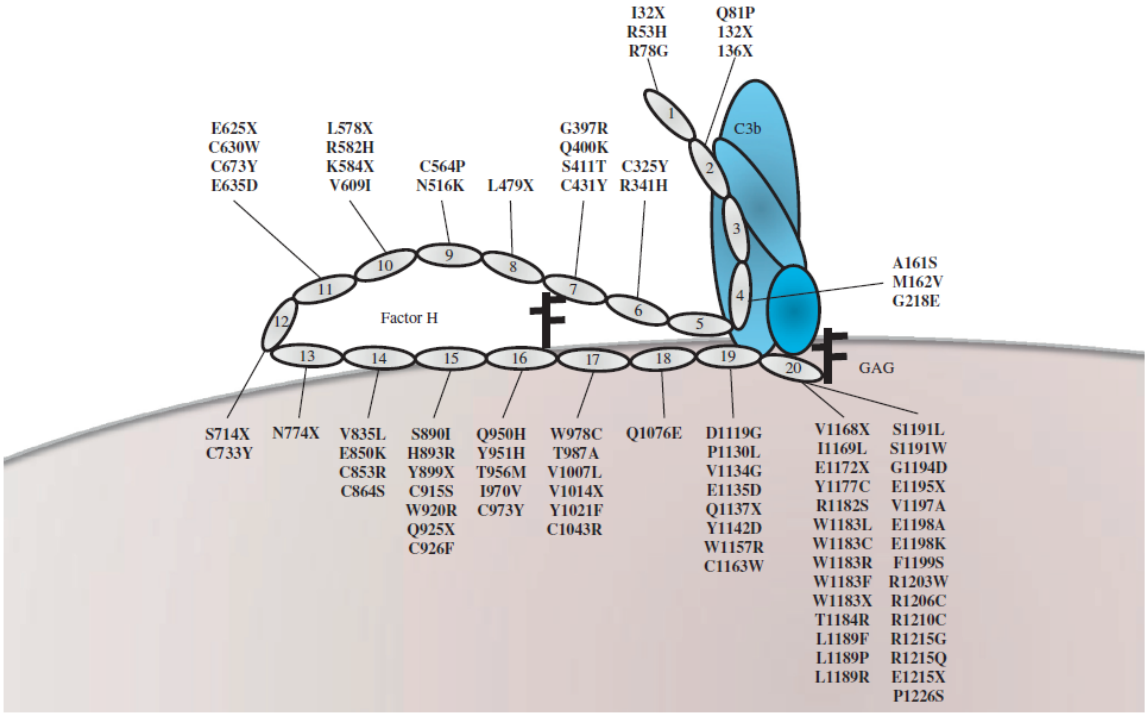
COMPLEMENT DYSREGULATION RELATED RENAL DISEASES



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This is to acknowledge that Bekir Tanriover, M.D. has disclosed that he has served for the Speakers Bureau of the Alexion Pharmaceuticals. Dr Tanriover will be discussing off-label uses in his presentation.

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Interests: Renal transplantation, induction immunosuppression, antibody mediated rejection and aHUS.

Purpose and Overview:

This presentation aims to review tremendous advances made in our understanding of the dysregulation of complement alternative pathway in regards to renal disease mechanisms, heterogeneous phenotypic presentations, and potential therapeutic approaches.

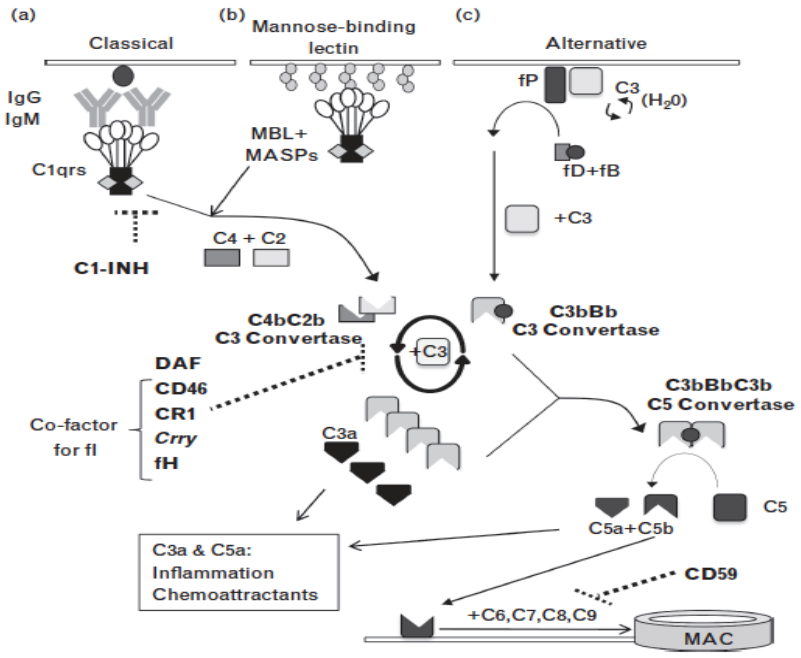
Educational Objectives:

1. Review the complement system; focus on the alternative pathway (AP).
2. Review pathogenesis of kidney diseases caused by inappropriate activation of the AP: C3 glomerulopathies (C3GN and Dense Deposit Disease) and atypical HUS [aHUS]).
3. Review sites of dysregulation of the AP.
4. Review renal outcomes of C3 glomerulopathies and aHUS.
5. Differentiate aHUS from TTP.
6. Discuss new therapeutic options in kidney diseases caused by inappropriate activation of the AP.
7. Review renal transplantation outcomes in patients with HUS as a cause of ESRD

INTRODUCTION TO THE COMPLEMENT SYSTEM:

The complement system (CS) plays a role in serving as essential part of innate immunity (the first line defense against infections), providing an interface between the innate and adaptive immunity (augmentation of antibody response, enhancement of T-cell response to antigen presenting cells, and reduction of Treg function), contributing immune surveillance by clearing foreign, malignant and apoptotic cells.(1) The CS is comprised of more than 30 soluble and surface-expressed proteins. Complement activation can be initiated through three pathways (the classic, lectin and alternate pathways), (Figure 1). The alternative pathway (AP) is capable of auto-activation by a mechanism called tick over of C3, always spontaneously active at a low rate. In all three pathways, the key step is the cleavage of C3 to C3a and C3b via C3 convertase. C3b initiates terminal complement cascade (C5b-9, Membrane Attack Complex [MAC]) by the formation of C5 convertase. The C3 convertase amplification loop requires tight control to prevent unintended tissue damage. Some regulatory proteins reside **on the cell surface** and provide cytoprotection, whereas others exist in **plasma** limiting (**fluid-phase**) complement activation.(2) Important steps involved in the CS are: 1) initiating complement activation; 2) amplifying complement activation; 3) performing effector functions ; 4) regulating the cascade .

Figure 1. The Complement cascade and its regulators.(3)



PATHOGENESIS OF KIDNEY DISEASES (C3 GLOMERULOPATHIES & ATYPICAL HUS) CAUSED BY DYSREGULATION OF THE AP:

Kidney diseases caused by genetic or acquired dysregulation of the AP are classified as C3 glomerulopathies (C3 glomerulonephritis [C3GN] and Dense Deposit Disease [DDD])(4), atypical hemolytic uremic syndrome (aHUS), and atypical post-infectious GN. Inappropriate activation or alteration of the C3 convertase is the pathophysiologic process common to all of these diseases. The C3 glomerulopathies are described by C3 accumulation without or sparse Ig deposition on immunofluorescence (IF) microscopy and electron dense deposits in mesangium and along GBM and capillary walls on electron microscopy (EM).(4) aHUS is a thrombotic microangiopathy (TMA due to endothelial swelling and disruption) defined by triad of AKI, hemolytic anemia, and thrombocytopenia. Atypical post-infectious GN indicates a clinical course persistent glomerular damage without resolution.(5)

Why disorders of the AP target kidney more is incompletely understood, but it might be related to the presence of the fenestrae continuously exposing subendothelial tissues to complement activators, a lower baseline expression of complement regulators, and/or differences in the glycocalix (endothelial surface layer). The glycocalyx is a highly interactive matrix covering the luminal side of vascular endothelial cells and consists of glycosaminoglycans, proteoglycans and glycoproteins, which have an important role in maintaining homeostasis of the vasculature. The surface-bound glycocalyx glycosaminoglycan constituent heparan sulfate is crucial for CFH binding and function, both in recognition of host tissue and prevention of spontaneous complement activation via the alternative pathway.(6)

Transitions between glomerulopathies and aHUS can occur during disease course (7), after kidney transplantation (8) or among members of same family members.(9) The cause of phenotypic variation is currently unknown and adds another layer of complexity to AP pathophysiology, more examples regarding this issue outlined below Table1.

Table 1. Examples of variable phenotypic expression of CFH mutations.

Mutation in CFH	Phenotype	Reference
Prol621Thr	Patient with C3 glomerulopathy later develops aHUS	Vaziri-Sani et al.(10)

Tyr899Stop	Patient with aHUS develops C3 glomerulopathy in transplant kidney	Boyer et al.(11)
Ala161Ser; Arg1210Val; Arg53Cys	Identified in patients with aHUS and C3 glomerulopathy	Servais et al.(12)
Asn1117Ser	Crescentic and necrotizing GN in the region where aHUS mutations cluster	Fervenza et al.(13)

Associated complement gene mutations are most often present in heterozygosity, and cause either deficiency or abnormal function of the protein product. For the reasons that remain unclear, **penetrance of the disease phenotype is mostly incomplete (~50%).** Presence of a **trigger** (over activation of the AP: infection, pregnancy, autoimmune disorders, vaccinations, immunosuppressive or antineoplastic drugs, etc.) and several genetic defects (a rare genetic variant [CFH, CFI, MCP, C3, CFB mutations, etc.] and a **modifier** / common genetic variant [at-risk haplotype in a complement gene] and/or an autoantibody [directed against CFH or C3 convertase –C3 nephritic factor C3Nefs]) may be required to initiate clinical disease.(14)

The expression of disease may be determined by the site of the defect in the AP. C3 glomerulopathies are typically characterized by uncontrolled activation of the AP in the fluid phase (circulation) and/or at the tissue surfaces that lack membrane-anchored complement regulators.(15) However, aHUS generally results from AP dysregulation at the level of cell membrane with impaired cell surface protection against complement activation. The renal microvascular endothelium is generally targeted thereby leading to a TMA.(16)

CFH is single-polypeptide chain glycoprotein and consists of 20 short consensus repeats (SCRs) with two main functional domains positioned at the opposite ends of the protein. **The N terminus (SCRs 1–4)** is responsible for the fluid–phase complement regulatory functions (**Regulatory Domain** playing role in the C3b binding, cofactor activity and decay-accelerating activity). The mutations at N terminus result in uncontrolled complement activation in the fluid phase and GBM. The phenotypic expression is that of a **proliferative GN**.(5, 12, 17, 18) **The C terminus (SCRs 19 and 20)** mediates the recognition of ligands and binding to cell surfaces and tissue matrices, thus distinguishing self from non-self. The CFH mutations in **aHUS** mainly affect the surface

recognition sites in SCRs 19 and 20, C terminus (**Recognition Domain** playing role in surface binding, cell surface recognition, C3b binding). CFH is the most frequently mutated gene in aHUS with a disease-causing mutations identified in ~25% of sporadic and 40% of familial cases. More than 160 mutations in CFH are currently identified (www.FH-HUS.org) resulting deficiency or dysfunction of CFH.(19-22)

SITES OF COMPLEMENT PATHWAY DYSREGULATION:

A. Loss of CFH inhibition
    1. Deficiency or dysfunction of CFH. Mutations that lead to complete absence of CFH (type I mutation) or a CFH that is expressed in plasma but lacks complement regulatory activities (type II mutations).
    2. Functional inactivation of CFH by an autoantibody.

       The presence of an autoantibody directed against CFH results in functional CFH deficiency and occurs in 6-25% Europeans with aHUS.(23) These antibodies bind with C-terminal of CFH, thus affecting surface binding and recognition.
B. CFH deregulation (mutations affecting CFH related proteins – [CFHR])

   Generation of mutant CFHR proteins by internal duplication or gene fusions leads to unusual CFHR protein dimers and multimers with enhanced avidity for ligands, enabling the CFHR protein to outcompete CFH and amplify degree of CFH deregulation (meaning CFHR proteins acting as competitive antagonist of CHF).
C. Stabilization of the C3 Convertase
    1. Gain-of-function mutations in CFB
    2. Gain-of-function mutations in C3
    3. C3 Nephritic Factor (C3Nefs)

       C3Nefs are composed of IgG and IgM autoantibodies that bind directly to the C3 convertase or its components, thereby providing resistance to spontaneous or CFH or CFI mediated decay.
D. Impaired inactivation of C3b to iC3b
    1. Mutations in CFI
    2. Mutations in MCP

RENAL OUTCOMES C3 GLOMERULOPATHIES AND aHUS:

Outcomes of C3 glomerulopathies and aHUS are outlined in Figure 2 and Table 2.

Figure 2. ESRD free survival among patients with C3 glomerulopathies vs. MPGN 1.(12)

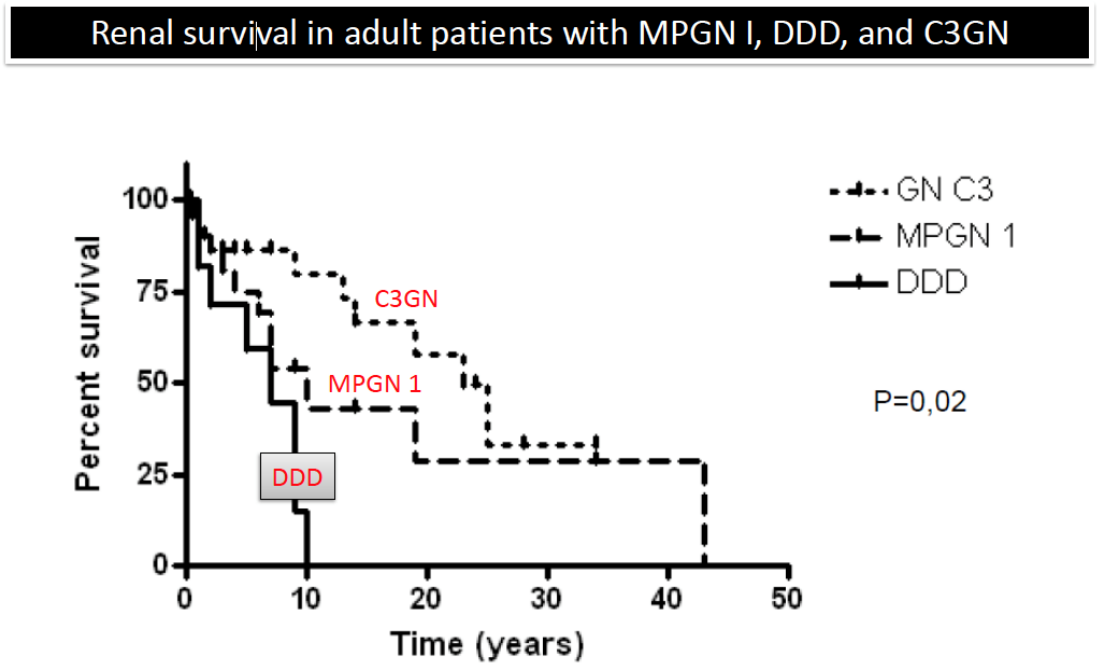


Table 2. Inherited and acquired causes of aHUS and their kidney outcomes prior to eculizumab therapy (M Noris & G Remuzzi, Comprehensive Clinical Nephrology 2015).

Affected Gene	Affected Protein	Frequency in aHUS (%)	Rate of remission with Plasma exchange (%)	Mortality (5-10 yr) or ESRD (%)	Rate of recurrence after kidney transplant (%)
CFH	Factor H	30	60 (dose and timing dependent)	70-80	60-70
CFHR1-CFHR3	CFHR1-3 (anti-CFH Ab)	5-10	70-80 combined with immunosuppression	30-40	40
MCP	Membrane cofactor protein	10-15	No indication for plasma exchange	<20	15-20
CFI	Factor I	4-10	30-40	60-70	70-80
CFB	Factor B (C3 convertase stabilization)	1-2	30	70	One case report
C3	C3 (resistance to C3b inactivation)	8-10	40-50	60	40-50
THBD	Thrombomodulin (reduced C3b inactivation	4-5	60	60	One case report

DIFFERENCES BETWEEN aHUS vs. TTP

Differences between aHUS and TTP are outlined in the Table 3 and 4.

Table 3. Different types of microvascular pathology associated with microangiopathic hemolytic anemia.(24)

Pathology	Key Features	Examples
VWF-platelet thrombosis	Thrombus with VWF and platelets Endothelium and vessel wall are intact	Thrombotic thrombocytopenic purpura
Fibrin-platelet thrombosis	Thrombus with fibrin and platelets Endothelium and vessel wall are intact	Disseminated intravascular coagulopathy, heparin-induced thrombocytopenia, paroxysmal nocturnal hemoglobinuria, catastrophic antiphospholipid antibody syndrome, HELLP syndrome
Microvascular cancer cells	Intravascular clusters of cancer cells	Metastatic neoplasm
Vasculitis	Inflammatory cell infiltration Fibrinoid necrosis Fibrous proliferation Disruption of internal elastic lamina	Autoimmune diseases, certain infections
Thrombotic microangiopathy	Endothelial swelling or disruption Subendothelial expansion/cell proliferation Thrombosis present or absent Internal elastic membrane is intact Interstitial edema Cavitary fluid accumulation	Atypical hemolytic uremic syndrome, hemolytic uremic syndrome due to shiga toxins or microbial neuraminidases, catastrophic antiphospholipid antibody syndrome, drugs*

Table 4. Comparison of aHUS and TTP.(24)

Disorder	Thrombotic Thrombocytopenic Purpura		Atypical Hemolytic-Uremic Syndrome	
Molecular defects	ADAMTS13 inhibitors	*ADAMTS13* mutations	CFH antibody	Genetic defects in C′ activators or regulators
Mode of transmission	Acquired	Autosomal recessive	Acquired	Autosomal dominant**
Age	Teens - adults	Children - adults	Children - adults	
Gender (female vs male)	2-3:1	~ 1:1	~ 1:1	
Incidence (relative)	1	<5%	1/3 - 1/2	
Pathology	Microvascular VWF-platelet thrombosis		Thrombotic microangiopathy	
Common presentation	Fatigue, headache, dizziness Focal deficits, confusion Petechiae Abdominal pain		Fatigue, headache, dizziness Chest pain, dyspnea Abdominal pain, vomiting, diarrhea Somnolence, confusion	
Thrombocytopenia	Generally precedes symptoms and signs		May not reflect disease severity	
Advanced renal failure	Rare	Occasional	Common	
Hypertension	Rare	Occasional	Common	
↑Vascular permeability Cerebral edema, PRES pulmonary edema, effusions, ascites, anasarca	Rare	Rare	Common	
Mortality with plasma therapy	~10%	Very low	20%-30%	
Treatment of choice	Plasma exchange	Plasma infusion	Eculizumab	
Long-term prognosis*	Relapse Stroke	Relapse Stroke Renal failure	Relapse End-stage renal disease in 30%-40%	

THERAPEUTIC OPTIONS FOR C3 GLOMERULOPATHIES and aHUS:

A. Non-specific treatment:

The clinical presentation of C3 glomerulopathies is more heterogeneous than that of aHUS and is generally slowly progressive. Nonspecific treatment measures can be used based on the data from other chronic GNs. In C3 glomerulopathies RAS blockade was associated with better renal survival (12), and RAS blockade with immunosuppression was better than either agent alone.(25)

1. BP control,
2. Proteinuria reduction (RAS blockade)
3. Lipid lowering

B. Replace deficient gene product

1. Plasma infusion:

Patients with type I mutations (complete absence of the complement regulatory proteins) may benefit from replacement of the deficient factor. Since there is no recombinant CFH is currently available, functionally intact CFH can only be administered through plasma therapy. There are reports attesting successful outcomes with long-term intermittent plasma infusion with patients with C3 glomerulopathy or aHUS caused by complete CFH deficiency. (26, 27) Plasma therapy is ineffective in patients with a single MCP mutation (membrane bound protein).(28) In patients with gain-of-function mutations in complement activation proteins, plasma infusion may be ineffective or detrimental, because it provides additional substrate for the hyper functioning mutant protein.(29, 30)

2. Liver transplantation:

Since CFH, CFI, CFB and C3 are predominantly synthesized by the liver, to prevent thrombosis or recurrent disease in the transplant kidney, combined liver kidney transplant can correct the genetic abnormality in patients with ESRD. To avoid perioperative hepatic failure (~15%) resulting from uncontrolled complement activation, the patients are prepared for the surgery with intensive plasma exchange and

eculizumab.(31-33) The overall success (both graft function and cure of disease) rate is around 80% in experienced centers.

C. Eliminate autoantibodies and/or mutant proteins

1. Plasma exchange (PE):

The rationale for PE is to replace deficient or defective regulatory proteins, to remove autoantibodies and/or mutant proteins, to enable the administration of higher volumes of plasma. Before the availability of C5 inhibitors, PE was the therapeutic mainstay of aHUS, although controlled trials showing its effectiveness are lacking. Overall, there is no difference in response to plasma infusion compared to PE.(20) With the availability of specific complement inhibitors, the role of PE will likely be restricted to bridging the period between clinical presentation and initiation of targeted therapy.(34, 35)

2. Immunosuppression (IS):

Direct effect of IS on the AP components have not been shown. Main idea behind this approach is that acquired antibodies contribute to the pathophysiology of the disease and that production of these antibodies can be decreased by immunosuppression (prednisone, rituximab, cyclophosphamide, MMF, and azathioprine). Combination of immunosuppression with PE benefits the outcome along with the sustained reduction of the anti-CFH antibody titers.(36, 37) Subsequent maintenance therapy with prednisone and MMF/azathioprine reduces the risk of relapse up to 90%.(37)

D. Inhibit complement activation

1. Inhibition of C5 (eculizumab):

Eculizumab is a recombinant, fully humanized monoclonal antibody that binds with high affinity to the human C5, and effectively blocks C5 cleavage, and generation of the MAC. Mainly given as rescue therapy because of resistance to or complications with plasma therapy, eculizumab resulted in complete remission in 21 out of 24 patients from the literature (11 children and 13 adults).(38) A French study retrospectively identified 19

adults with aHUS that received eculizumab as first-line or rescue therapy.(39) The risk of reaching ESRD was reduced by one half compared with recent historical controls. Finally, in a prospective phase II trial in 37 patients (31 adults and 6 adolescents) with aHUS, eculizumab was associated with substantial renal recovery.(40) These data have led to the recommendation of eculizumab for aHUS indication as a first-line therapy.

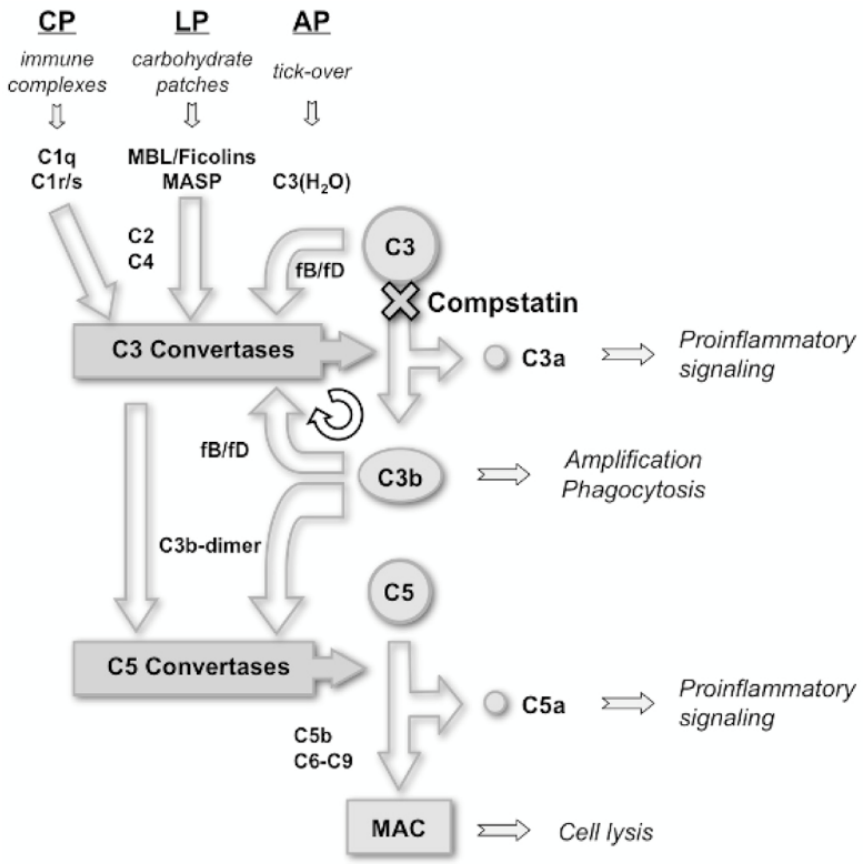
The phenotypic expression (DDD or C3GN) does not seem to predict the response to treatment, although biomarker studies have suggested a greater MAC activity in C3 GN compared with DDD.(41) Elevated sMAC was found to be a marker of response in accordance with the mechanism of action of eculizumab on terminal pathway activation.(42) Because C3 glomerulopathies are mainly characterized by persistent fluid–phase C3 convertase activity without predominant contribution of the terminal complement pathway, eculizumab may not be the therapy of choice in the majority of patients.

~15% of patients with aHUS are refractory to eculizumab. It is currently unclear whether it is caused by mutation in C5 (as shown in PNH (43), disease dominantly driven by C3 convertase cleavage products, or abnormalities in the coagulation cascade. After treatment with eculizumab, renal biopsies shows IgG-k staining of the glomeruli, tubular basement membrane, and vessel wall, consistent with deposition of eculizumab itself.(44) The long-term clinical effects of such binding of eculizumab to renal tissue are unclear.

2. Inhibition of the C3 convertase

a. **Compstatin** is a synthetic peptide that potently binds to C3 and its active fragment C3b (at the level of the C3 convertase and its components), Figure 3. It is currently in clinical trial for age-related macular degeneration (AMD), PNH, and C3 glomerulopathy.(45-47)

Figure 3. Effect of Compstatin on the complement system.



b. **The monoclonal antibody (mAb 3E7-H17),** 3E7, and its humanized chimeric counterpart, H17, bind C3b, C3b(H2O), and iC3b, but not native C3 or C4/C4b.(48) The mAbs compete with fB and fH for binding to C3b, preventing formation of C3 convertase and generation of iC3b, respectively; however, their precise mechanism of action is unclear. In an in vitro model of PNH, they inhibited deposition of C3b and abolished the hemolysis of PNH erythrocytes. Inhibition was due to mAb binding to fluid phase C3(H2O), preventing AP tickover and deposition of C3b on the surface of the endothelium.

3**. Soluble recombinant complement receptor 1 (CR1):**

Complement receptor 1 (CR1) regulates both the C3 and C5 convertases and is the only cofactor of CFI that can cleave iC3b into smaller fragments (C3c and C3dg). A soluble form of CR1 prevented dysregulation of the C3 convertase when administered in vitro to sera of patients with DDD with and without C3Nef.(49) In a murine model of C3

glomerulopathy, the soluble form of CR1 restored serum C3 levels to normal and cleared iC3b from the GBMs.

RENAL TRANSPLANTATION & HUS:

Renal transplantation has distinctive features that may trigger HUS in genetically susceptible recipients. These include donor kidney injury due to brain death with autonomic storm and procurement injury, warm/cold ischemia, ischemia-reperfusion injury, acute rejection, medications (calcineurin inhibitors [CNI]: cyclosporine and tacrolimus; mTOR inhibitors: sirolimus and everolimus), induction agents (alemtuzumab), and severe hypertension.

We studied a cohort of pediatric (N=447) and adult (N=786) renal transplant recipients with a diagnosis of HUS as their etiology of ESRD in the US between 1987 and 2013 using propensity score (PS) matching for accurate comparison.(50) The pediatric HUS patients had similar outcomes when compared with the PS matched controls, however adult patients with HUS had significantly lower graft survival and higher mortality. Rate of HUS recurrence after transplantation in both age groups was low (10%). When HUS recurred after transplantation, regardless of age group, it resulted in excessive allograft failure and significant elevation in mortality (approximately 33% at three-years).

Our findings highlight several important points which may affect clinical practice: 1) HUS recurrence, regardless of age group, has dire consequences including increased mortality and excessive graft loss. We think that an aggressive strategy of risk minimization pre-transplant (by avoiding complement amplifying conditions) and early treatment of HUS recurrence post-transplant (possibly with anti-complement treatment) may alter the course of disease and outcomes; 2) acute rejection is one of the most preventable triggers of HUS recurrence. We speculate that modification of immunosuppressive protocol (using an induction therapy followed with CNI based maintenance immunosuppression) and utilizing sensitive HLA testing may be necessary to diminish risk of rejection episodes; 3) CNIs do not seem to increase HUS recurrence post-transplant. We suggest that the benefit of using CNIs (cyclosporine or tacrolimus) in reducing acute rejection rate outweighs the risk of developing HUS resulting from CNIs.

De novo TMA occasionally occurs in the post-transplant setting; the reported incidence varies between 0.8% and 14% in different studies.(51) TMA is confined to the renal allograft in 38% of the cases without sign of systemic microangiopathic hemolysis and/or thrombocytopenia.(52) The etiology may be difficult to identify on the basis of renal biopsy but certain other findings may help to determine the underlying inciting event. Differential diagnosis includes acute antibody mediated rejection (AMR), immunosuppressive agents (CNIs and mTOR inhibitors, 1-15%), complement mediated-HUS, infections, anti-phospholipid syndrome, and de novo cancers. AMR appears to be the most common cause of TMA in renal allografts. Presence of glomerular arteriolar thrombi, peri-tubular capillary C4d staining, glomerulitis, endarteritis, and presence of donor specific antibody are typical findings for AMR. Satoskar et al reported that the incidence of de novo TMA was 6.1% (13.6% in C4d positive cases [N=243] and 3.6% in C4d negative cases, [N=715]) and AMR related TMA were mostly responsive to plasmapheresis therapy.(51) Complement regulatory protein mutations were also found to be an important risk factor for post-transplant TMA. Le Quintrec reported that 29% patients with de novo post-transplant TMA carried the mutations in CFH and CFI proteins.(53)

Conclusion:

Tremendous progress in our understanding of the AP dysregulation has made clear that distinct disease mechanisms underlie C3 glomerulopathies and aHUS. This may enable the optimal therapeutic approach.

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