Antibody Mediated Rejection in Kidney Allograft:

Role of Complement

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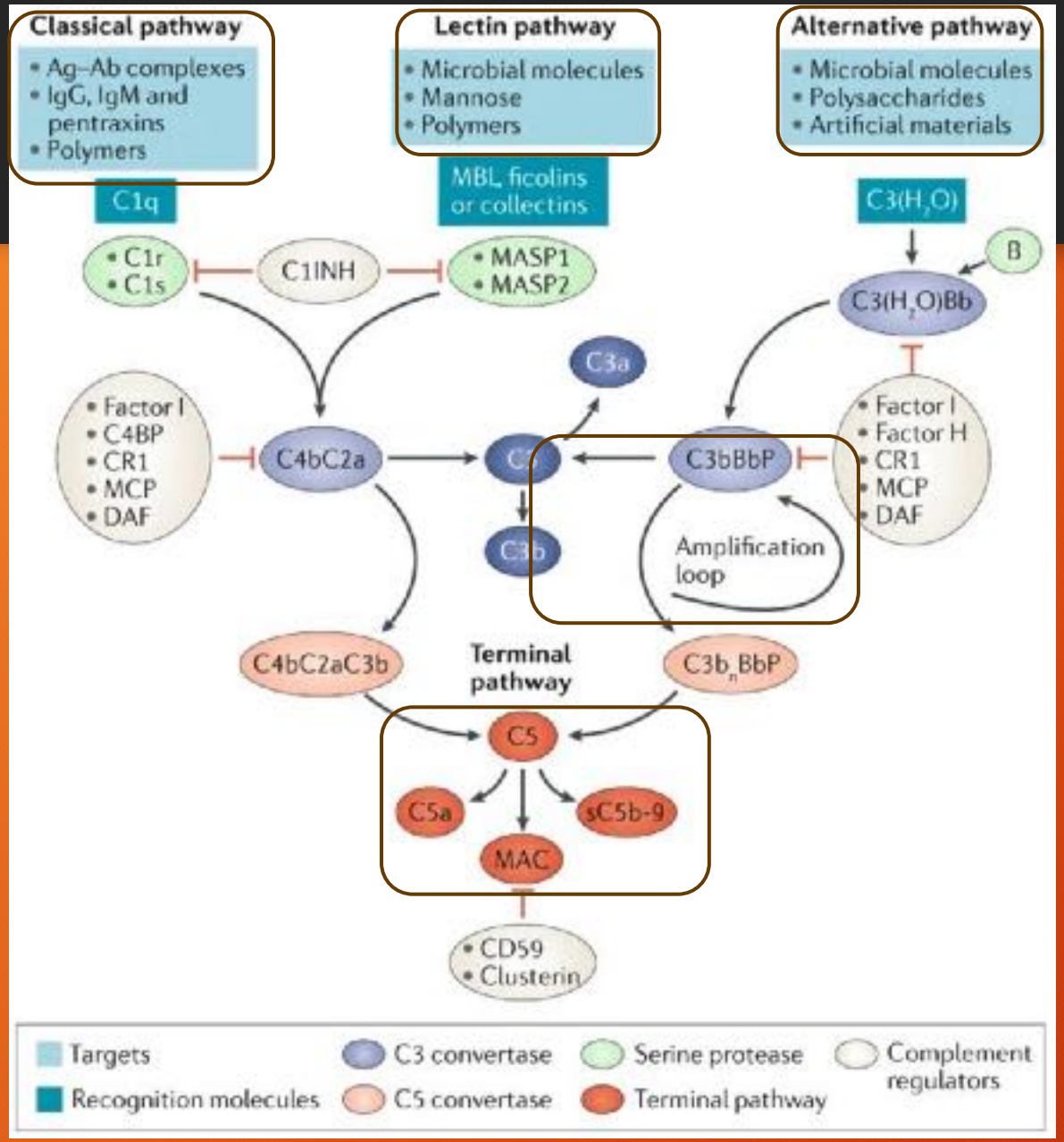
- Kidney transplantation (KT) is the optimal available therapeutic option for children with ESKD.
- Antibody-mediated rejection (AMR) remains the main immunological complication after KT and the leading cause of kidney graft loss.
- The main cause of AMR is the presence of preformed or de novo donor-specific antibodies (DSAs) against HLA
- DSA can mediate kidney graft injury primarily in a complement-dependent manner
- Non-HLA Abs [Angiotensin II receptor type 1 (AT1R), Endothelin-1 receptor type A (ETAR), MHC class I polypeptide-related sequence A (MICA)]have been shown to induce graft injury either by complement-dependent or -independent mechanisms.

Classic, lectin & alternative pathways complement activation

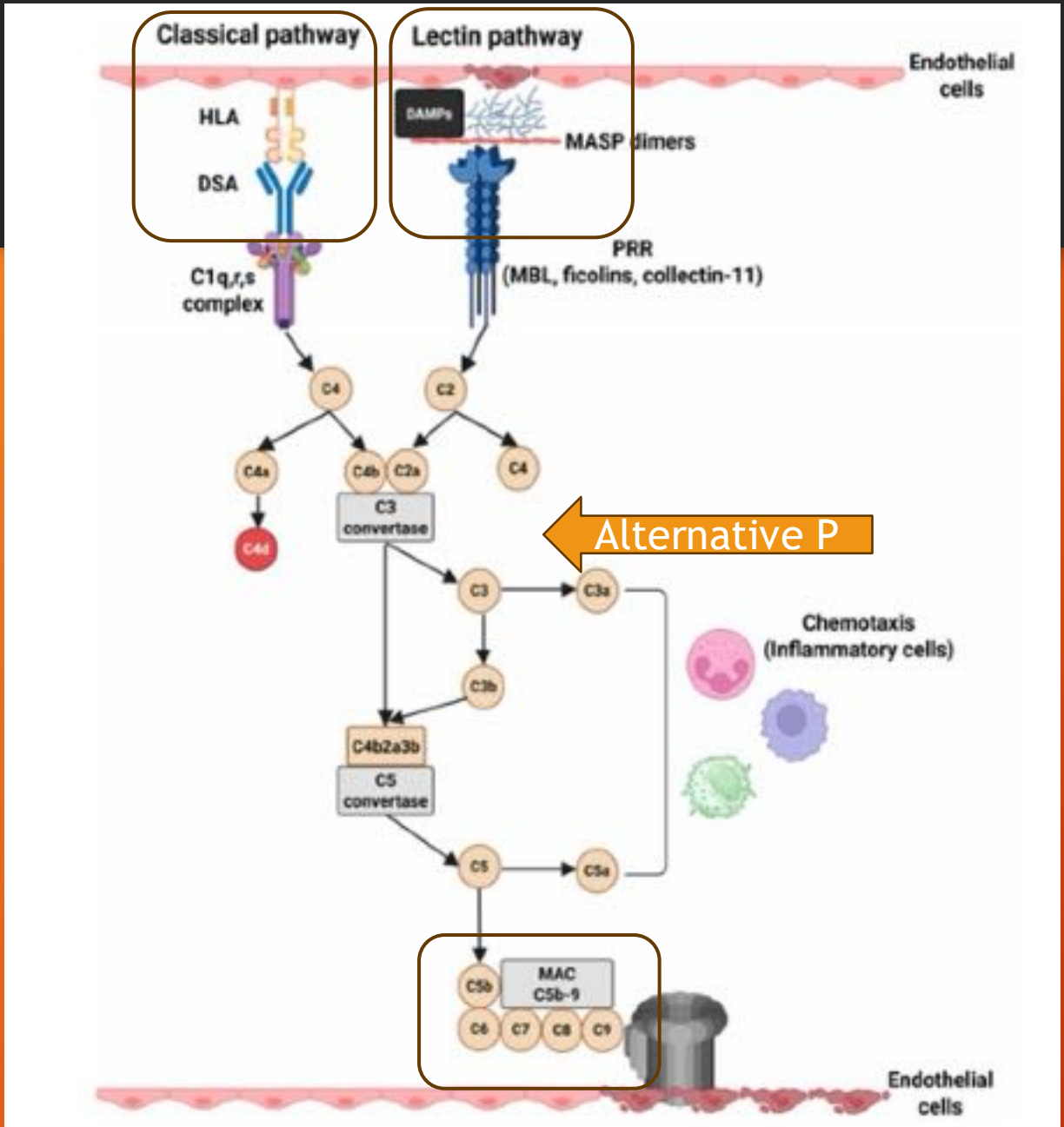


Recognition molecules within the 3 pathways are different and initiate the assembly of the C3 convertases C4bC2a (classical and lectin pathways) and C3bBbP (alternative pathway)

The alternative pathway includes a potent amplification loop that increases the generation of C3bBbP.

The 3 activation pathways converge into a common terminal pathway of which the first step is the proteolytic activation of C5

Nat Rev Nephrol 2018;14, 767–781 .



Classic & Lectin pathways activation in AMR

The classical pathway (CP) is the primary pathway involved in AMR, initiated when DSA (mainly IgG1 and IgG3 subclasses) bind to HLA on the donor graft endothelium.

Lectin pathway is triggered by recognition molecules (PRMs) like Mannose-Binding Lectin (MBL), bind to damaged carbohydrates on the ischemic graft endothelium.

The alternative pathway act as an amplification loop for classic, lectin pathways activation via formation of the C3 convertase C3bBb, which ultimately leads to formation of the membrane attack complex (MAC).

J. Clin. Med. 2025, 14, 2810

Agenda

- Multifaced Role of complement in KT
- Pathophysiology of complement mediated AMR
- Diagnosis of AMR
- Role of C4d in AMR pathophysiology & diagnosis
- Prevention and non-invasive diagnosis of AMR
- Children are immunologically different from adults
- Treatment of AMR

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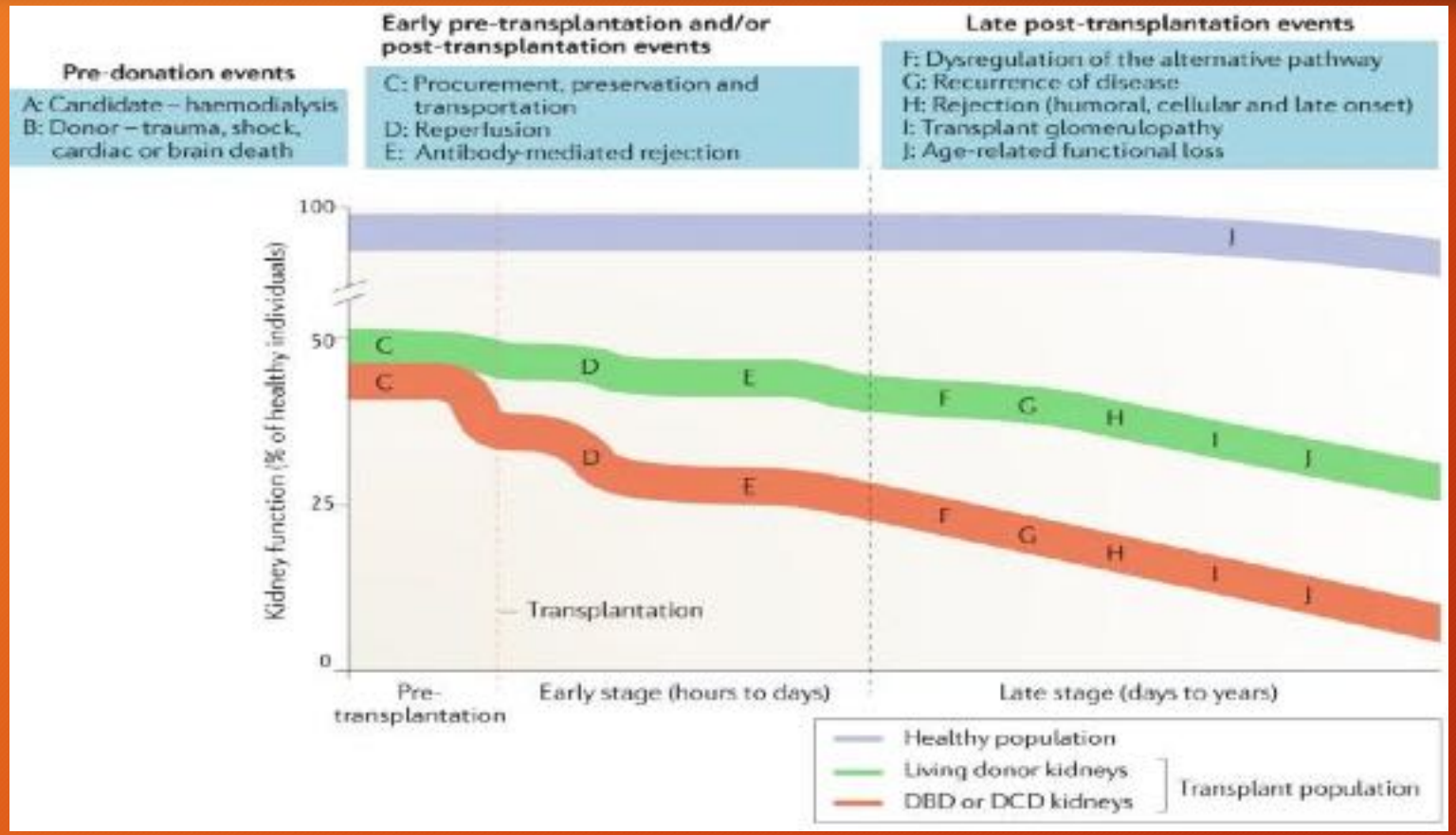
Hypothetical model of sequential events causing complement activation in kidney graft function.



A: HD
B:Donor type
C: Graft ischemia
D: Reperfusion
E:Presence of DSA
F:Long term AP dysregulation…original pathology
G: aHUS, C3G
H:Rejection
I:Transplant glomerulopathy

Complement likely has a role in all of these processes with the exception of age-related decline in kidney function

Nat Rev Nephrol 2018;14, 767–781

Table 1. Potential factors prior to or during KT that may increase complement activity in the transplanted kidney.

Recipient-Related Factors	Donor-Related Factors	Transplant-Related Factors
Comorbidities • Diabetes • HTN • Smoking	*Donor type* • Cadaveric donors (DBD, DCD)	*Graft preservation* • Cold storage • Normothermic machine perfusion
Cause of CKD • Atypical hemolytic uremic syndrome • C3 glomerulopathy • Lupus nephritis • AAV	*Comorbidities* • HTN	*Ischemia times* • Increased WIT • Increased CIT
Type of dialysis • Hemodialysis		*Immunosuppression* • CNI toxicity

HTN—hypertension; CKD—chronic kidney disease; AAV—ANCA-associated vasculitis; WIT—warm ischemia time; CIT—cold ischemia time; DBD—donation after brain death; DCD—donation after circulatory death; CNI—calcineurin inhibitors.

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1) Pre-operative (Decreased donors)

- Complement activation was reported to present in the kidneys of brain-death and circulatory-death donors prior to implantation.

Transplantation 2015, 99, 1293–1300

- In brain-death donor kidneys, complement activation via AP occurs both locally and systemically, contributing to inflammation and graft injury after implantation.
- Immunofixation for C3 has been observed in vascular endothelial cells and the glomerular area of brain-death donor kidneys .

Mol. Immunol. 2017, 84, 77–83.

- Elevated levels of C3a, C5a, and C5b-9 have been detected in the sera of deceased donors

Transplant. Proc. 2018, 50, 1697–1700

2) Intra-operative (Ischemia Reperfusion Injury)

IRI is unavoidable in KT. This process involves a two-stage injury, one determined by ischemia and the other by reperfusion

- **During ischemia:** hypoxia, which results in an acidic environment and a switch to anaerobic cell metabolism. With ATP depletion, release of damage-associated molecular patterns (DAMPs), and architectural damage to endothelial and renal tubular cells.
- **During reperfusion**: cellular damage is exacerbated by the generation of ROS. Damage of endothelial cells decreases surface complement regulation, favors complement activation, and promotes an inflammatory environment that sustains the process.

Clinical application: Prolonged cold ischemia time should be avoided, normothermic perfusion machines could be used or even prophylactic use of complement inhibitors in risky recipients

3) Post-operative (Infection, level of IS)

- Infections, underimmunosuppression, are both associated with complement activation after KT
- Infections such as CMV or BK virus can trigger complement activation and promote the development of DSA, which can culminate in AMR.

- Underimmunosuppression due to non-compliance or even a reduction in immunosuppression, in the context of severe infections, could lead to DSA formation, complement activation, and an increase in the risk of AMR.

The wise management of immunosuppression & infections in an individualized manner is decisive for preventing C-mediated graft injury.

Transplantation 2023, 107, 584–595.

4) Long-term (Chronic graft dysfunction)

- Chronic allograft injury can have both immune and nonimmune causes.
- In response to chronic injury, the affected structures respond by tissue remodeling and repair processes, which leads to IFTA, glomerulosclerosis, thickening of GBM, and vascular lumens. This is associated with kidney graft function decline and failure.
- The involvement of complement activation in IFTA development has been documented in basic research.

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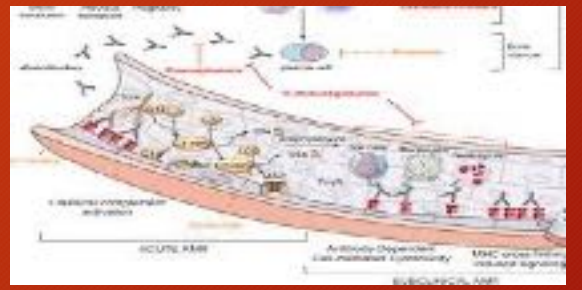
The Role of Complement in the Pathophysiology of AMR

DSA against HLA can cause antibody-mediated injury through complement-dependent and complement-independent mechanisms

- The vascular endothelium is the interface between the kidney graft and the recipient blood. It is the site of antibody–antigen interactions in AMR .



Complement-dependant mechanism

Presence of DSA with fixing-complement properties against HLA or non-HLA
The formation of immune complexes on the surface of the endothelium and complement activation by the CP

Complement-independent mechanisms

Direct DSA injury of the endothelium or indirect injury by the recruitment of inflammatory cells
More frequently in AMR with non-HLA antibodies

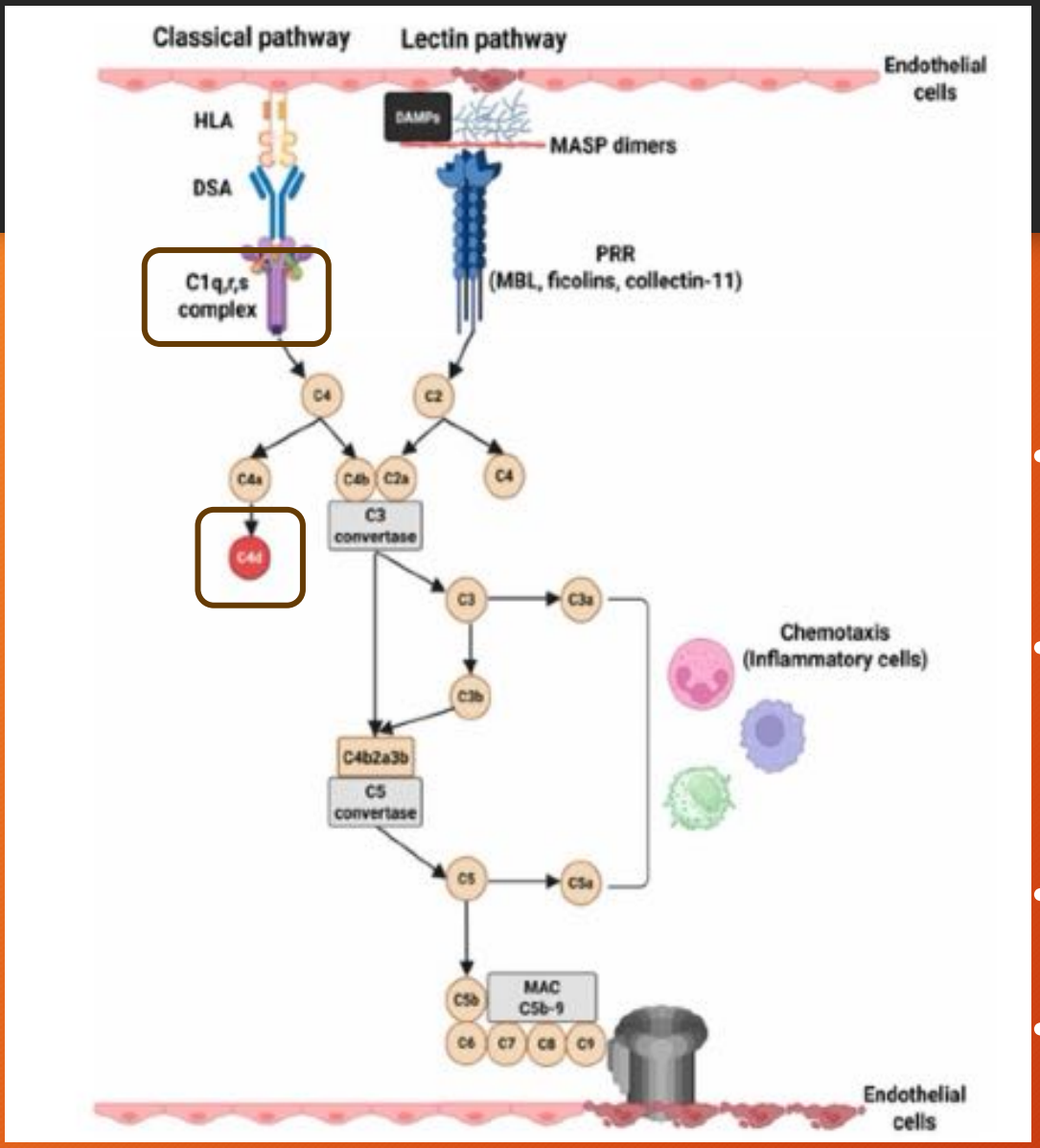
AMR is varying in severity

- DSAs have specificity, strength, and IgG subclass can modulate the AMR phenotype

Clin. J. Am. Soc. Nephrol. 2018, 13, 182–192.

- DSA with higher mean fluorescence (MFI) intensity and increased capacity for binding C1q and C3d, belonging to the IgG3 subclass, are highly pathogenic and are associated with negative graft outcomes

Immunol. 2019, 112, 240–246



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It begins when immune complexes bind circulating C1q

Subsequently, serine proteases C1r and C1s are activated and become capable of cleaving C4 into C4a and C4b fragments.

- C4a can activate protease-activated receptors 1 and 4, leading to stress fiber formation in endothelial cells and increased endothelial permeability.
- C4b on the one hand participates in the formation of the C3 convertase of CP and is subsequently cleaved to form C4d, an important histopathological feature of AMR.
- During complement activation, anaphylatoxins (C3a and C5a), C3b, and C5b-9 are generated.
- C3a and C5a determine the production of chemotactic cytokines and chemokines (interleukin-1, interleukin-6, interleukin-8, chemokine C-C motif ligand

AMR is a continuous process with varying degrees of active and chronic lesions

- AMR often evolves as a chronic progressive process, and it has been proven that insidious preformed or de novo DSA are involved in late onset AMR with chronic features.

Transplantation 2020, 104, 911–922.

- Longstanding activity of DSA and persistence of MVI create a pattern of repeated injury on the vascular endothelium, which leads to the occurrence of chronic lesions such as transplant glomerulopathy or peritubular capillary basement membrane multilayering.

J. Transplant. 2012; 2012, 193724.

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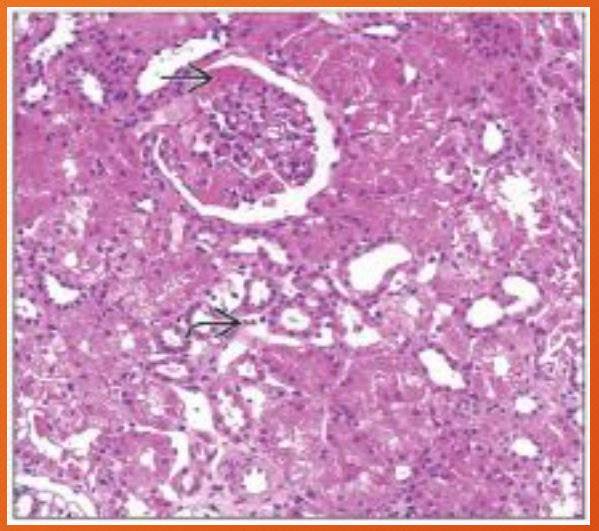
- Children are immunologically different from adults
- Treatment of AMR (Role of complement blockade)

AMR is a clinicopathological entity

Active AMR

Depending on the presence of active and chronic histological lesions, there are three subtypes of AMR: active AMR, chronic active AMR, and chronic AMR



Active AMR (3 mandatory features)

1) Histological evidence of acute tissue injury
2) Evidence of current/recent antibody interaction with the vascular endothelium
3) Serologic evidence of circulating DSA against HLA and non-HLA.

Am. J. Transplant. 2020, 20, 2318–2331

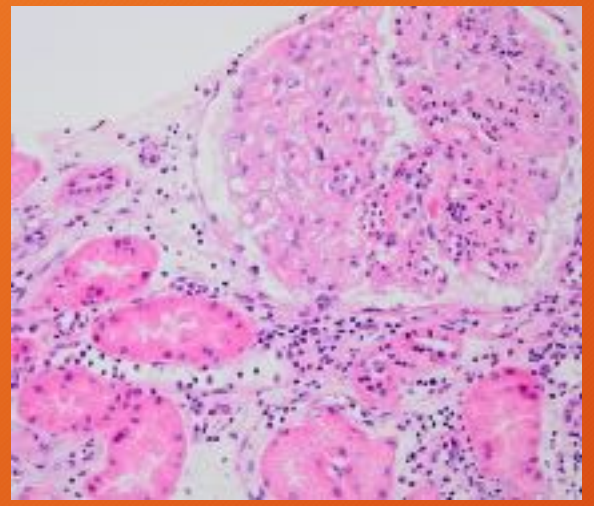
1) At least one of:
- MVI
- Intimal transmural arteritis
- Acute TMA

2) At least one of the following:
- Linear C4d staining in peritubular capillaries or medullary vasa recta
- At least moderate MVI
- Increased expression in the biopsy tissue of gene transcripts/classifiers strongly associated with AMR

AMR is a clinicopathological entity

Chronic active AMR

Depending on the presence of active and chronic histological lesions, there are three subtypes of AMR: active AMR, chronic active AMR, and chronic AMR



Chronic active AMR (3 mandatory features)

1) Morphological evidence of chronic tissue injury
2) Evidence of current/recent antibody interaction with the vascular endothelium
3) Serologic evidence of circulating DSA against HLA and non-HLA.

Am. J. Transplant. 2020, 20, 2318–2331

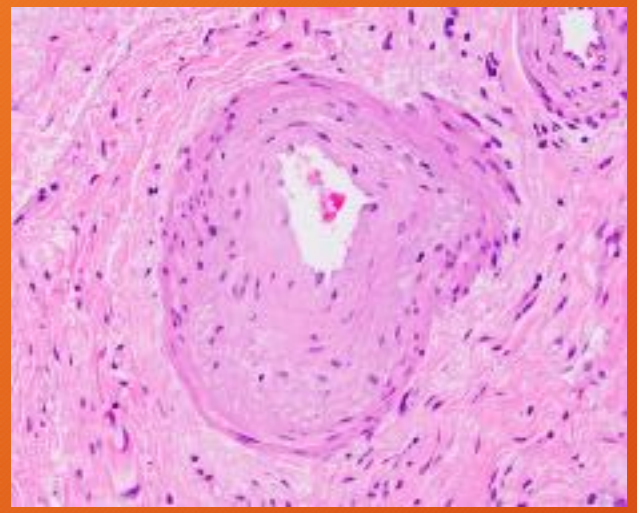
1) Presence of transplant glomeruopathy or severe peritubular capillary basement membrane multilayering .

2) At least one of the following:
- Linear C4d staining in peritubular capillaries or medullary vasa recta
- At least moderate MVI
- Increased expression in the biopsy tissue of gene transcripts/classifiers strongly associated with AMR

AMR is a clinicopathological entity

Chronic AMR

Depending on the presence of active and chronic histological lesions, there are three subtypes of AMR: active AMR, chronic active AMR, and chronic AMR



Chronic AMR (3 mandatory features)

1) Morphological evidence of chronic tissue injury
2) Absence of evidence of current/recent antibody interaction with the vascular endothelium
3) Prior documented diagnosis of AMR and/or documented evidence of DSA

Am. J. Transplant. 2020, 20, 2318–2331

1) Presence of transplant glomeruopathy or severe peritubular capillary basement membrane multilayering .

2) At least one of the following:
- Linear C4d staining in peritubular capillaries or medullary vasa recta
- At least moderate MVI
- Increased expression in the biopsy tissue of gene transcripts/classifiers strongly associated with AMR

That was The Banff 2019 Kidney Meeting Report After the Banff 2022 Kidney Meeting Report:

- Acute tubular injury was removed from the list of key histological features of AMR. It has been considered that its presence as a solitary feature is too common and remains to be reported along with other AMR lesions to further support the diagnosis of AMR

- Arterial intimal fibrosis of new onset, as a solitary lesion, was removed from the diagnostic features of AMR due to its nonspecific status

Am. J. Transplant. 2024, 24, 338–349.

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C4d is considered a footprint of antibody–antigen interaction on the surface of endothelial cells

- The link between AMR and complement by identifying the presence of complement cleavage product C4d in the peritubular capillaries of kidney graft biopsies was first demonstrated in 1991

Clin. Exp. Immunol. 1991,86, 464–470

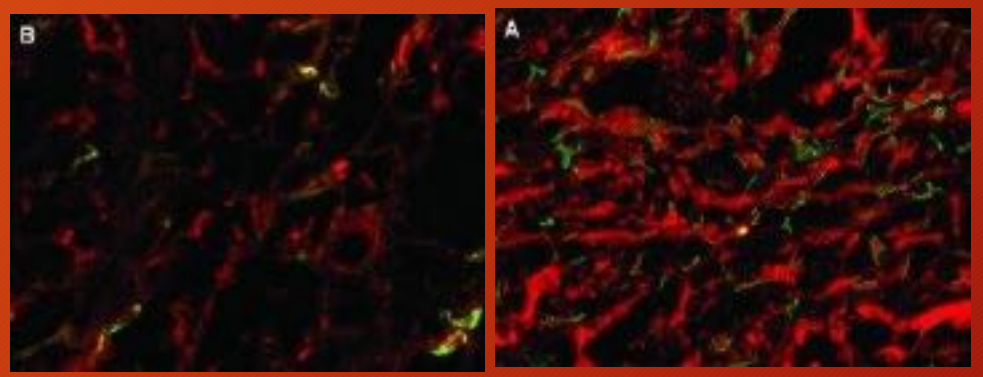
- C4d's structure allows it to form strong and durable covalent bonds with the vascular endothelium

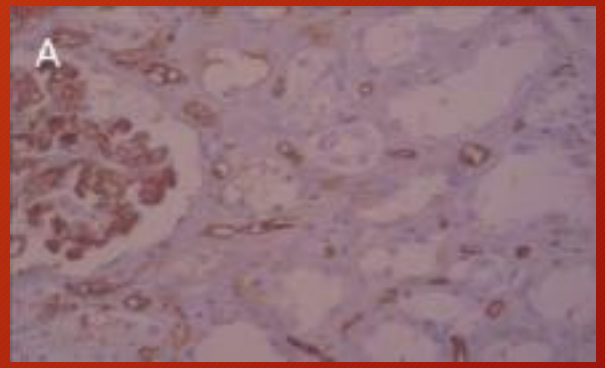
Kidney Int. 2012, 81, 628–639

- It was hypothesized that the endothelium of peritubular capillaries is the major site of complement activation because there are fewer anti-complement protective pathways than the endothelium of glomerular capillaries

J. Am. Soc. Nephrol. 1999, 10, 2208–2214.

- **By immunofluorescence,** C4d staining should be focal (–10–50% of peritubular capillaries and medullary vasa recta) or diffuse (>50% of peritubular capillaries and medullary vasa recta) in a linear pattern to be considered a criterion for current/recent antibody interaction with the vascular endothelium or an equivalent for serologic criterion (DSA).
- **By immunohistochemistry,** minimal staining for C4d (>0% but <10% of peritubular capillaries and medullary vasa recta) is sufficient to fulfill the aforementioned criteria





Transplantation 2018, 102, 1795–1814

C4d is NOT a universal marker for AMR diagnosis

Currently, C4d positivity is no longer a mandatory feature for AMR diagnosis and can be replaced by the presence of MVI or by molecular diagnosis of gene transcripts/classifiers

Am. J. Transplant. 2020, 20, 2318-2331

C4d-negative AMR

C4d-negative AMR

Lesions of MVI associated with the presence of DSA in absence of C4d

C4d Significance

- Banff Criteria
- Prognostic value
- Acute or chronic

C4d Limitations

- Staining can be inconsistent
- Intensity & location of staining
- TTT with C inhibitors may not always eliminate c4d deposition
- Not useful in non-HLA ab

It is not known whether the C4d-negative cases of AMR are caused by non-complement-mediated injurious effects of the DSA, or whether it simply reflects variability in the ability to detect the C4d

Front. Immunol. 2019;10:2380.

Thus, moderate microvascular inflammation (MVI) together with DSA are the mainstay in differentiating between:

- **AMR**
- Key 2022 Banff update is the term **"Probable AMR"** (MVI below threshold (g + ptc $\geq$ 2), DSA positive, C4d negative) and the exclusion of non-HLA
- Entity of **MVI**, DSA-negative, C4d-negative

according to the Banff 2022 classification

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Prevention & Non-invasive diagnosis of AMR

- **Why prevention of AMR in children is essential??????**
- Due to a higher risk of long-term immunologic complications related to life-long IS and a higher probability of multiple transplantations, especially in the absence of established treatment.
- **How to prevent AMR in children?????**
- Good organ matching, adequate IS, and the motivation of good compliance remain priorities in pediatric transplantation. At the same time, the recognition of early forms of AMR is essential

Clinical Transplantation. 2022;36:e14608.

Prevention & Non-invasive diagnosis of AMR

- Algorithms based on urinary metabolic patterns have been developed as screening for indolent forms of AMR.
- These methods are becoming current and, in the future, should permit to avoid systematic biopsy in children.
- Promising results obtained by using urinary CXC-Chemokine
- 133 urinary metabolite patterns with an excellent negative predictive value, but a low positive predictive value.
- An overlapping was observed of some key metabolites between AMR and TCMR..... biopsy confirmation before treatment will still be necessary

Clinical Transplantation. 2022;36:e14608.

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AMR in children different from adults

(1)The immaturity of the immune system and its variability with age.

For example, the capacity of mononuclear cells to synthesize interleukin 2 (IL2) is lower compared to adults at least until 12 years of age, entailing a lower stimulation of T cells proliferation following an exposition to alloantigen and possibly contributing to a better graft survival rate in children of <10 years of age compared to older transplanted patients.

Am J Transplant. 2021;21(S2):21-137

. (2) Lower exposure to sensitizing agents.

As multiple blood transfusions, during previous transplantation or pregnancy could increase the role of non-anti-HLA antibody (nHLA) mediated rejection, possibly contributing to an increased risk of graft deterioration, eventually with more undetected chronic subclinical AMR

Pediatr Nephrol. 2021;36(8):2473-2484

AMR in children different from adults

(3) The lower exposure to viral infection

Lower rate of CMV and EBV seropositivity, increases the risks associated with a primo-infection and the risk of presenting PTLD. At the same time, one-third of children have a positive BK bacteriuria, and the increased immunosuppression used in AMR could increase the risk of BK nephropathy.

Pediatr Nephrol. 2019;34(7):1155-1166

(4) More prolonged use of IS and the choice of more potent IS Drugs for AMR treatment increase the long-term risk of infections and malignancy

Clinical Transplantation. 2022;36:e14608.

Adults' protocols must be critically adapted to the single situation, and rational use of potent immunosuppressants is a priority in pediatric transplantation.

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Treatment of AMR is a major challenge

- Strategies have been addressed to preserve graft function, improve/reduce histologic lesions, and decrease antibody load depending on the clinical-histologic phenotypes of rejection .
- Therapeutic plasma exchange/ immuno-adsorbtion, intravenous immune globulins, anti-CD20 monoclonal antibodies, proteasome inhibitors, and interleukin-6 inhibitors.
- Unfortunately, these therapeutic regimens are not always effective, and sometimes evolution leads to persistence or recurrence of AMR and ultimately to chronic lesions and graft loss.
- Given the significant role of complement activation in AMR, terminal and proximal complement blockades have gained recognition in the treatment and prevention of AMR

C5 Inhibitors and C1 INH

- Eculizuimab has been successfully used in desensitization protocols for patients with a positive crossmatch, reducing the risk of AMR from an expected rate of 41%–7.7% at 3 months.

Am J Transplant. 2011;11:2405-2413.

- A cheaper alternative is C1-inhibitor (C1-INH), targeting the proximal part of the classical complement pathway. Originally used as a replacement therapy for patients with hereditary angioedema
- Data available on the use of C1-INH on AMR is limited to a few small trials showing encouraging trends but failing to show an improvement in graft survival compared to historical control.

Am J Transplant. 2016;16(5):1596-1603.

- Till now evidence for the use of complement inhibitors on AMR is poor and essentially based on case reports or small RCT

Clinical Transplantation. 2022;36:e14608.

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Home Message

- Complement plays a crucial role in the entire kidney transplant process, particularly in the onset and progression of AMR.
- There is a balance between complement-dependent and -independent mechanisms in the development of rejection lesions, which vary from patient to patient
- Classic and lectin pathways are involved in this process. C4d positivity is no longer a mandatory feature for AMR diagnosis but remains an independent predictor of negative outcomes.
- Current evidence regarding AMR treatment is limited and of a low quality
- The use of complement inhibitors in AMR, whether targeting the proximal or terminal pathways, should be part of a personalized approach as an adjunctive therapy to standard therapy

Thank you