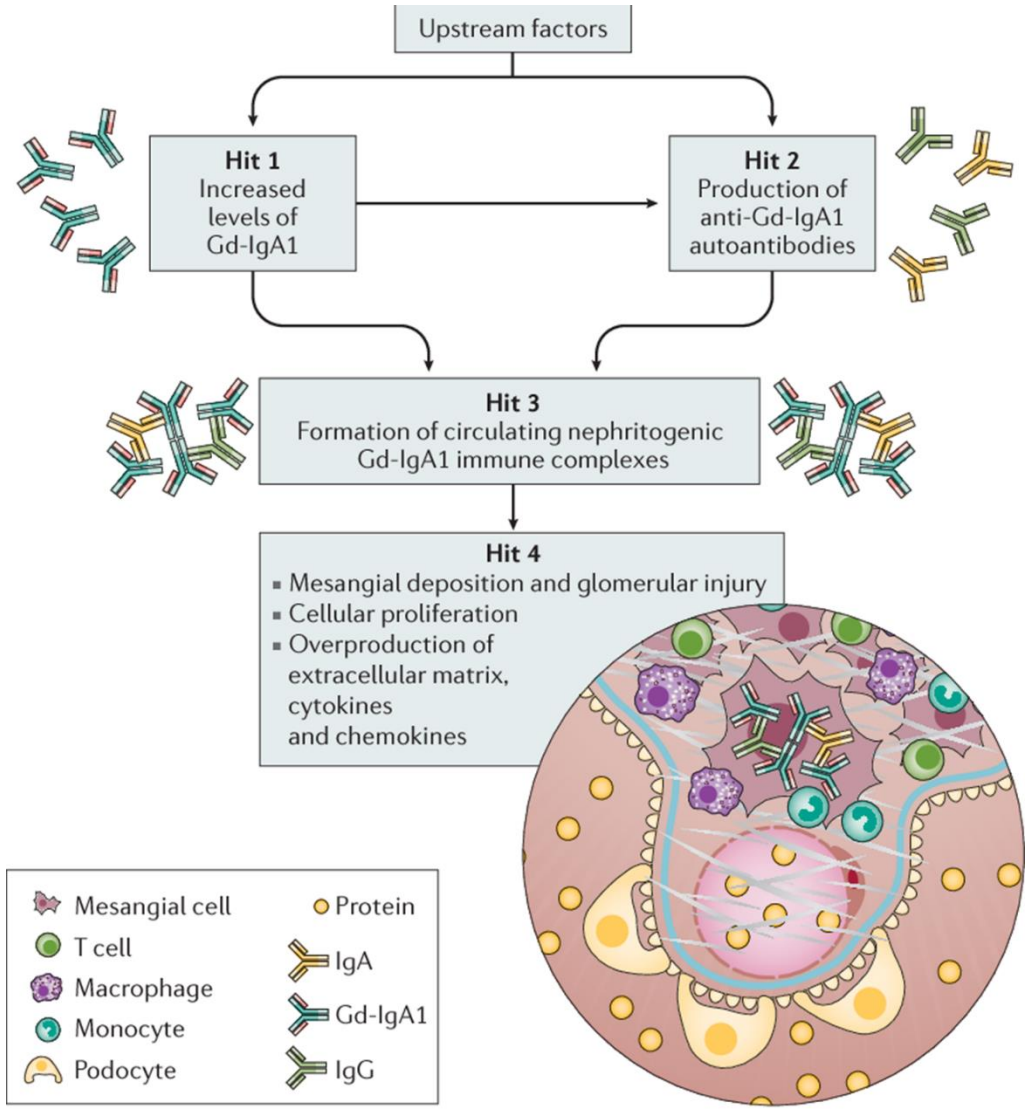
GN example (2b): IgA nephropathy (Berger's disease)





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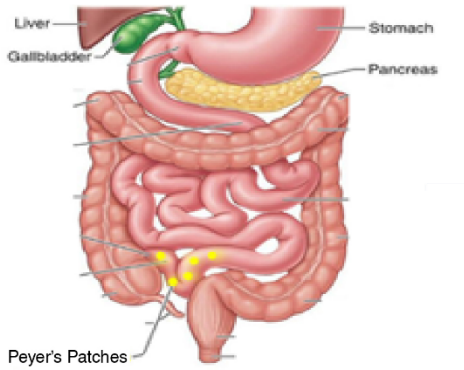
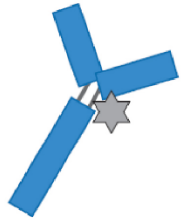
GN example (2b): IgA nephropathy (Berger's disease)

Peyer´s patches



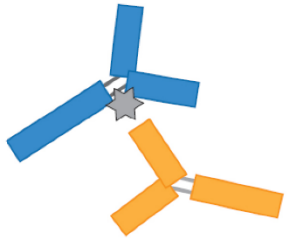
- Follicles of lymphatic tissue, know as Peyer's patches, in the distal part of the small intestine (ileum) produce secretory IgA antibodies

1 Galactose-deficient IgA



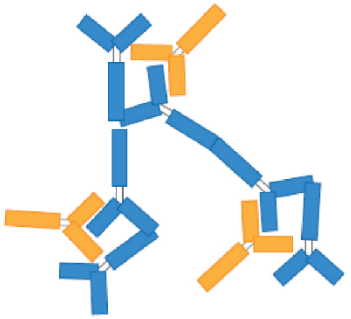
- Patients with IgAN have an increased appearance in the blood of secretory IgA antibodies that lack galactose units (galactose-deficient IgA) in the hinge region, which makes the antibody immunogenic

2 Immune recognition



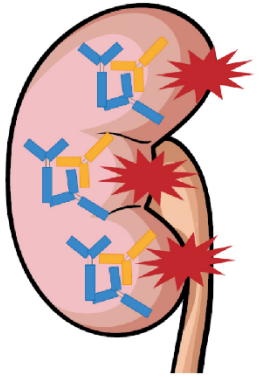
- The galactose-deficient IgA antibodies trigger autoantibody production Autoantibodies bind to and form complexes with the galactose-deficient IgA autoantibodies

3 IgA – dependent immune complex



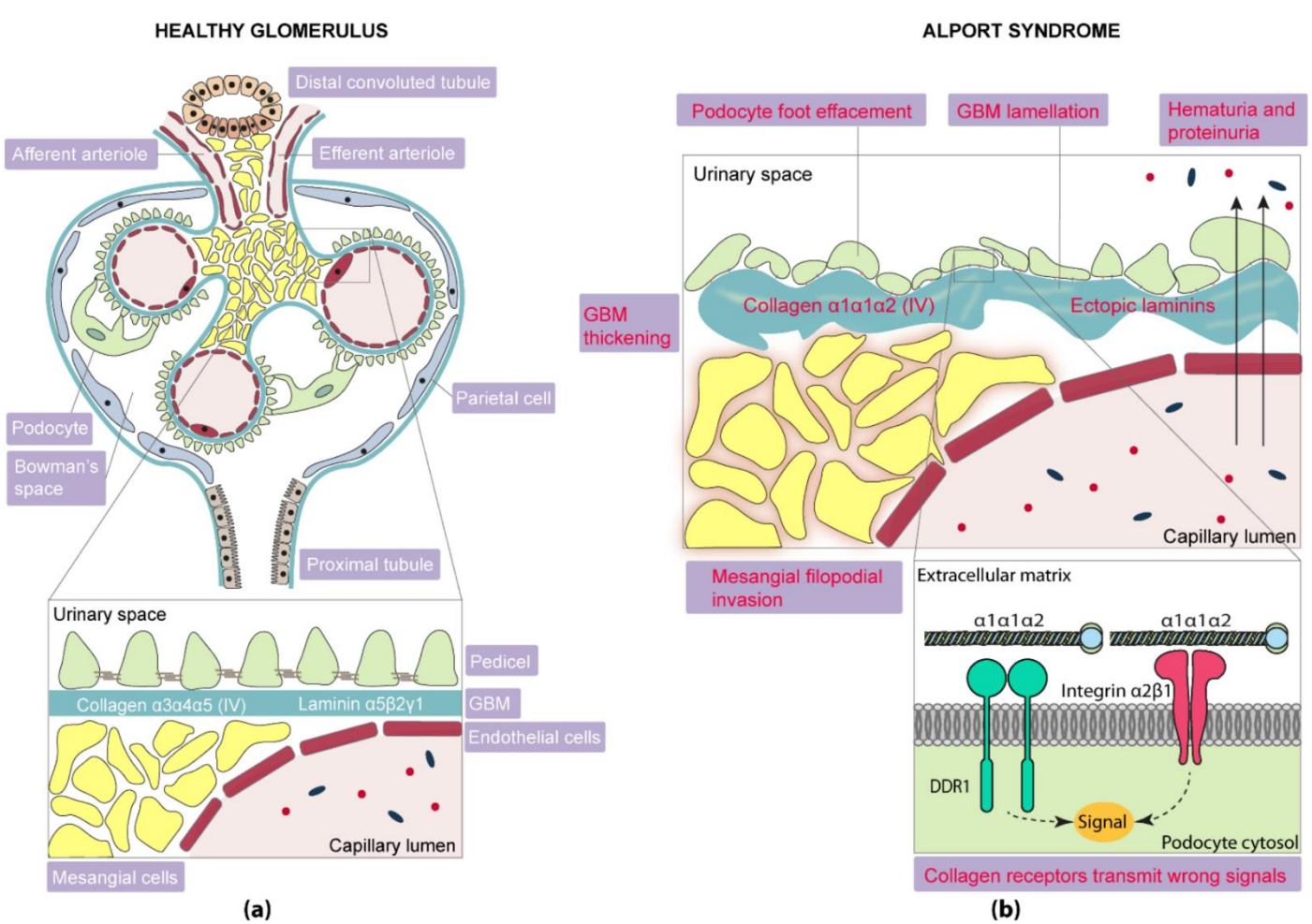
- These aggregates form pathogenic immune complexes that deposit in the glomeruli, which are the filtration apparatus of the kidney

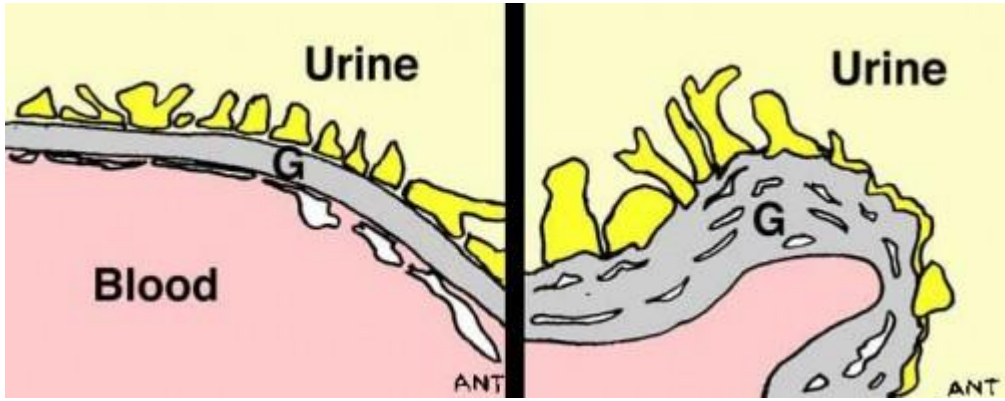
4 IgA nephropathy

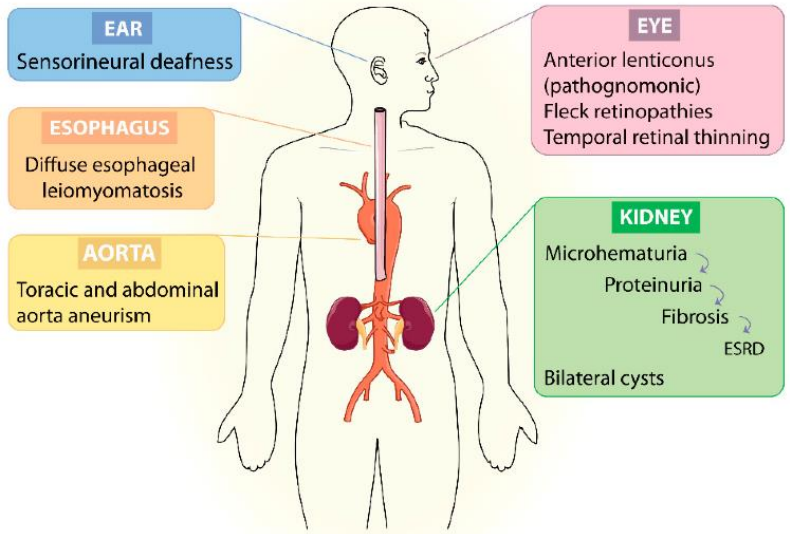


- The deposits of immune complexes in the glomeruli cause an inflammatory cascade that destroys the glomeruli, reducing the kidney's ability to remove waste products from the blood and eventually may result in ESRD

GN example (2c): Alport syndrome







- (a) Healthy glomerulus presents podocyte foot processes with slit diaphragms. The mature GBM is composed of collagen α3α4α5 and laminin α5β2γ1. Albumin does not filtrate pathologically into the urinary space.
- (b) In Alport glomerulus, podocyte foot effacement disrupts the podocyte structure and slit diaphragms disappear. Immature forms of collagen and laminins are expressed in the GBM as a compensatory mechanism. Albumin is lost pathologically due to increased permeability.

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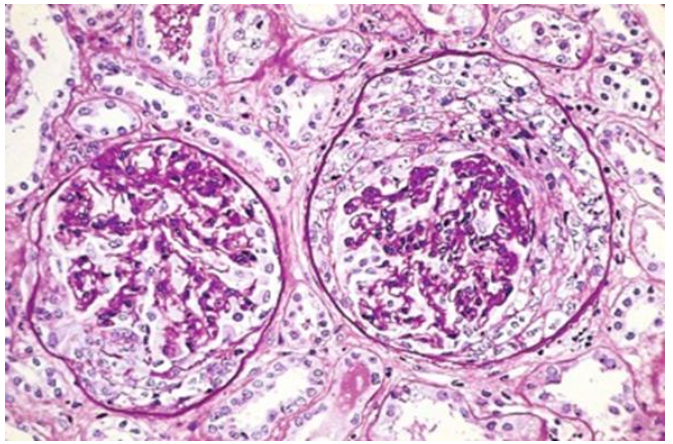
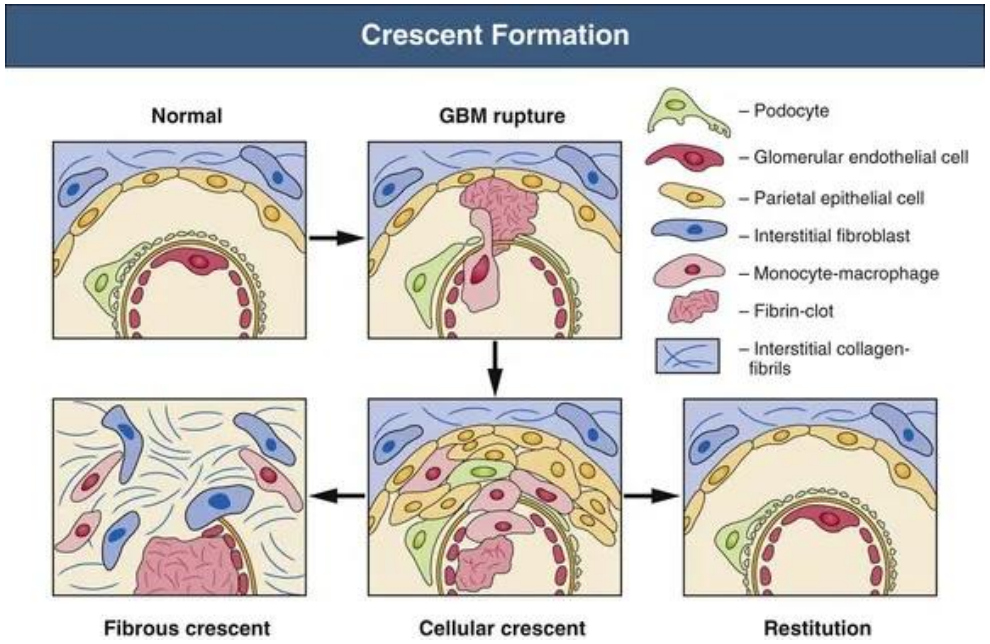
GN example (2d): Rapidly progressive (crescentic) GN

- RPGN is characterised **severe glomerular injury with poor prognosis**
  - not a specific GN but a specific clinical (time course) and morphological term
  - often as a consequence of anti-GBM (type I), immune-complex mediated GN (type II) or ANCA (type III)
  - or loss of kidney function (GFR<50%) over a short time period
  - if not treated, this will progress to acute renal failure and death within weeks
    - renal replacement therapy necessary



- glomerular crescents formation (on LM)
  - crescents are classical histopathological lesions found in severe forms of rapidly progressive glomerulonephritis
  - **develop from activated parietal epithelial cells** (PECs) residing along Bowman's capsule and their formation has as a consequence the decline of GFR
  - development
    - **rupture of glomerular capillary**
    - **leakage** and extravasation of fibrin, macrophages, fibroblasts, … within the Bowman space
    - **proliferation** of parietal epithelial cells



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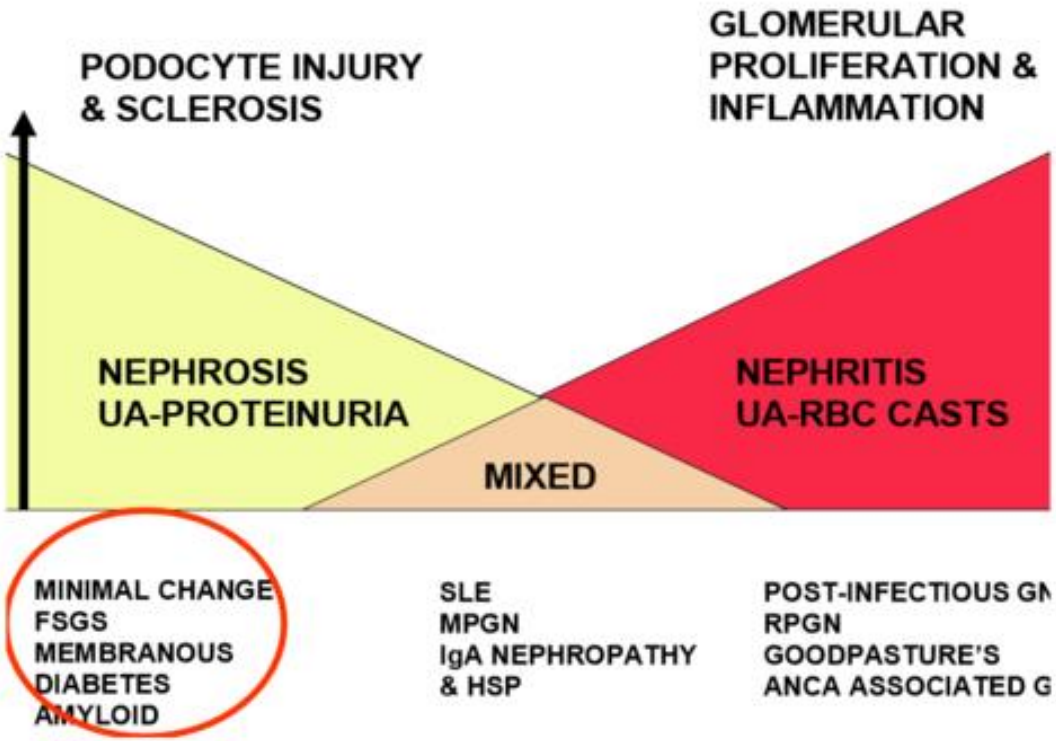
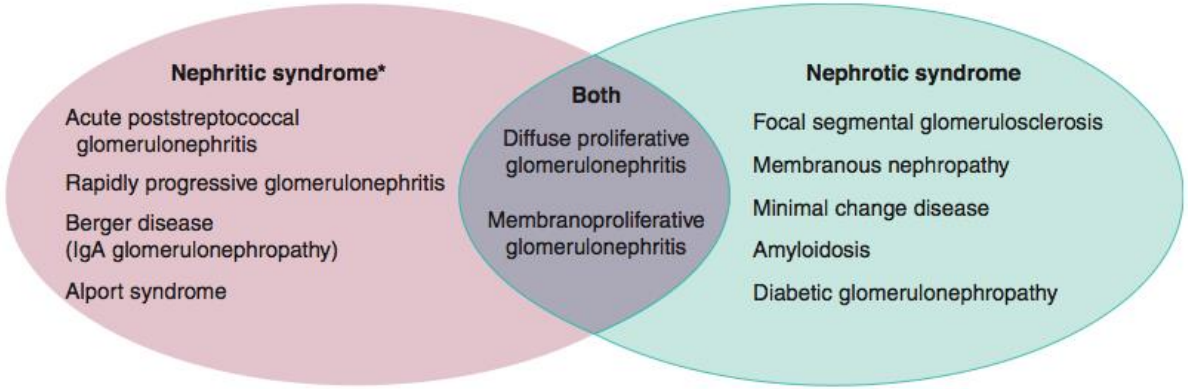
Single disease – uniform clinical manifestation?





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Nephritic vs Nephrotic Syndrome

Step 1: If the patient has hematuria and/or proteinuria,
think about nephrotic/nephritic syndromes.

Step 2: Does the patient have glomerular hematuria (RBC casts or dysmorphic RBCs in urine)?

Yes: Think Nephritic!

Is there suspected glomerulonephritis? aka evidence of significant proteinuria (> 0.5 g/day), worsening renal function, or evidence of a systemic disease?

Eval suspected GN:
Renal consult
Labs + Urine
Biopsy

N → **Isolated glomerular hematuria**

Y ↓

Glomerulonephritis

Pathology subtype confirmed by biopsy

Immunofluorescensce	Disease
Linear	Anti-GBM
Immune complex (granular)	Low C3/C4 PSGN Diffuse proliferative GN (like in SLE) Membranoproliferative Cryoglobulins Endocarditis Normal C3/C4 IgA Nephropathy IgA Vasculitis (aka HSP)
Pauci-Immune (Neg IF)	ANCAs: GPA, eGPA, MPA Drugs

No: Think Nephrotic!

Is it mostly albumin?
- N → **Non-glomerular proteinuria** ex MM: get SPEP, SFLC
- Y ↓

Is it A LOT of protein (>3-3.5g/day)?
- N → Repeat and if still present workup **non-nephrotic proteinuria**
- Y ↓

Does the pt have peripheral edema and hypoalbuminemia?
- N → Consider:
  - **Early nephrotic syndrome**
  - **Nephritic syndrome**
  - **FSGS** may be more subacute
  - causes of non-nephrotic proteinuria
- Y ↓

Eval suspected NS:
Renal consult
Labs + Urine
+/- **Biopsy**

Nephrotic syndrome

Pathology confirmed by biopsy

Pathology Pattern	Disease
1. Foot process effaced	1. Minimal Change 2. FSGS
2. Immune complex deposition	1. Membranous 2. Membranoproliferative
3. Systemic disease affecting glomerulus	1. DM 2. Amyloidoisis

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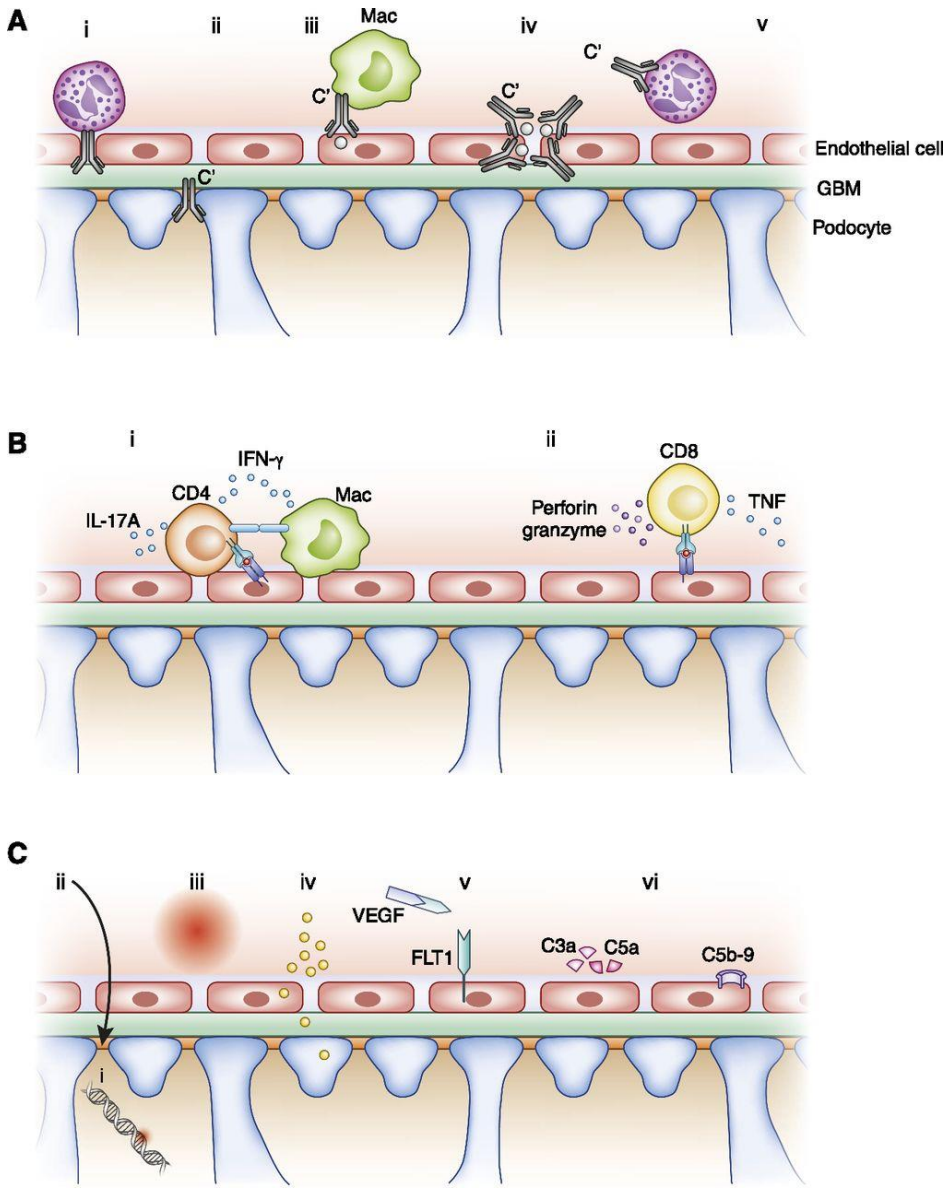
Overview of various mechanisms of glomerular injury



- (A) Antibody-mediated glomerular injury
  - (i) neutrophils (shown) and macrophages induce injury after anti-α3(IV)NC1 autoantibodies bind to the GBM in anti-GBM GN
  - (ii) in membranous glomerulopathy autoantibodies against PLA2R1 (and other antigens) on podocytes are deposited subepithelially, with the involvement of complement
  - (iii) antibodies can bind to antigens lodged in the glomerulus (grey dots) with recruitment of macrophages (shown) and neutrophils, and the activation of complement
  - (iv) circulating immune complexes can be deposited in glomeruli, activate complement, and recruit leukocytes
  - (v) ANCA (with complement) activates neutrophils and enables their recruitment to the glomerulus
  - (vi) not shown, but important, is IgA deposition in mesangial areas
- (B) Cell-mediated immune mechanisms
  - (i) Effector CD4+ cells (often Th1 or Th17 type) recognize antigens that can be intrinsic to or planted in the glomeruli. This occurs via their T cell receptor recognizing MHC class II peptide complexes (several cell types could possibly be involved in this process). Activated T cells produce cytokines (IL-17A and IFN-γ as examples) that have direct effects on intrinsic kidney cells and activate, together with costimulatory molecules (e.g., CD154/CD40), innate leukocytes such as macrophages
  - Not shown are interactions between intrinsic renal cells and T cells that include costimulation and cytokines. (ii) CD8+ cells can recognize antigenic peptides with MHC class I on intrinsic cells and secrete cytokines or induce cell death.
- (C) Metabolic, vascular, and other mechanisms of injury
  - Podocyte and foot process injury and dysfunction occurs due to
  - (i) genetic abnormalities of slit diaphragm proteins and
  - (ii) in minimal change disease and FSGS due to circulating permeability factors
  - (iii) haemodynamic factors such as systemic and intraglomerular hypertension and
  - (iv) metabolic factors such as hyperglycemia and its consequences are common, and affect both the cells and the structural components of the glomerulus
  - (v) both glomerular endothelial cell and podocyte injury are important consequences of preeclampsia, involved a number of mediators including soluble fms-like tyrosine kinase-1
  - C3 glomerulopathy, as well as some types of atypical hemolytic uremic syndrome (vi), can be induced by autoantibodies to, or genetic abnormalities in, complement regulatory proteins, resulting in complement activation. α3(IV)NC1, the non-collagenous domain of the α3 chain of type IV collagen;
- Abbreviations: FLT1, fms-like tyrosine kinase-1; GBM, glomerular basement membrane; Mac, macrophage; M-type PLA2R1, phospholipase A2 receptor 1; Th, T helper; VEGF, vascular endothelial growth factor.

A. Richard Kitching, and Holly L. Hutton CJASN 2016;11:1664-1674

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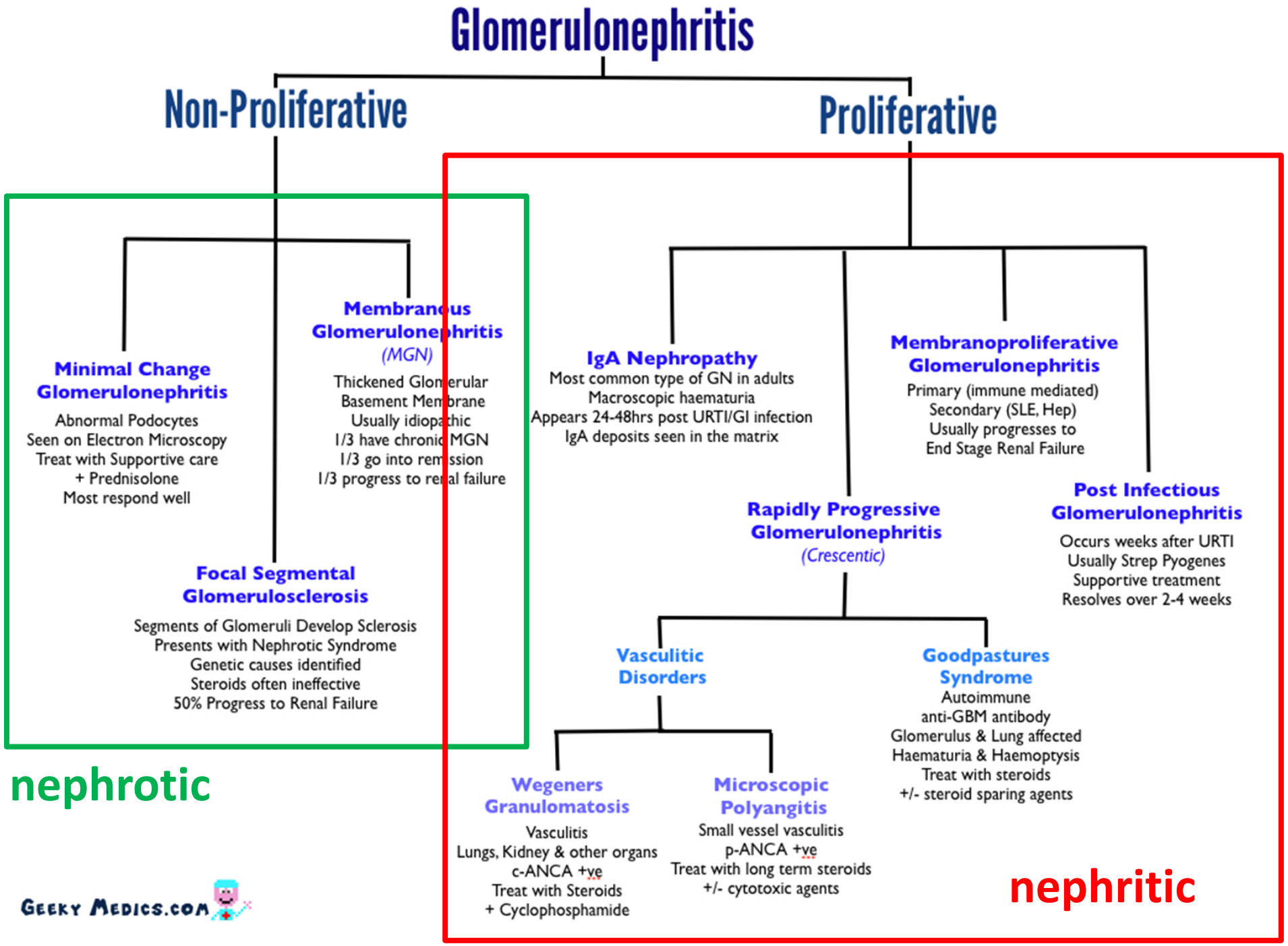

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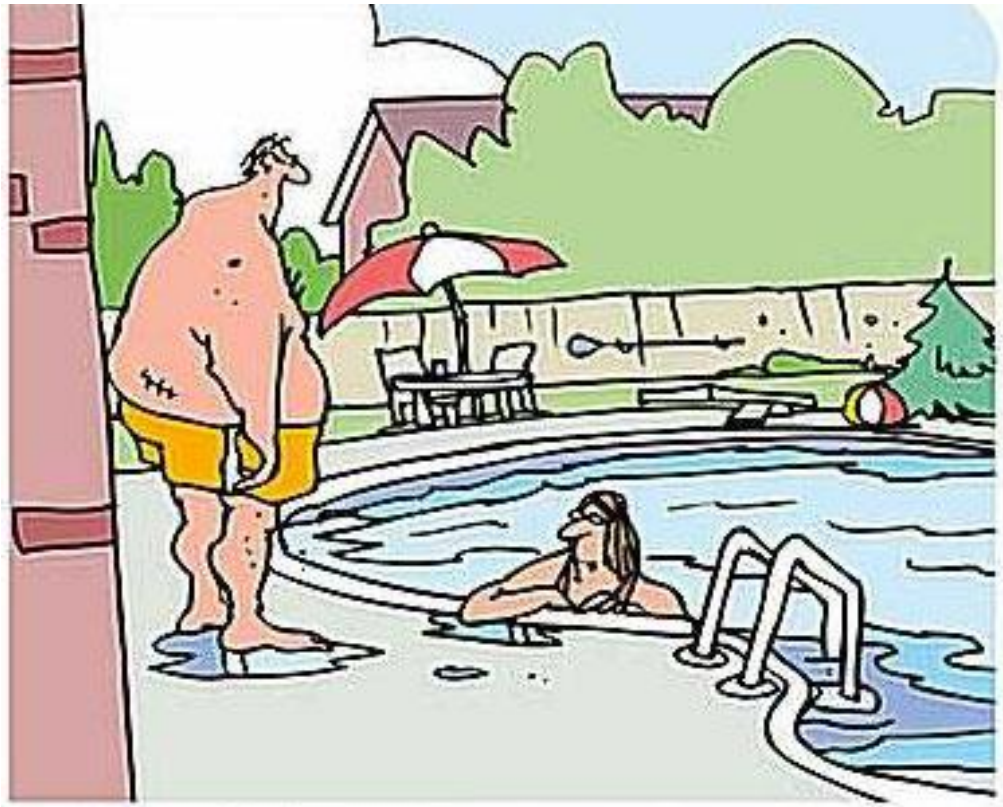

"The kidney shape was a cool idea.
Reminds us what you sold to pay for it."

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