Transplant rejection and other types of transplant pathology

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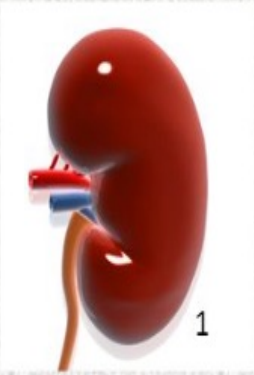
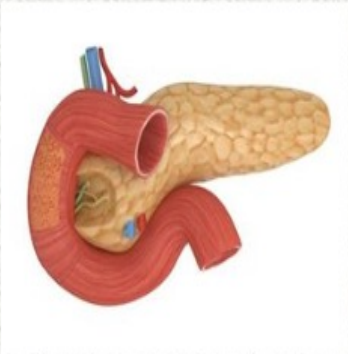
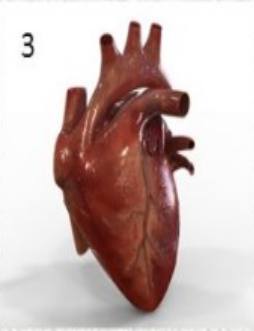
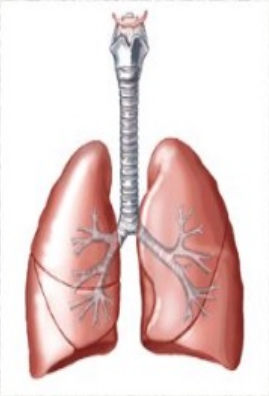
What can be transplanted?

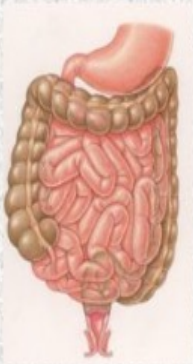


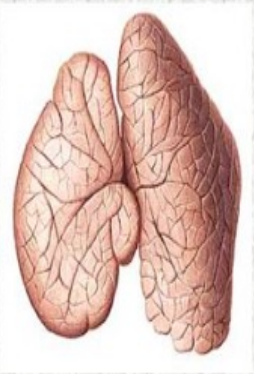


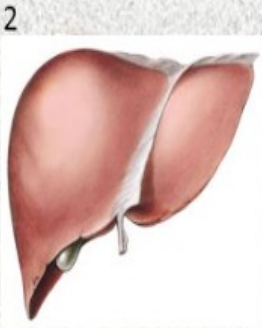


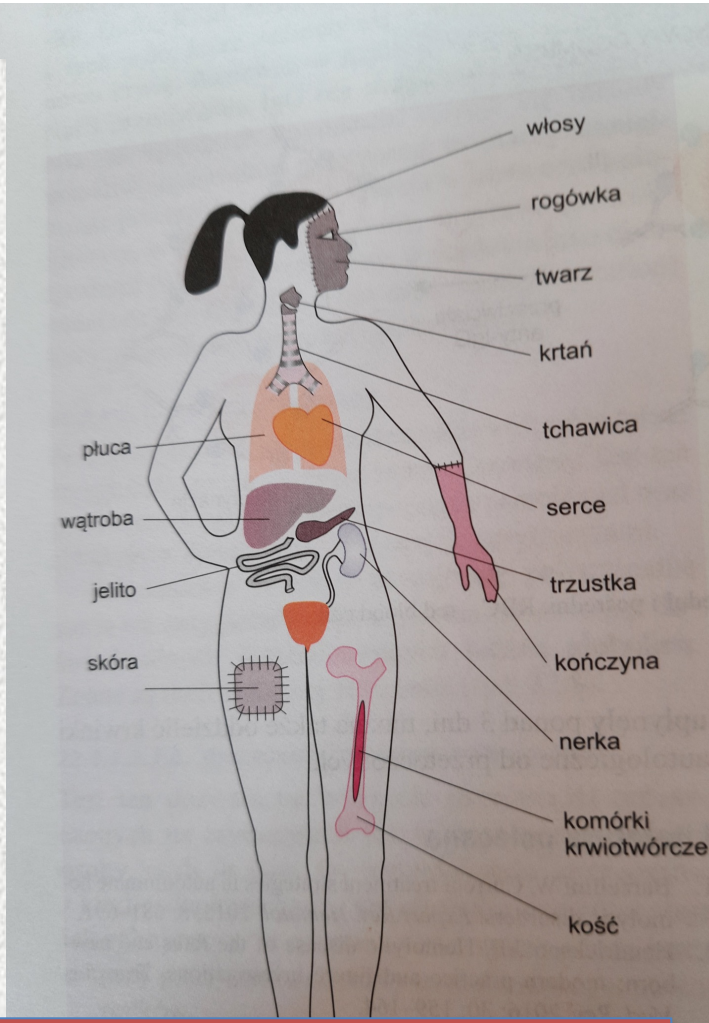
Organs











1. Solid organ
2. Tissue/tissue complex (vascularised tissue allograft)
3. HSCT/BMT

Kidney transplant pathology

- ATN and ischemic-reperfusion injury
- Rejection (acute, chronic, late-onset)
- Infection (BKV, CMV)…
- Thrombotic microangiopathy
- CNI nephrotoxicity (tacrolimus, CsA)
- Reccurrence of GN, *de novo* GN
- PTLD
- Lesions inherited from the donor

  (arteriosclerosis, glomerulosclerosis, tubular atrophy, interstitial fibrosis)

Deterioration of kidney graft function

Prerenal

- Hypovolemia, diarrhea, heart failure…

Renal (parenchymal)

- Rejection
- Reccurrence of primary disease, *de novo* glomerulopathy
- Urinary track infection
- Drugs (CNI, mTOR-i, NSAID, ACE-I, aminoglycosides)
- PVN (BKV) – SV40 in biopsy

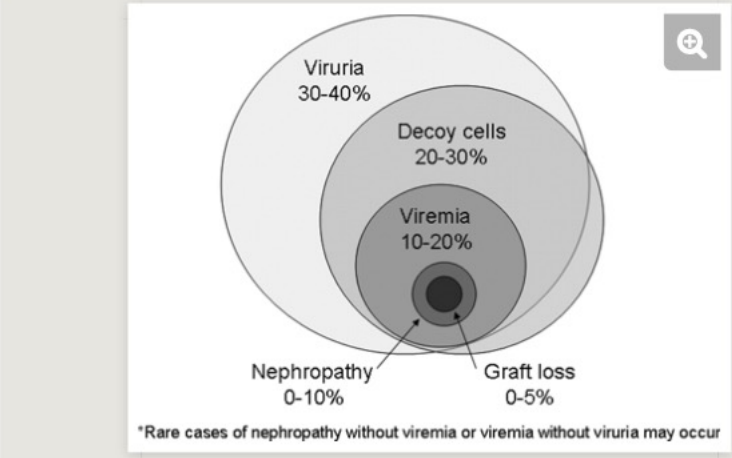


Figure 1. Download figure | Open in new tab | Download Powerpoint

Type and prevalence of BK virus (BKV) infections in kidney transplant recipients.

Deterioration in renal graft function

Postrenal

- Urine obstruction

calculi, ureteral stricture, BPH, neoplasms, hematoma, lymphocele, RPF…

+ hypertension, proteinuria, odema and raised SCr in clinical presentation

Kidney transplant pathology

Differential diagnosis of renal allograft dysfunction.

Differential Diagnosis of Renal Allograft Dysfunction

Week 1 Post-Transplantation

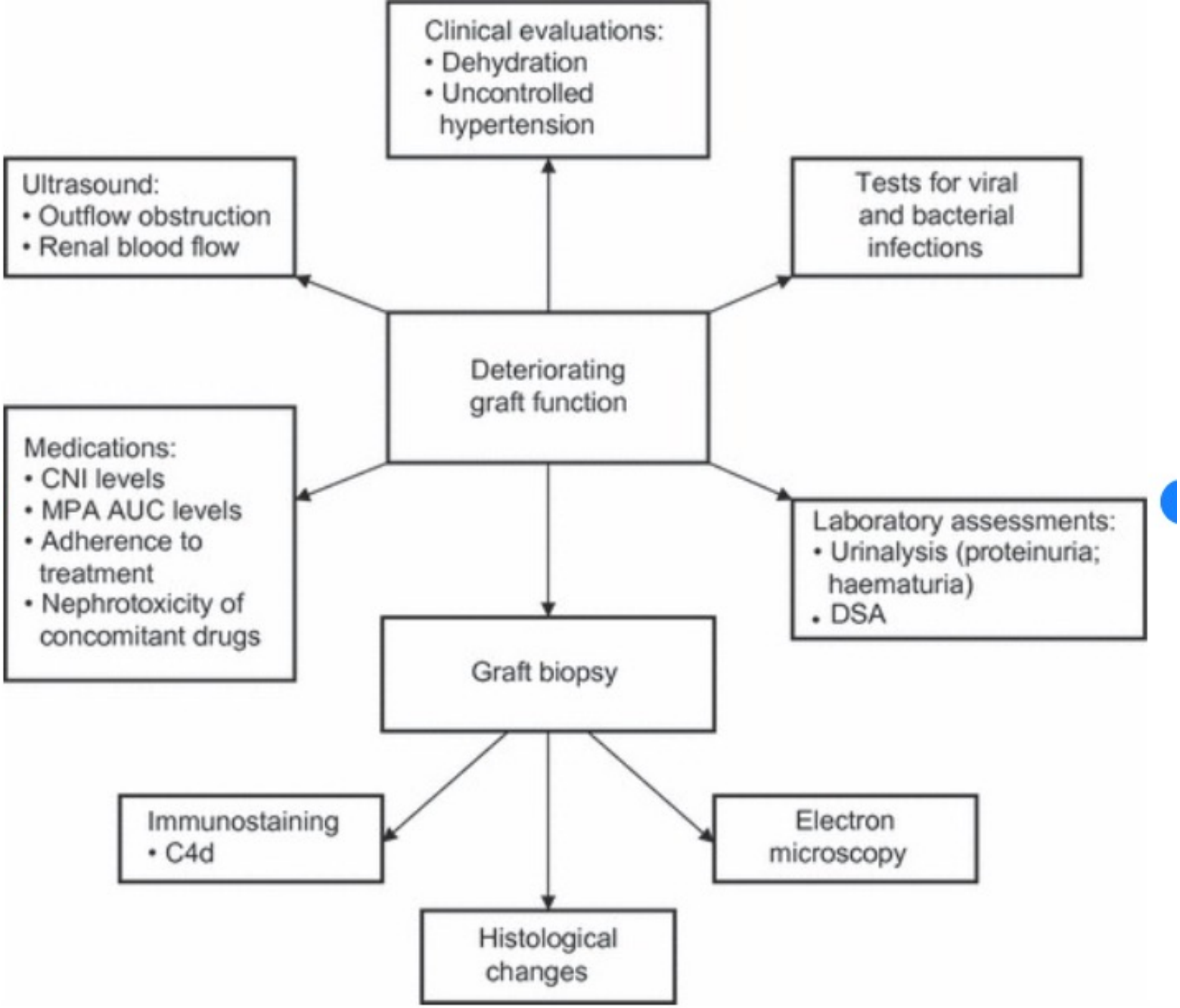
- Acute tubular necrosis
- Hyperacute or accelerated rejection
- Urologic
  - Obstruction
  - Urine leak
- Vascular thrombosis
  - Renal artery
  - Renal vein
- Volume contraction

<12 Weeks after Transplantation

- Acute rejection
- Calcineurin inhibitor toxicity
- Volume contraction
- Urologic
  - Obstruction
- Infection
  - Bacterial pyelonephritis
  - Viral infections
- Interstitial nephritis
- Recurrent disease

>12 Weeks after Transplantation

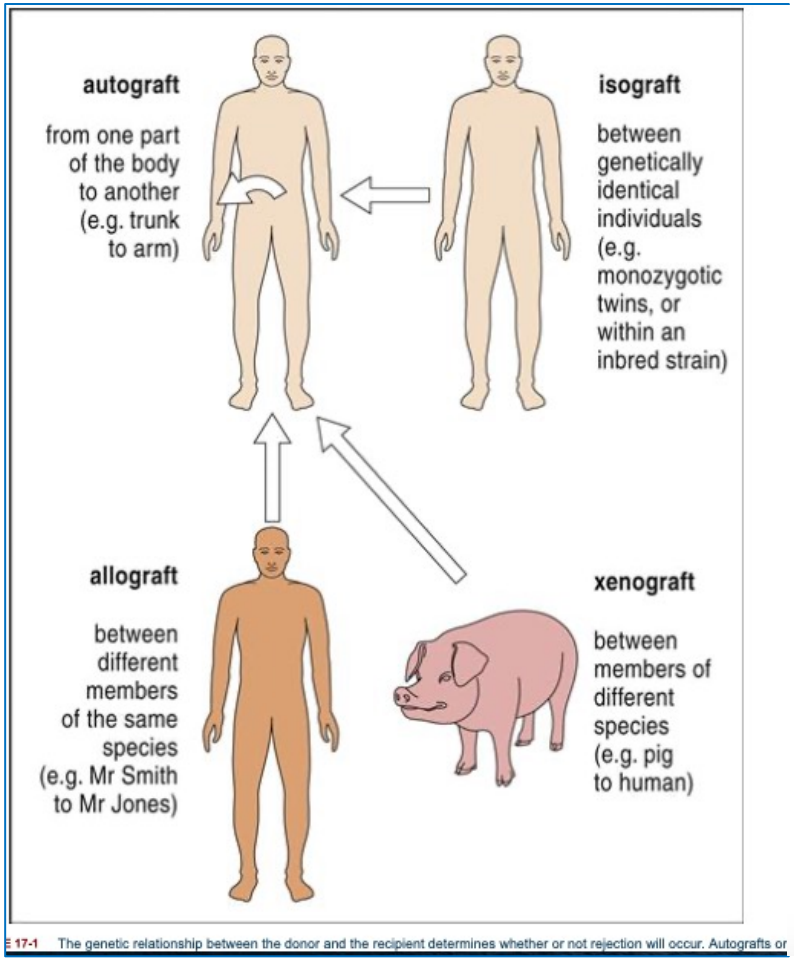
- Acute rejection
- Volume contraction
- Calcineurin inhibitor toxicity
- Urologic
  - Obstruction
- Infection
  - Bacterial pyelonephritis
  - Viral infections
- Chronic allograft nephropathy
- Recurrent disease
- Renal artery stenosis
- Post-transplantation lymphoproliferative disorder



Overview of investigations to determine the cause of kidney disease in patients with deteriorating graft function.
AUC, area under the curve; DSA, donor-specific antibodies; MPA, mycophenolic acid.

Transplant immunology

Types of transplants



E 17-1 The genetic relationship between the donor and the recipient determines whether or not rejection will occur. Autografts or

Box 100-1
Graft terminology.

Graft Terminology

Autograft (autologous graft): A graft from one part of the body to another. Examples include skin and vascular grafts. No rejection occurs.

Isograft (isogenic or syngeneic graft): A graft from one member of a species to a genetically identical member of the same species. Examples include grafts between identical twins and between members of the same inbred rodent strain. No rejection typically occurs.

Allograft (allogeneic graft): A graft between nonidentical members of the same species. Examples include grafts between unrelated or related nonidentical humans and between members of different inbred rodent strains. Rejection occurs by lymphocytes reactive to alloantigens on the graft (i.e., alloresponse).

Xenografts (xenogeneic grafts): A graft between members of different species. Examples include pig or baboon to human, and rat to mouse. Rejection occurs by lymphocytes reactive to xenoantigen on the graft (i.e., xenoresponse).

Transplant immunobiology

1. The principal function of the immune system
   is to defend against infection

2. Fundamental to this function is the capacity of the immune system
   to discriminate between self and non-self antigens

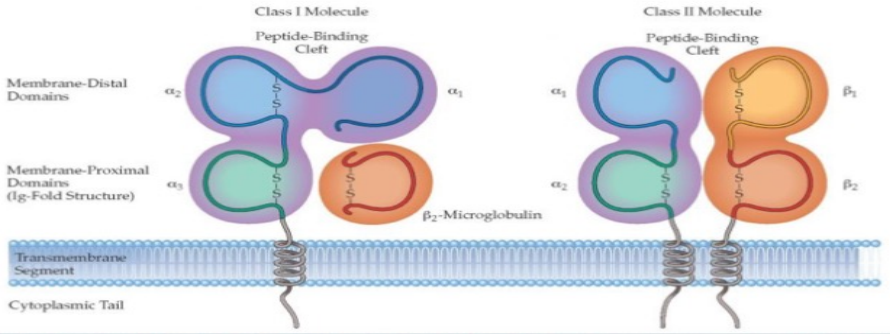
3. The immune response to allograft can be divided into:

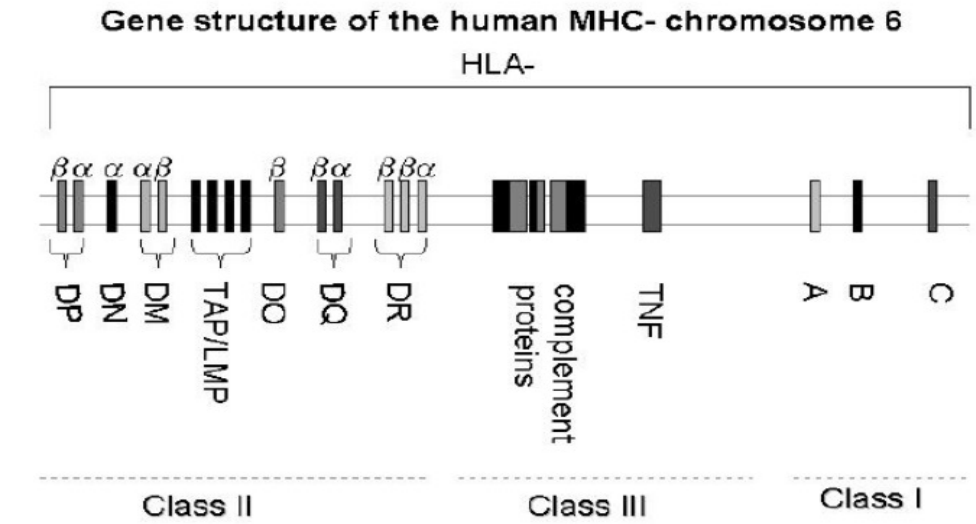
- recognition of foreign (non-self) antigens
- activation of antigen-specific lymphocytes
- the effector phase of graft rejection

MHC structure

1. The genes that determine the rejection or acceptance of graft are present in a locus on chromosome number 6
2. The MHC class I molecule is composed of a polymorphic alpha chain (3 domains) noncovalently attached to a nonpolymorphic beta2-microgobulin chain
3. The MHC class II consist of 2 alpha chains (two domains) and 2 beta chains (two domains), both polymorphic
4. Both class I and class II bind a peptide in their polymorphic region (peptide-binding groove)

	HLA complex							
MHC class	MHC I			MHC II			MHC III	
Region	A	B	C	DP	DQ	DR	C4, C2, BF	
Gene products	HLA-A	HLA-B	HLA-C	DP, αβ	DQ, αβ	DR, αβ	C' Protein	TNF-α TNF-β





Gene structure of the human major histocompatibiltiy complex (MHC)

Pathways of allorecognition

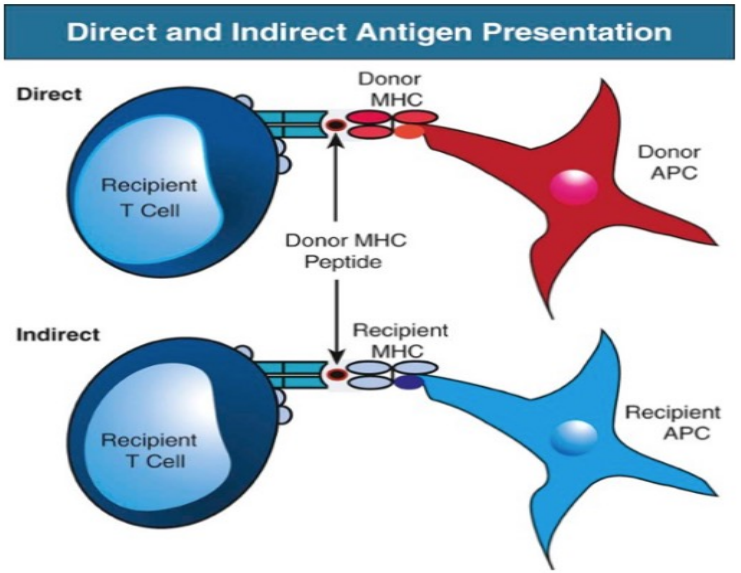
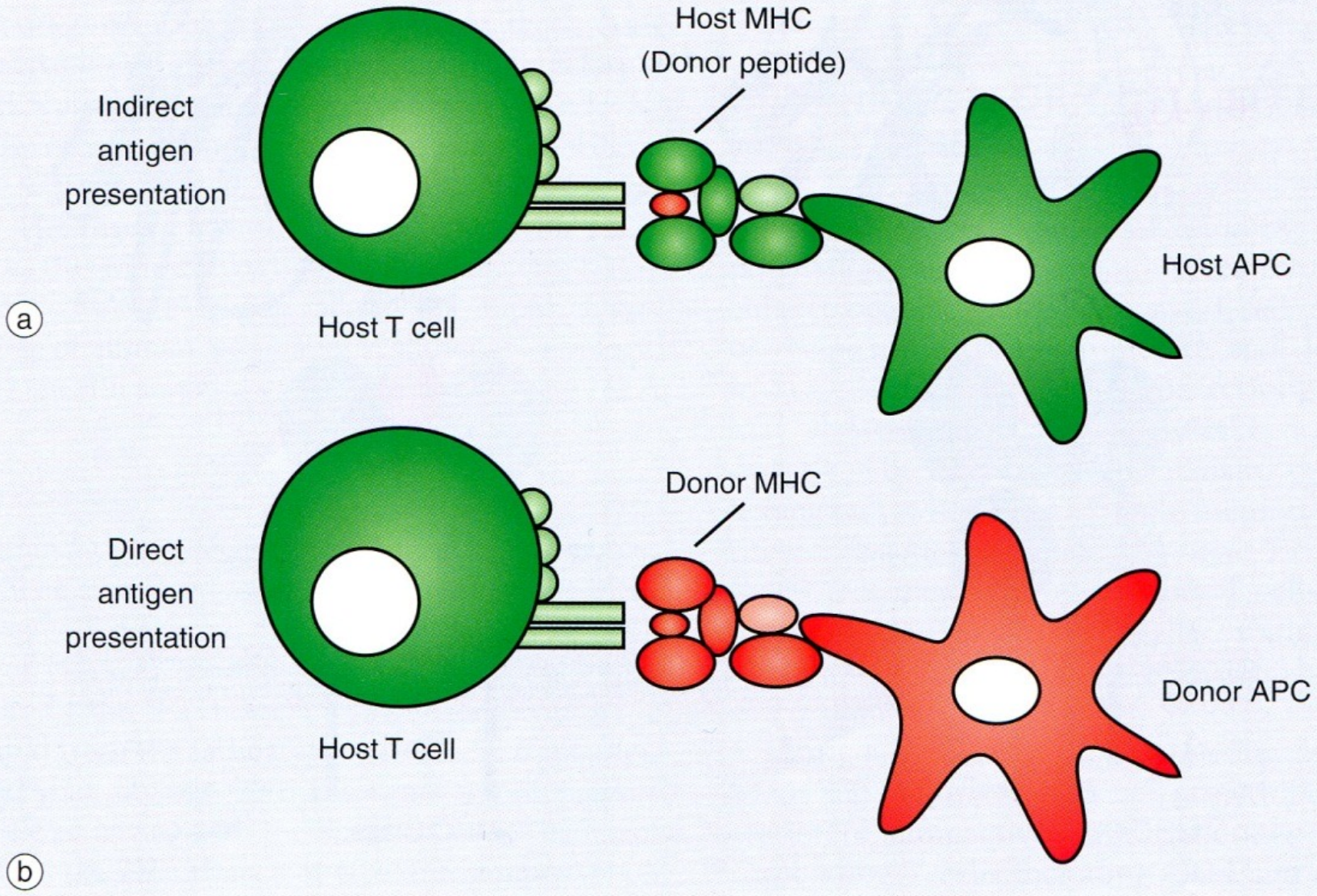


FIGURE 100-2 **Direct and indirect antigen presentation.** In direct allorecognition, don...

Indirect antigen recognition is the physiologic mechanism of foreign antigen presentation. Foreign antigen is taken up by APCs, processed intracellularly, and then presented as peptides on MHC molecules. During indirect allorecognition, donor MHC molecules are shed from the graft and processed by recipient APCs, where they are presented as peptides to recipient T cells in the context of recipient MHC molecules.[13] Because donor MHC molecules are continually shed from the graft and presented by recipient APCs, indirect allorecognition may play a larger role in the late alloresponse, including chronic rejection. However, the relative contribution of direct versus indirect allorecognition to the alloresponse at different time points after transplantation remains the subject of debate.

Direct and indirect antigen presentation



Three signal model of T cell acitvation

Signal 1 (stimulation, allorecognition)

- naïve T cells recognize alloantigen; nonself antigen is recognized by APC and presented in a complex with MHC.
- T-cell receptors recognize MHC-Ag complex on APC

Signal 2 (co-stimulation)

- is provided by the triggering of CD28 on the T cell by CD80/CD86 molecules on DC
- B7 family - CD28 and CD152(CTLA4)

Signal 3 (proliferation)

- T cells differentiate into various effector phenotypes Th1, Th2, secrete cytokines, infiltrate the graft
- activation of B cell depends on CD4

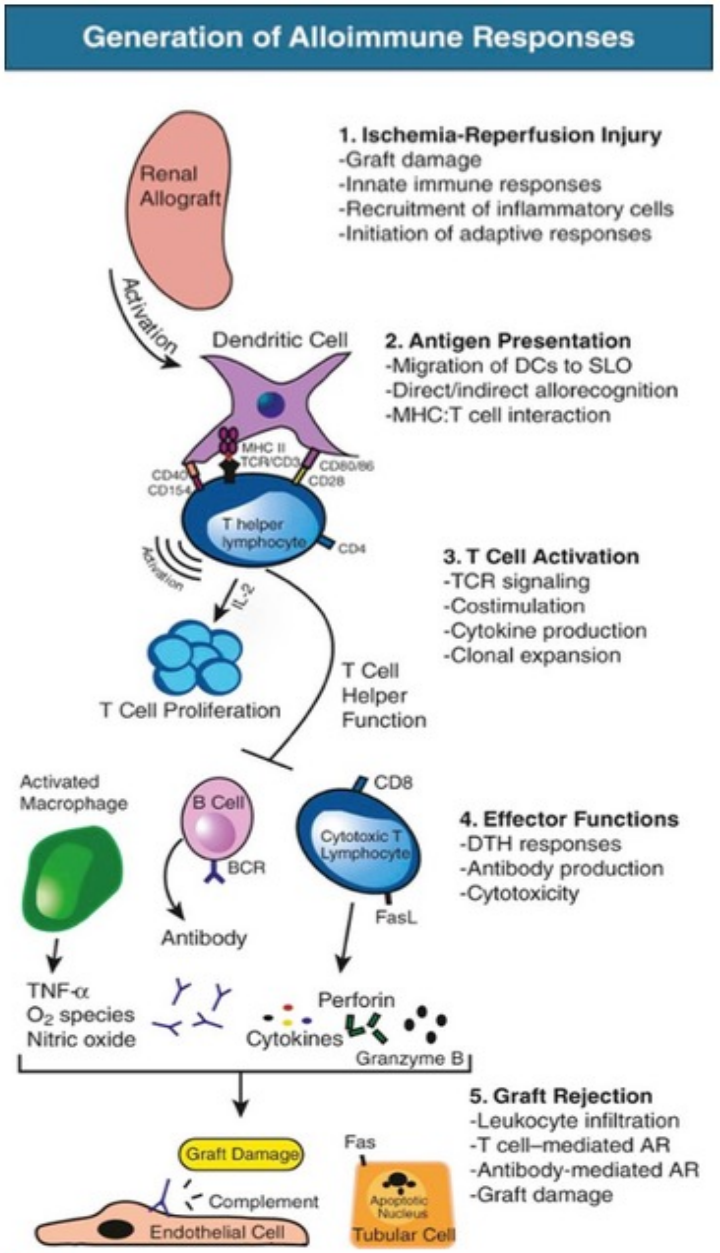
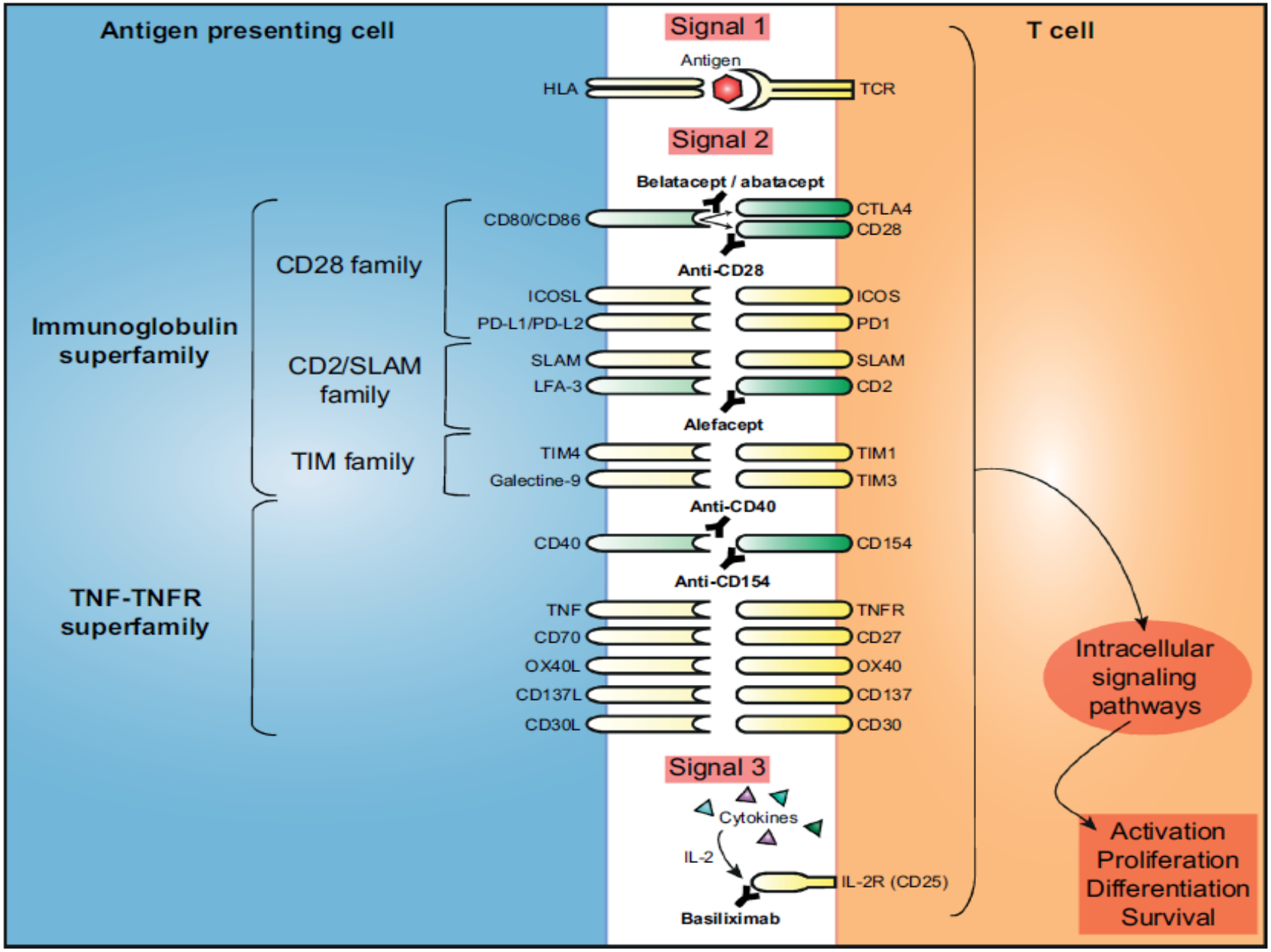
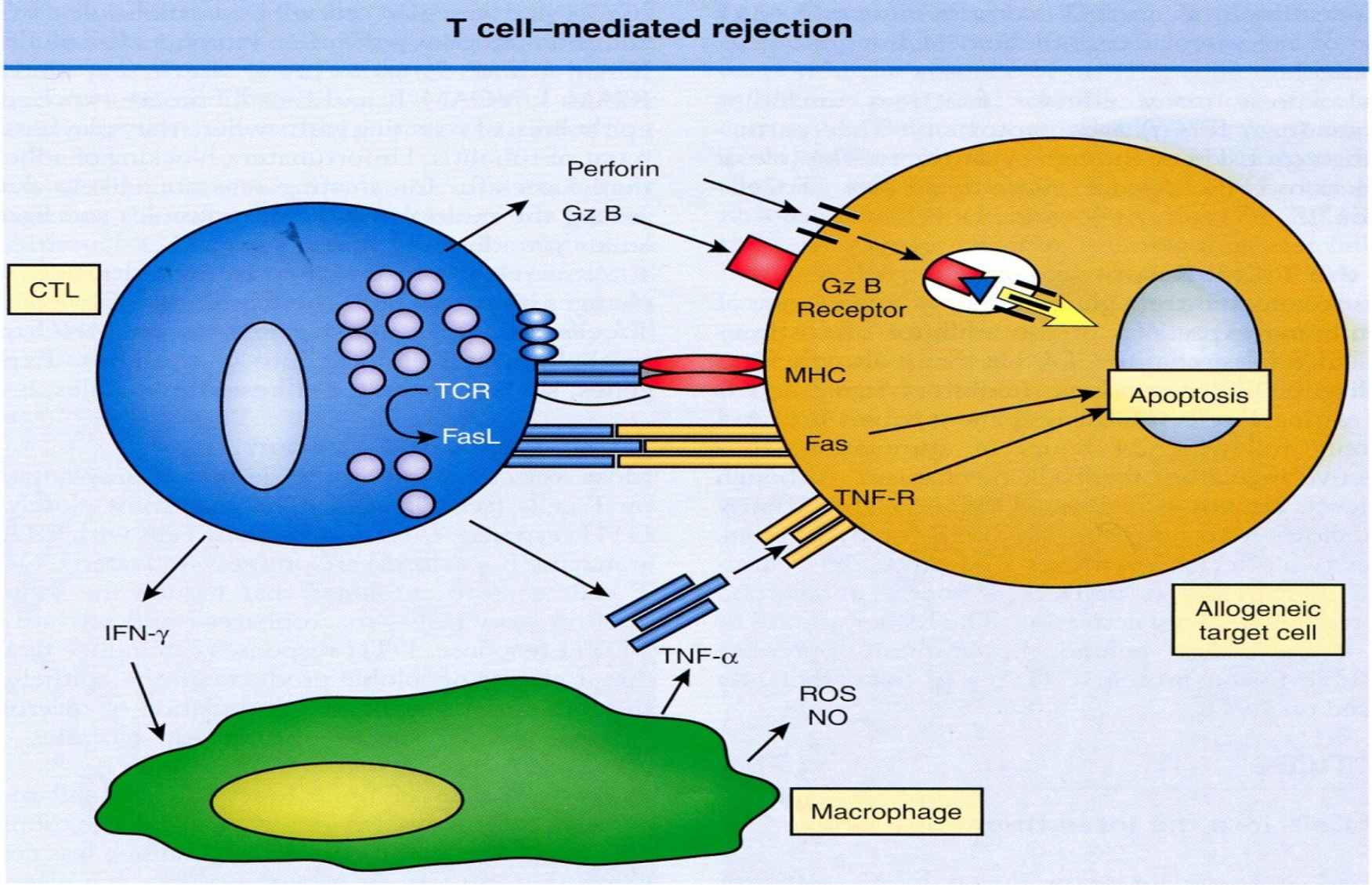
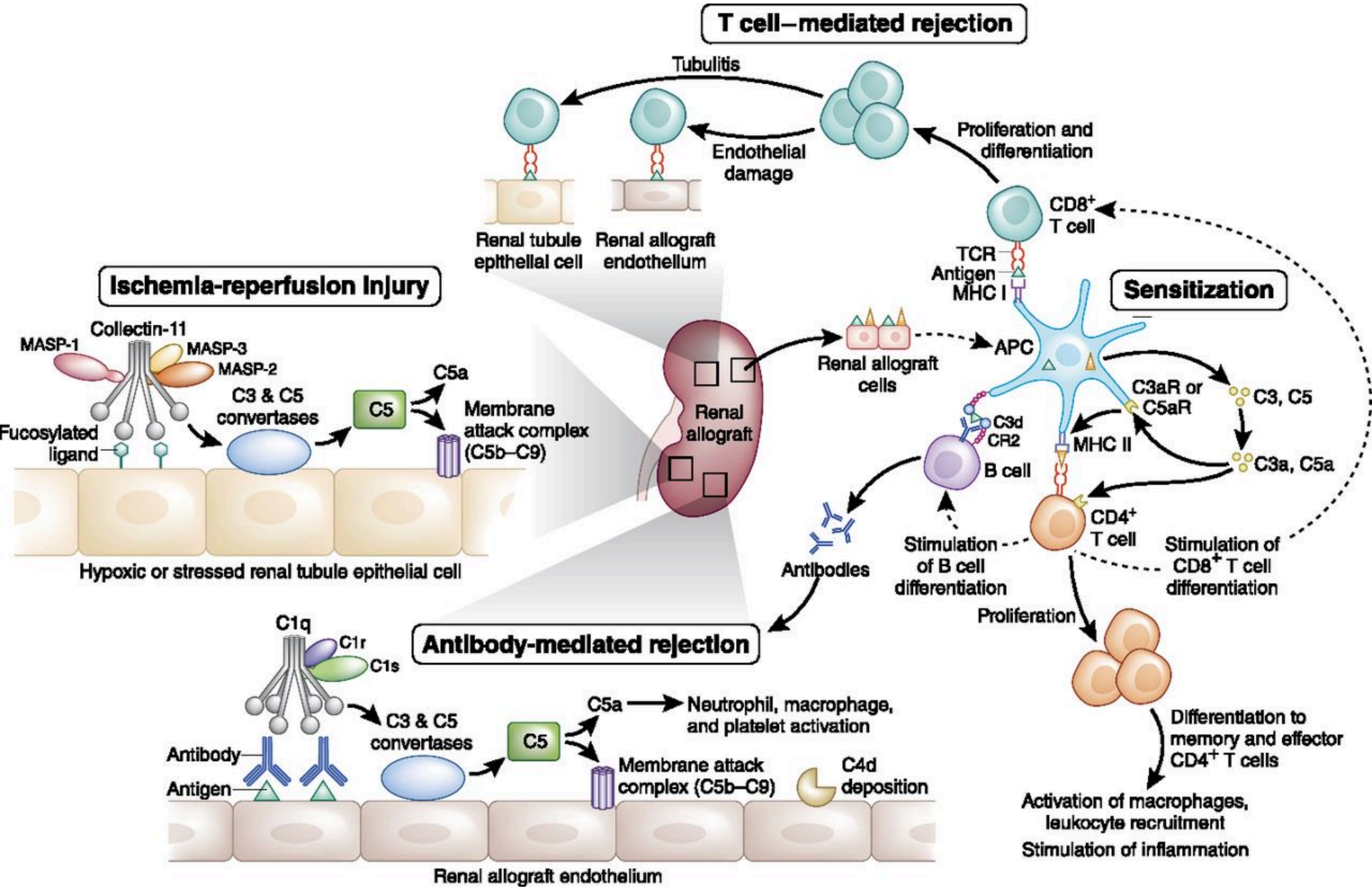


FIGURE 100-1 **Generation of alloimmune responses.** Immunologic responses after re.

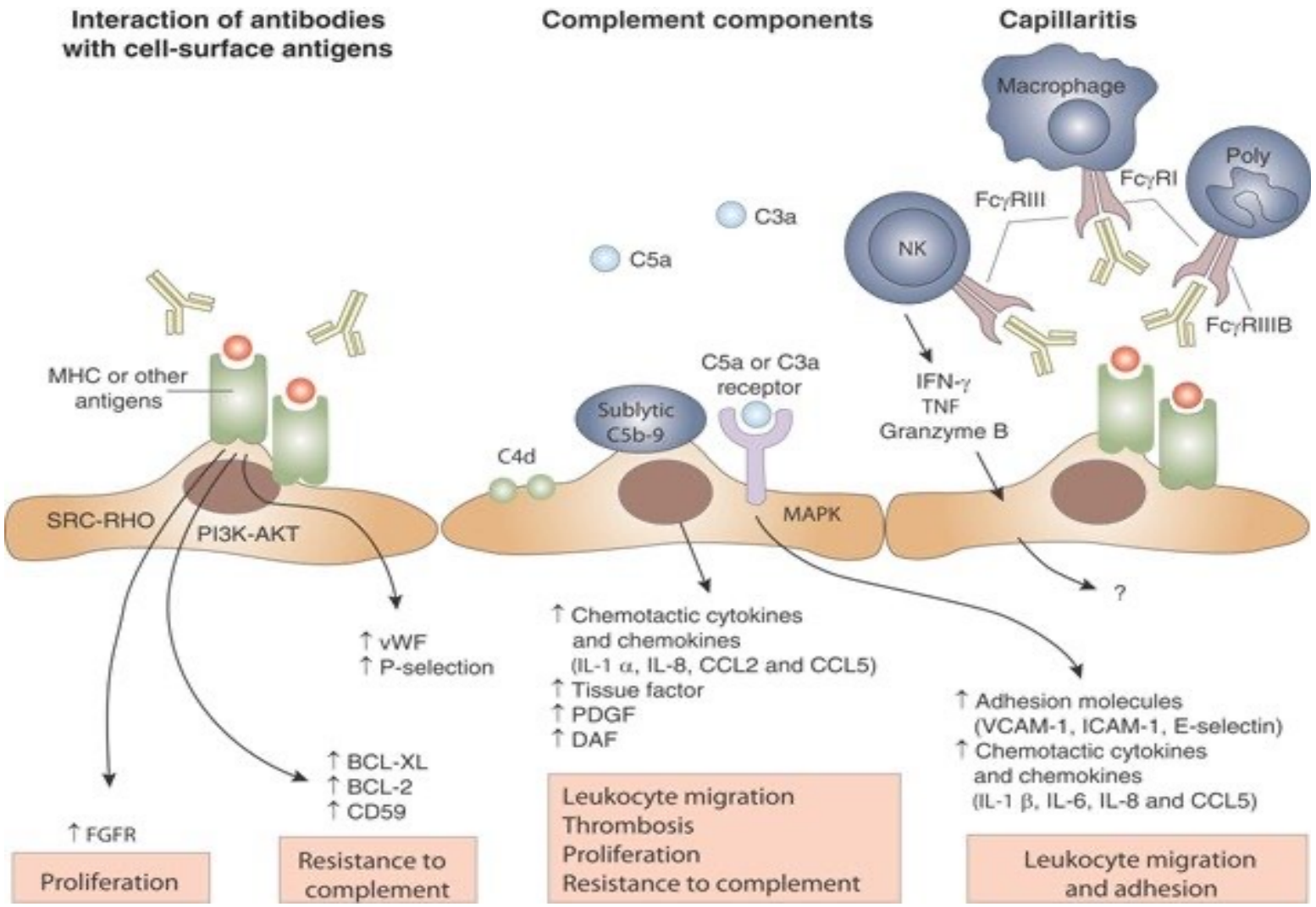

Antigen presenting cell
Signal 1
T cell
Antigen
HLA
TCR
Signal 2
Belatacept / abatacept
CD80/CD86
CTLA4
CD28
Anti-CD28
Immunoglobulin superfamily
CD28 family
ICOSL
ICOS
PD-L1/PD-L2
PD1
CD2/SLAM family
SLAM
SLAM
LFA-3
CD2
Alefacept
TIM family
TIM4
TIM1
Galectine-9
TIM3
Anti-CD40
CD40
CD154
Anti-CD154
TNF-TNFR superfamily
TNF
TNFR
CD70
CD27
OX40L
OX40
CD137L
CD137
CD30L
CD30
Signal 3
Cytokines
IL-2
IL-2R (CD25)
Basiliximab
Intracellular signaling pathways
Activation
Proliferation
Differentiation
Survival



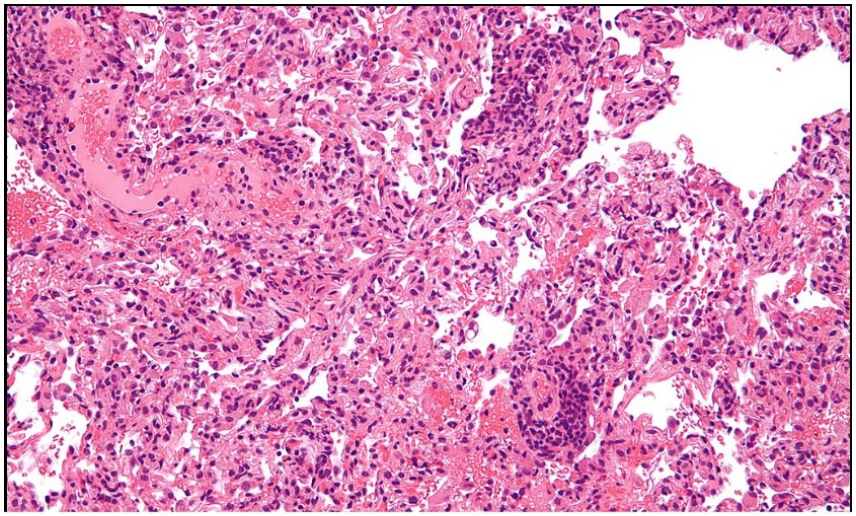
Cytotoxic **CD8 T cells** carry out their killing function by releasing two types of preformed cytotoxic protein: **the granzymes**, which seem able to induce apoptosis in any type of target cell, and **the pore-forming protein perforin**, which punches holes in the target-cell membrane through which the granzymes can enter


T cell–mediated rejection
Tubulitis
Endothelial damage
Proliferation and differentiation
Renal tubule epithelial cell
Renal allograft endothelium
CD8+ T cell
TCR
Antigen
MHC I
Sensitization
Ischemia-reperfusion Injury
Collectin-11
MASP-1
MASP-3
MASP-2
C3 & C5 convertases
C5
C5a
Membrane attack complex (C5b–C9)
Fucosylated ligand
Renal allograft
Renal allograft cells
APC
C3aR or C5aR
C3, C5
C3d
CR2
B cell
MHC II
C3a, C5a
CD4+ T cell
Antibodies
Stimulation of B cell differentiation
Stimulation of CD8+ T cell differentiation
Hypoxic or stressed renal tubule epithelial cell
Proliferation
C1q
C1r
C1s
Antibody-mediated rejection
C3 & C5 convertases
C5
C5a
Neutrophil, macrophage, and platelet activation
Antibody
Antigen
Membrane attack complex (C5b–C9)
C4d deposition
Differentiation to memory and effector CD4+ T cells
Activation of macrophages, leukocyte recruitment
Stimulation of inflammation
Renal allograft endothelium

Antibody-mediated rejection



Transplant rejection



graph of **lung transplant rejection**. Lung biopsy. H&E stain. Features: Perivascular lymphocytic infiltrate. +/-
s. +/- Neutrophils. Related images Intermed. mag. Very high mag.

- transplant rejection is an immune inflammatory reaction
- graft is rejected by the recipient`s immune system that leads to graft dysfunction

Types of rejection

<u>According to the alloimmune response in time</u>

- Hyperacute – within minutes/hours after engraftment; anti-HLA Ab`s in R, ABO incompatibility;

  excluded by biological XM
- Acute – within few days-months (cellular, humoral, mixed)
- Chronic > 1 year following tx (cellular, humoral, mixed)

  within weeks-months
- GvHD in BMT (allo-HSCT)

<u>According to the damage pattern</u>

- Cellular rejection (T-cell mediated)
- Antibody-mediated rejection (ABMR)

Risk factors for acute rejection

Risk Factors for Acute Rejection

High Risk

- Sensitization (high panel reactive antibody percentage)
  - Previous transplantation
  - Pregnancy
  - Transfusion
- Delayed graft function
  - Deceased donor source
  - Increased donor age
  - Prolonged ischemic time
  - Donor brain death
  - Donor acute renal dysfunction
- HLA mismatching
- Positive pretransplantation B cell crossmatch
- ABO incompatibility
- Corticosteroid minimization
- Infection
  - Bacterial pyelonephritis
  - Cytomegalovirus
- Adolescent recipient
- African American recipient
- Previous rejection episode

Low Risk

- Zero HLA mismatch
- Elderly recipient of young donor kidney
- Preemptive transplantation
- Living donor source
- First transplant

Acute rejection

- T-cell mediated AR (tubulitis or endarteritis)
- Antibody-mediated rejection
  (microcirculation inflammation, PTC-itis with C4d /+/ or C4d /-/)

- Within first 3 months after tx, graft function deterioration (AKI) or subclinical rejection with stable graft function

- Clinical symptoms?
  -decrease in urine output, hypertension
  pain in the area of the graft, low-grade fever
  -subclinical

- **Graft biopsy!**

Graft biopsy

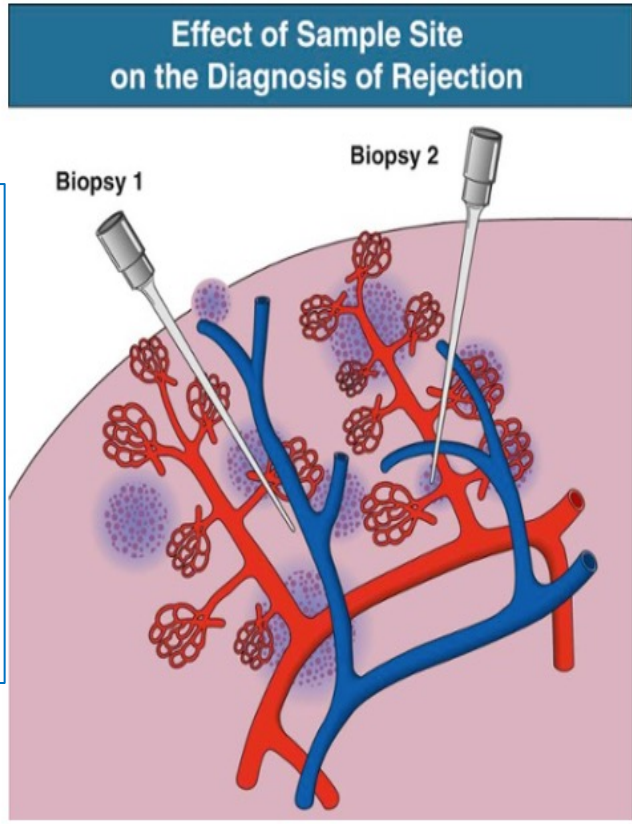
70% cellular rejection

In sensitized recipients – ABMR 40- 90%!

In non-sensitized ABMR is 5-7%

Acute ABMR is combined with cellular rejection in 25%

An isolated form of ABMR are rare



...osis of rejection. Acute

Rejection (both acute and chronic) is defined by histologic findings after kidney transplant biopsy. A biopsy considered adequate for analysis involves sampling of at least 10 glomeruli and two small arteries, stained with hematoxylin-eosin (HE), periodic acid-Schiff (PAS) or silver, and trichrome stains; a biopsy with seven to nine glomeruli and one artery is considered of marginal adequacy. When biopsy is performed for clinical indications (renal dysfunction), two separates cores should be obtained because the findings of rejection are often patchy in distribution (Fig. 104-2).[3] There is not a consensus of opinion regarding the number of cores required when biopsies are performed for nonclinical indications (e.g., in protocol-driven practice), although adequate tissue sampling defined by the previously described criteria is preferable if performed.

Protocol renal graft biopsy

- Subclinical rejection may be treated before kidney function deteriorates
- Early therapeutic intervention, modification of IS regimen prevents from irreversible chronic lesions
- Biopsy reveals:
  - Subclinical Tcell-AR
  - Subclinical ABMR
  - IF/TA
  - Recurrence of glomerulonephritis
  - PVN (BKV)
- Limitation – invasive procedure

Classification of cellular rejection

T Cell Mediated

ACUTE

Mononuclear cell interstitial inflammation and tubulitis and/or arteritis

- IA: More than 25% interstitial infiltration, 4 to 10 mononuclear cells/tubular cross section
- IB: More than 25% interstitial infiltration, >10 mononuclear cells/tubular cross section
- IIA: Intimal arteritis, mild to moderate (0% to 25% of luminal area)
- IIB: Intimal arteritis, severe (>25% of luminal area)
- III: Transmural arteritis and/or fibrinoid change and necrosis of medial smooth muscle cells with accompanying lymphocyte inflammation

CHRONIC

Arterial intimal fibrosis with mononuclear cell infiltration in fibrosis, formation of neointima

BORDERLINE

Presence of 10% to 25% interstitial infiltration, <4 mononuclear cells/tubular cross section

Classification of humoral rejection

Classification of rejection. *ATN*, Acute tubular necrosis.

BANFF Classification of Rejection

Antibody Mediated

ACUTE

C4d$^+$, presence of circulating antidonor antibodies and acute tissue injury

I. ATN-like (minimal inflammation)
II. Capillary and/or glomerular inflammation and/or thromboses
III. Arterial inflammation

CHRONIC

C4d$^+$, presence of circulating antidonor antibodies and chronic tissue injury

(1) Glomerular double contours, (2) peritubular capillary basement membrane multilayering, (3) tubular atrophy or interstitial fibrosis, and/or (4) fibrous intimal thickening in arteries

TABLE 4 Updates of 2019 Banff classification for ABMR, borderline changes, TCMR, and polyomavirus nephropathy. All updates in boldface type[a]

Category 1: Normal biopsy or nonspecific changes

Category 2: Antibody-mediated changes

Active ABMR; all 3 criteria must be met for diagnosis

1. Histologic evidence of acute tissue injury, including 1 or more of the following:
- Microvascular inflammation (g > 0 and/or ptc > 0), in the absence of recurrent or de novo glomerulonephritis, although in the presence of acute TCMR, borderline infiltrate, or infection, ptc ≥ 1 alone is not sufficient and g must be ≥ 1
- Intimal or transmural arteritis (v > 0)[b]
- Acute thrombotic microangiopathy, in the absence of any other cause
- Acute tubular injury, in the absence of any other apparent cause

2. Evidence of current/recent antibody interaction with vascular endothelium, including 1 or more of the following:
- Linear C4d staining in peritubular capillaries or medullary vasa recta (C4d2 or C4d3 by IF on frozen sections, or C4d > 0 by IHC on paraffin sections)
- At least moderate microvascular inflammation ([g + ptc] ≥2) in the absence of recurrent or de novo glomerulonephritis, although in the presence of acute TCMR, borderline infiltrate, or infection, ptc ≥ 2 alone is not sufficient and g must be ≥1
- Increased expression of gene transcripts/classifiers in the biopsy tissue strongly associated with ABMR, if thoroughly validated

3. Serologic evidence of circulating donor-specific antibodies (DSA to HLA or other antigens). C4d staining or expression of validated transcripts/classifiers as noted above in criterion 2 may substitute for DSA; however thorough DSA testing, including testing for non-HLA antibodies if HLA antibody testing is negative, is strongly advised whenever criteria 1 and 2 are met

Chronic active ABMR; all 3 criteria must be met for diagnosis

1. Morphologic evidence of chronic tissue injury, including 1 or more of the following:

Transplant glomerulopathy (cg > 0) if no evidence of chronic TMA or chronic recurrent/de novo glomerulonephritis; includes changes evident by electron microscopy (EM) alone (cg1a)

Severe peritubular capillary basement membrane multilayering (ptcml1; requires EM)

Arterial intimal fibrosis of new onset, excluding other causes; leukocytes within the sclerotic intima favor chronic ABMR if there is no prior history of TCMR, but are not required

2. Identical to criterion 2 for active ABMR, above

3. Identical to criterion 3 for active ABMR, above, including strong recommendation for DSA testing whenever criteria 1 and 2 are met. **Biopsies meeting criterion 1 but not criterion 2 with current or prior evidence of DSA (posttransplant) may be stated as showing chronic ABMR, however remote DSA should not be considered for diagnosis of chronic active or active ABMR**

Chronic (inactive) ABMR

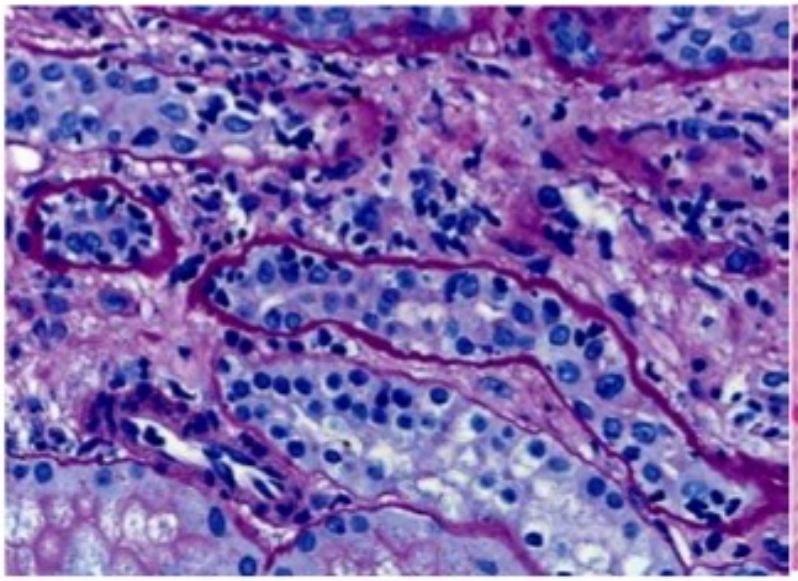
1. cg > 0 and/or severe ptcml (ptcml1)

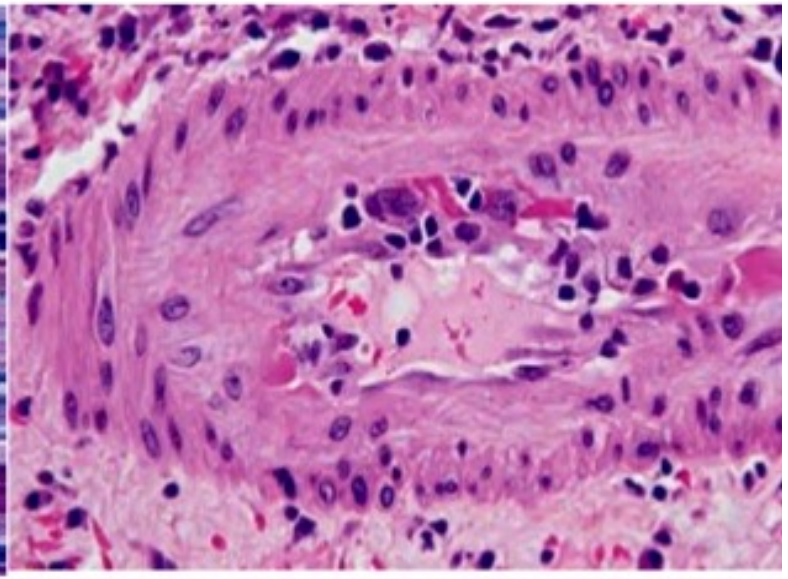
2. Absence of criterion 2 of current/recent antibody interaction with the endothelium

3. Prior documented diagnosis of active or chronic active ABMR and/or documented prior evidence of DSA

Category 3: Borderline (Suspicious) for acute TCMR
Foci of tubulitis (t1, t2, or t3) with **mild interstitial inflammation (i1)**, or mild (t1) tubulitis with moderate-severe interstitial inflammation (i2 or i3)
No intimal or transmural arteritis (v = 0)
Category 4: TCMR
Acute TCMR
Grade IA: Interstitial inflammation involving >25% of non-sclerotic cortical parenchyma (i2 or i3) with moderate tubulitis (t2) involving 1 or more tubules, not including tubules that are severely atrophic[d]
Grade IB: Interstitial inflammation involving >25% of non-sclerotic cortical parenchyma (i2 or i3) with severe tubulitis (t3) involving 1 or more tubules, not including tubules that are severely atrophic[d]
Grade IIA: Mild to moderate intimal arteritis (v1), with or without interstitial inflammation and/or tubulitis
Grade IIB: Severe intimal arteritis (v2), with or without interstitial inflammation and/or tubulitis
Grade III: Transmural arteritis and/or arterial fibrinoid necrosis involving medial smooth muscle with accompanying mononuclear cell intimal arteritis (v3), with or without interstitial inflammation and/or tubulitis
Chronic active TCMR[e]
Grade IA: Interstitial inflammation involving >25% of sclerotic cortical parenchyma (i-IFTA2 or i-IFTA3) AND > 25% of total cortical parenchyma (ti2 or ti3) with moderate tubulitis (t2 or **t-IFTA2**) involving 1 or more tubules, not including severely atrophic tubules[d]; other known causes of i-IFTA should be ruled out
Grade IB: Interstitial inflammation involving >25% of sclerotic cortical parenchyma (i-IFTA2 or i-IFTA3) AND > 25% of total cortical parenchyma (ti2 or ti3) with severe tubulitis (t3 or **t-IFTA3**) involving 1 or more tubules, not including severely atrophic tubules[d]; other known causes of i-IFTA should be ruled out
Grade II: Chronic allograft arteriopathy (arterial intimal fibrosis with mononuclear cell inflammation in fibrosis and formation of neointima). This may also be a manifestation of chronic active or chronic ABMR or mixed ABMR/TCMR

Acute T cell–mediated (cellular) rejection:





Tubulointerstitial pattern (Common) Inflammatory cells in the interstitium and between epithelial cells of the tubules (tubulitis)

Vascular pattern- Rejection vasculitis, with inflammatory cells attacking and undermining the endothelium (endotheliitis)

Treatment of cellular rejection

- First line treatment – methylprednisolone 500mg iv, infusion lasting 1 hour (250-1000mg iv) for 3 -5 days; If no response within 5 days– steroid resistant AR, give polyclonal antilymphocyte sera (lymphocyte-depleting Ab`s)
- >75% episodes of early AR response to initial treatment
- Raised maintanance immunosuppression based on CNI!
- Avoid steroid-free regimen
- GS+tac+MMF
- In high risk for CMV infection in pts treated with ATG - give ValGCV

KDIGO 2009

Chapter 6: Treatment of Acute Rejection

6.1: We recommend biopsy before treating acute rejection, unless the biopsy will substantially delay treatment. *(1C)*

6.2: We suggest treating subclinical and borderline acute rejection. *(2D)*

6.3: We recommend corticosteroids for the initial treatment of acute cellular rejection. *(1D)*

6.3.1: We suggest adding or restoring maintenance prednisone in patients not on steroids who have a rejection episode. *(2D)*

6.3.2: We suggest using lymphocyte-depleting antibodies or OKT3 for acute cellular rejections that do not respond to corticosteroids, and for recurrent acute cellular rejections. *(2C)*

6.4: We suggest treating antibody-mediated acute rejection with one or more of the following alternatives, with or without corticosteroids *(2C)*:

- **plasma exchange;**
- **intravenous immunoglobulin;**
- **anti-CD20 antibody;**
- **lymphocyte-depleting antibody.**

6.5: For patients who have a rejection episode, we suggest adding mycophenolate if the patient is not receiving mycophenolate or azathioprine, or switching azathioprine to mycophenolate. *(2D)*

OKT3, muromonab (anti–T-cell antibody).

to treatment with corticost
tion is defined by histopath
'suspicious for acute rejecti
sification schema (99). A re
unresponsive to treatment
return to baseline after the

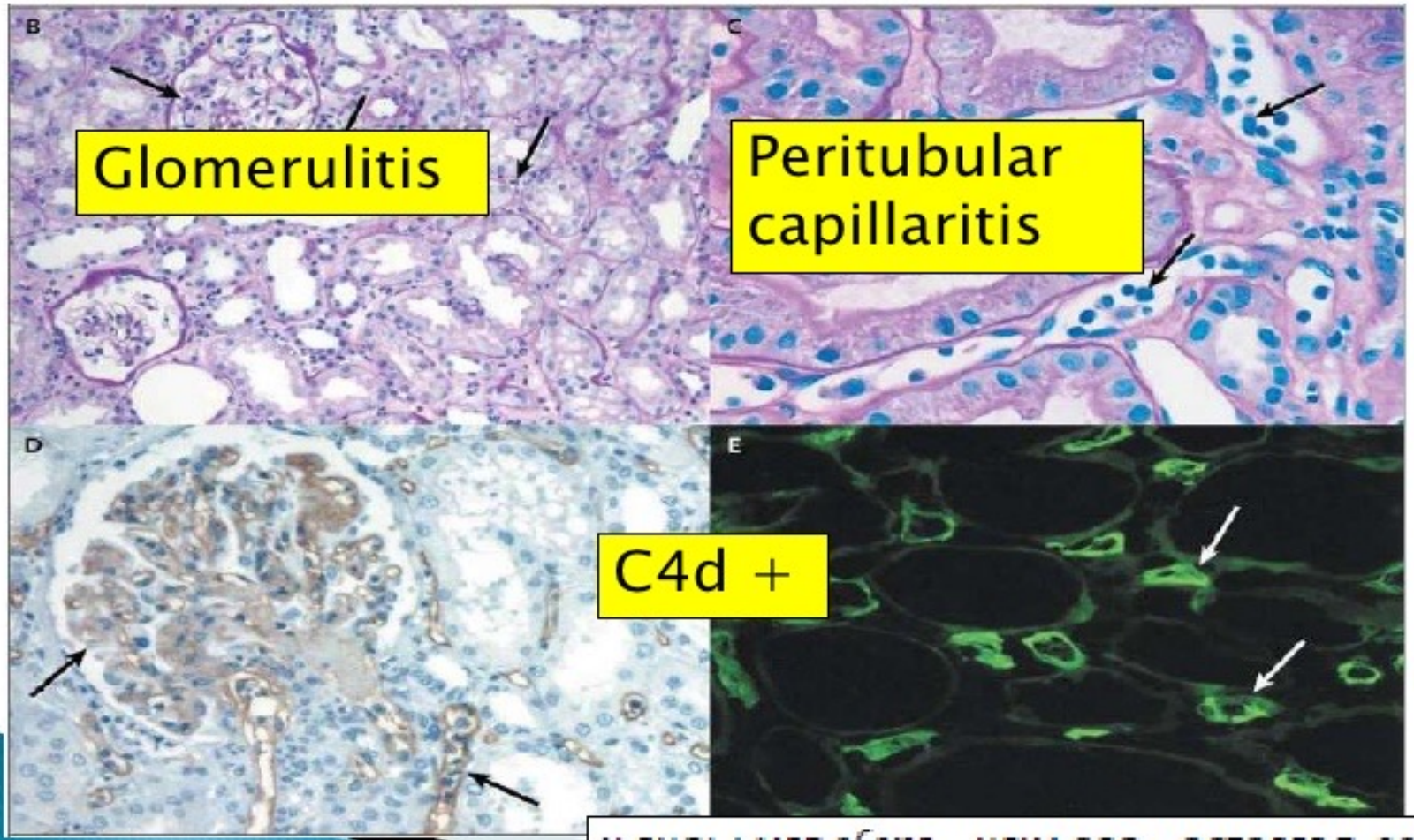
An antibody-mediated rejec
changes caused by a circul
antibody. The following crit
termine whether an acute r
specific antibody:

i) staining of peritubular ca
plement fraction);
ii) the presence of a circul
antibody and
iii) histological changes c
mediated rejection inclu
presence of polymorpho
illaries.

Rationale

- Several causes of decre
be distinguished from a
- Treatment of decreased

Pathology of Acute ABMR



N ENGL J MED 363;15 NEJM.ORG OCTOBER 7, 2010

Antibody-mediated rejection, T cell–mediated rejection, and the injury-repair response: new insights from the Genome Canada studies of kidney transplant biopsies

Philip F. Halloran[1,2], Jeff P. Reeve[1,3], Andre B. Pereira[1], Luis G. Hidalgo[1,3] and Konrad S. Famulski[1,3]

[1] Alber
[2] Depa
Univer

American Journal of Transplantation 2012; 12: 388–399
Wiley Periodicals Inc.

doi: 10.1111/j.1600-6143.2011.03840.

Understanding the Causes of Kidney Transplant Failure: The Dominant Role of Antibody-Mediated Rejection and Nonadherence

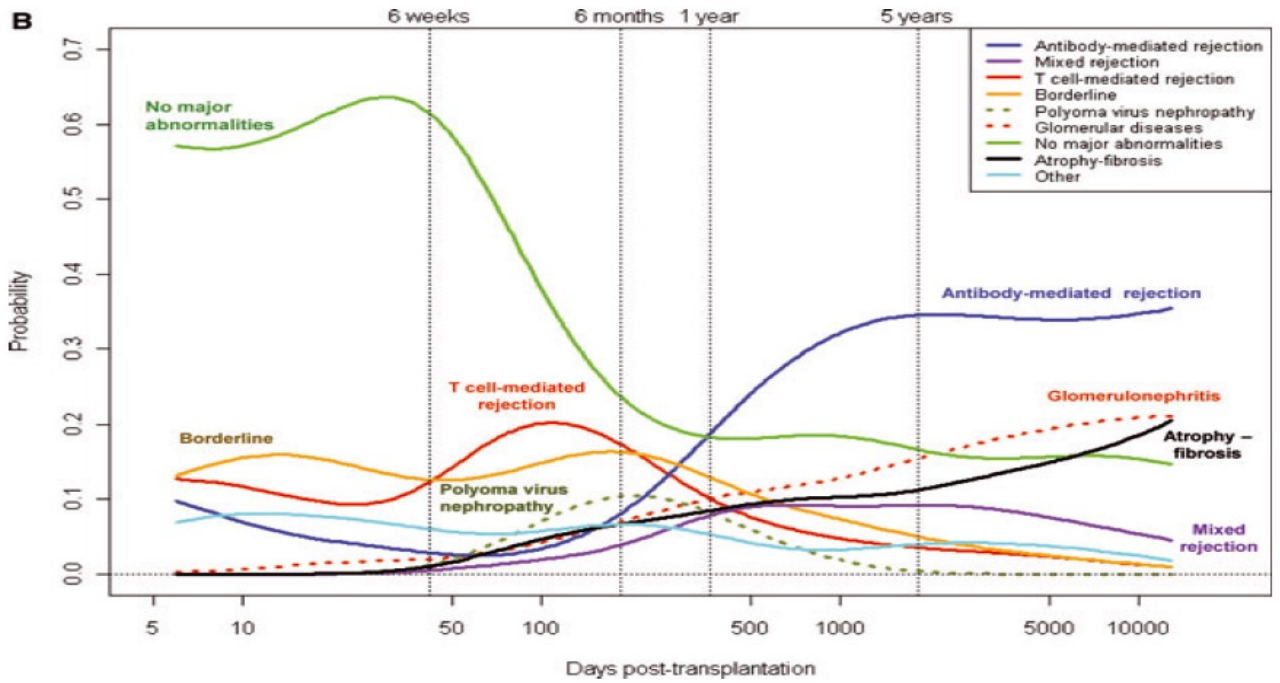


Figure 1: Distribution of histologic diagnoses and nonadherence according to time posttransplantation. (A) Histologic diagnoses and adherence status according to timing of the biopsy posttransplant. (B) Distribution of histopathology diagnoses and adherence status in biopsies expressed as probability plots conditional on the time of biopsy posttransplantation. The ABMR category includes C4d-positive ABMR, C4d-negative ABMR and probable ABMR.

rejection
ulonephri
ulonephri
oma viru

Graft loss

IS reduction (intended or unintended)

GS- free or CNI-free regimen

mTOR-I

non-adherence

↓

T, B activation

↓

DSA

↓

ABMR

↓

graft loss

A

Histological diagnosis	n	Attributed causes of allograft failure: Antibody-mediated rejection	Probable ABMR	Mixed rejection	Polyoma virus nephropathy	Glomerulone-phritis	Medical causes	Missing data	Non-adherence
Antibody-mediated rejection	28	26	–	–	–	–	2	–	11
Probable ABMR	2	–	2	–	–	–	–	–	1
Mixed rejection	6	2	–	3	–	–	1	–	2
T cell-mediated rejection	1	–	1	–	–	–	–	–	1
Borderline	1	–	1	–	–	–	–	–	1
Polyoma virus nephropathy	1	–	–	–	1	–	–	–	0
Glomerulonephritis	12	–	1	–	–	9	2	–	2
No major abnormalities	3	–	–	–	–	–	1	2	1
Atrophy-fibrosis	3	–	–	–	–	1	–	2	0
Other	3	–	–	–	3[a]	–	–	–	0
Total	60	28	5	3	4	10	6	4	19

[a] Patients whose biopsies showed histologic changes highly suggestive of polyoma virus nephropathy, although the IC/in situ hybridization was reported either inconclusive (n=1) or negative (n=2)

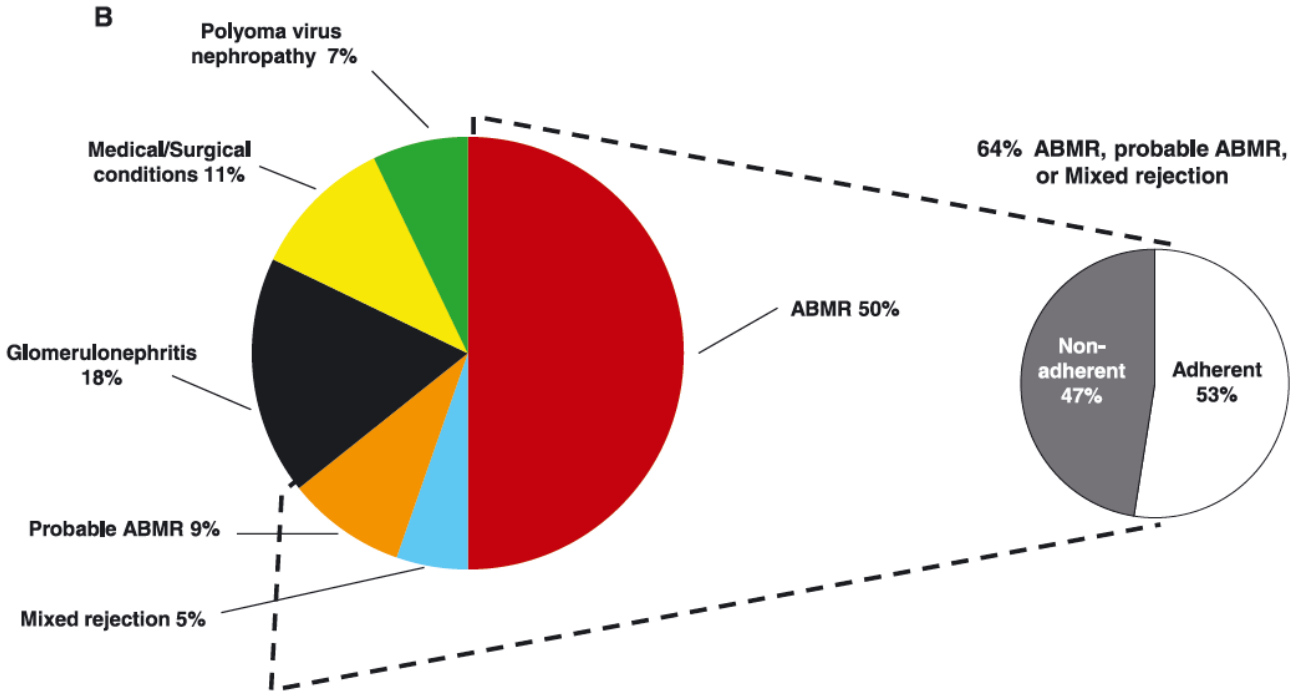
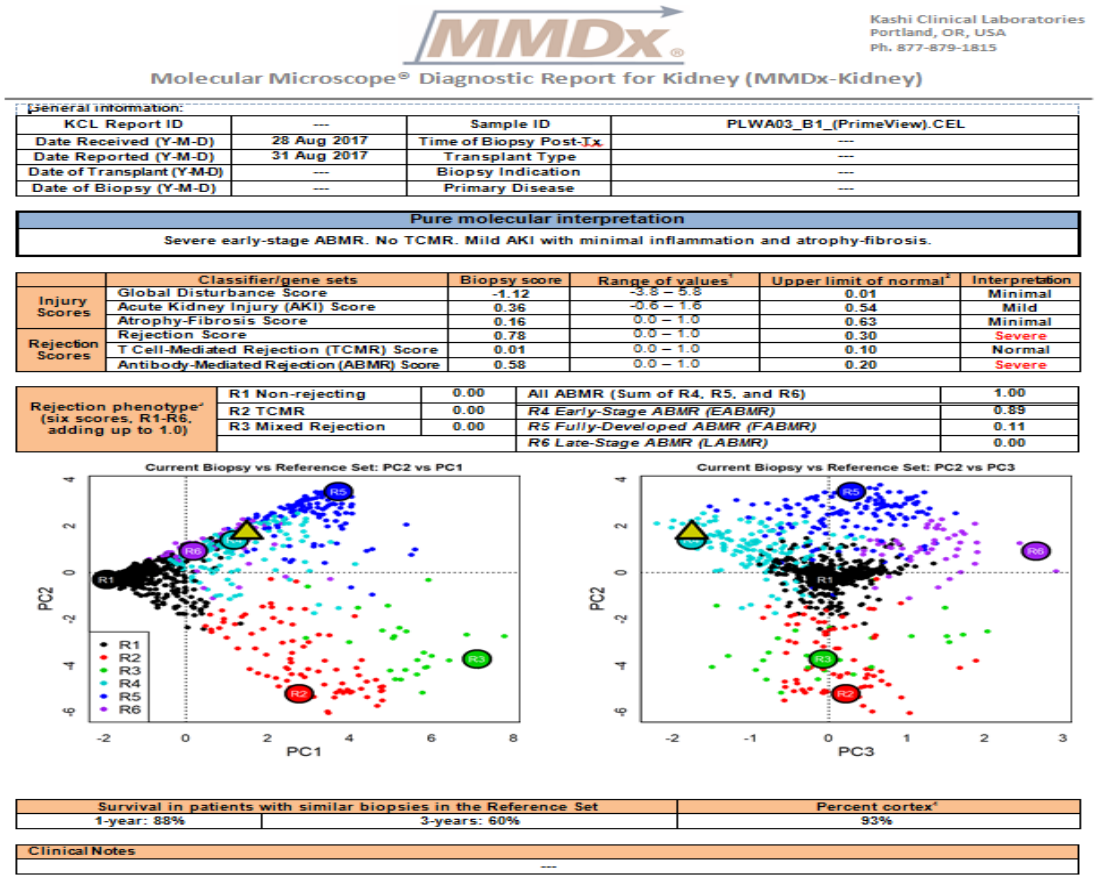


Figure 3: Attributed causes of graft failure in the biopsy-for-cause population. (A) Distribution of the attributed causes of failure (columns) according to the histological diagnosis in the last biopsy available per patient (rows). (B) Distribution of attributed causes of failure. Failures that could not be attributed due to missing clinical information are not represented (n = 4).

Molecular microscope



MMDx®

Kashi Clinical Laboratories
Portland, OR, USA
Ph. 877-879-1815

Molecular Microscope® Diagnostic Report for Kidney (MMDx-Kidney)

General information:

KCL Report ID	---	Sample ID	PLWA03_B1_(PrimeView).CEL
Date Received (Y-M-D)	28 Aug 2017	Time of Biopsy Post-Tx	---
Date Reported (Y-M-D)	31 Aug 2017	Transplant Type	---
Date of Transplant (Y-M-D)	---	Biopsy Indication	---
Date of Biopsy (Y-M-D)	---	Primary Disease	---

Pure molecular interpretation

Severe early-stage ABMR. No TCMR. Mild AKI with minimal inflammation and atrophy-fibrosis.

	Classifier/gene sets	Biopsy score	Range of values[3]	Upper limit of normal[3]	Interpretation
Injury Scores	Global Disturbance Score	-1.12	-3.8 – 5.8	0.01	Minimal
	Acute Kidney Injury (AKI) Score	0.36	-0.6 – 1.6	0.54	Mild
	Atrophy-Fibrosis Score	0.16	0.0 – 1.0	0.63	Minimal
Rejection Scores	Rejection Score	0.78	0.0 – 1.0	0.30	Severe
	T Cell-Mediated Rejection (TCMR) Score	0.01	0.0 – 1.0	0.10	Normal
	Antibody-Mediated Rejection (ABMR) Score	0.58	0.0 – 1.0	0.20	Severe

Rejection phenotype[4] (six scores, R1-R6, adding up to 1.0)				
	R1 Non-rejecting	0.00	All ABMR (Sum of R4, R5, and R6)	1.00
	R2 TCMR	0.00	*R4 Early-Stage ABMR (EABMR)*	0.89
	R3 Mixed Rejection	0.00	*R5 Fully-Developed ABMR (FABMR)*	0.11
			R6 Late-Stage ABMR (LABMR)	0.00

Current Biopsy vs Reference Set: PC2 vs PC1

Current Biopsy vs Reference Set: PC2 vs PC3

Survival in patients with similar biopsies in the Reference Set		Percent cortex[4]
1-year: 88%	3-years: 60%	93%

Clinical Notes

Molecular Microscope Diagnostics System (**MMDx**) is new microarray biopsy service. **MMDx** is a central diagnostic system that uses a MyGeneChip™ Custom Microarray from Thermo Fisher Scientific to measure transcript levels in biopsies, apply algorithms and compare algorithm results to a set of reference biopsies.

Antibodies in ABMR

- Anti-HLA Ab`s (*Donor Specific Antibodies –DSA)* class I, class II
- Pre-formed (preexisting) DSA
- *De novo* DSA

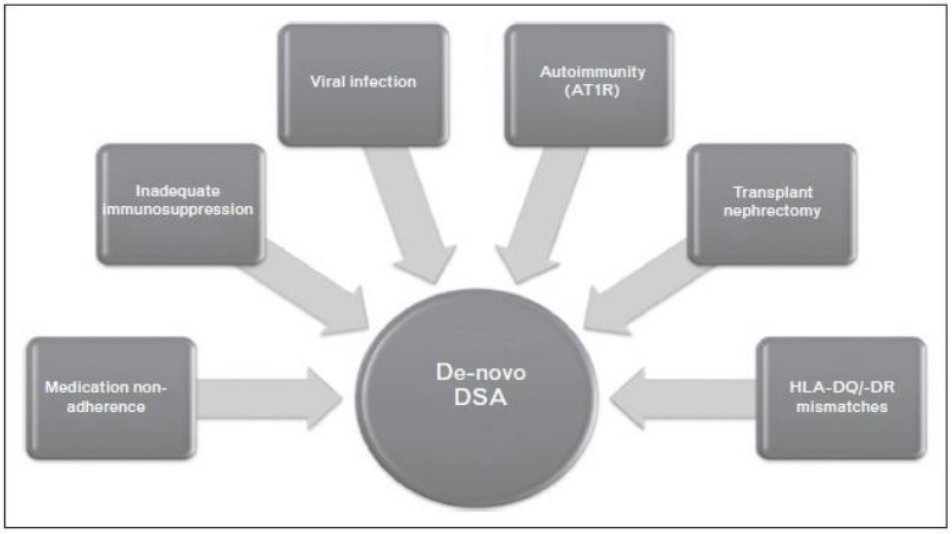


FIGURE 1. This figure shows the multiple factors that impact de-novo DSA development after renal transplantation. Among the most important are medication nonadherence and inadequate immunosuppression. Once DSAs develop, they result in ongoing injury to the allograft that limits the $^1/_2$ life and results in a sensitized patient who will be very difficult to retransplant. DSA, donor-specific antibody.

Antibodies in ABMR

- Anti-HLA Ab`s
- Anti-non-HLA Ab`s

  -Anti-MICA (MHC I chain related)

  -Anti-ATR1 (angiotensyn type 1 receptor)

  -Anti-endothelium

  -Anti-LG3

  -Anti-ABO

De novo DSA

- in 13-30% nonimmunized recipients
- mainly directed against class II
- appear in the 1st yr post tx

Risk factors for dn DSA

- Re-tx
- Preformed anti-HLA
- HLA MM between D and R (DR and DQ)
- Young age
- Non-adherence, non-compliance
- IS reduction
- Infection (CMV)
- Subclinical TCR
- Graftectomy

DSA are the risk factors for ABMR and graft loss

1929-2016

The humoral theory of graft rejection was proposed by P. Terasaki in 1969

The New England Journal of Medicine

Volume 280 APRIL 3, 1969 Number 14

SIGNIFICANCE OF THE POSITIVE CROSSMATCH TEST IN KIDNEY TRANSPLANTATION*

RAMON PATEL, M.R.C.P., AND PAUL I. TERASAKI, PH.D.

Abstract Crossmatch tests of the prospective kidney-transplant donor's lymphocytes with the serum of the prospective recipient in 225 transplants showed that eight of 195 with negative crossmatch failed to function immediately, in contrast to 24 of 30 with positive crossmatch (p less than 0.001). Immediate failure occurred in significantly higher numbers among patients with a higher risk of having antibodies, such as multiparous females and patients receiving secondary transplants. The effect was not a nonspecific one, for more immediate failures occurred among transplants from unrelated than among those from related donors. The corresponding frequency of positive crossmatch was also lower among related donors. The presence of preformed cytotoxic antibodies against the donor appears to be a strong contraindication for transplantation.

Preformed anti-HLA

- Developing prior to tx
- Previus exposure to antigens:
  - Blood transfusions
  - History of tx, raised DSA after graftectomy
  - Pregnancy 30% of women produce Ab`s
  - Infection

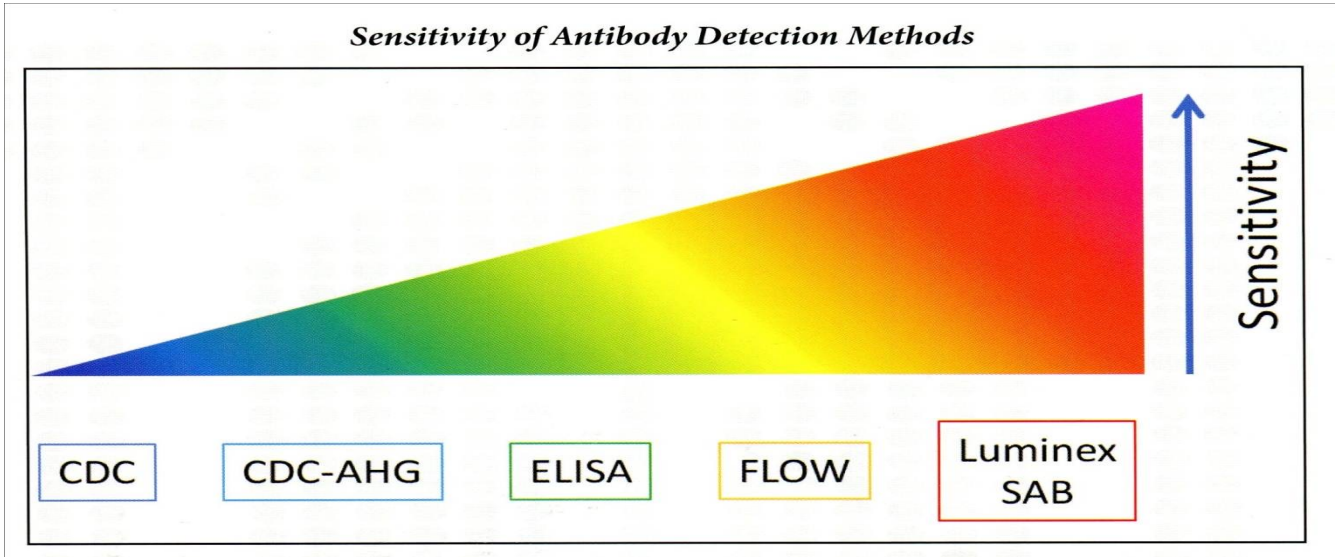
Detection of anti-HLA

- PRA-CDC (lymphocytotoxic test) complement-dependent, serologic test that detects complement-binding antibodies in serum (recipient); low sensitivity
- Flow cytometry (FCXM)- T , B cells, more sensitive, no specificity
- Solid phase tests- assess the specificity of DSA
  - Flow fluorimeter (Luminex) with beads (microspheres)

Luminex

- Sreening test – anti-HLA IgG, class I and class II
- Single antygen bead (SAB)- determines the specificity of the antibodies and mean fluorescent intensity (MFI)

- Advantage – high sensitivity and specificity



How antibodies destroy the graft?

•DSA binds to alloantigen on graft endothelium that activate the complement system on classic way, membrane attack complex (MAC)

Damage phenotype is ABMR

•DSA activates proliferation of endothelium, activates synthesis of growth factor, stimualtion of FGF receptor

Damage phenotype: transplant glomerulopathy and vasculopathy

• DSA activates antibody-dependent cytotoxicity

Innate immune response – neutrophiles, macrophages, NK bind to Fc DSA that stimulates macrophages degranulation, lytic enzymes destroy the graft

Damage phenotype: subclinical and chronic ABMR

DSA class

- HLA class I are expressed on all nucleated cells
- HLA class II (DR, DQ, DP) are present on APC (dendritic cells, B cells, macrophages) and can be expressed after inflammation ie. Ischemia-reperfusion injury, infection, rejection
- Preformed DSA can be in class I, class II or both
- Majority of dn DSA are class II (DQ). DSA class I are detected early post-tx, subclasses IgG1 and IgG3 (compelment-binding), responsible for acute ABMR and graft loss
- DSA class II are late-onset, complement non-binding, subclass IgG2 and IgG4, long-lasting, responsible for chronic ABMR and transplant glomerulopathy

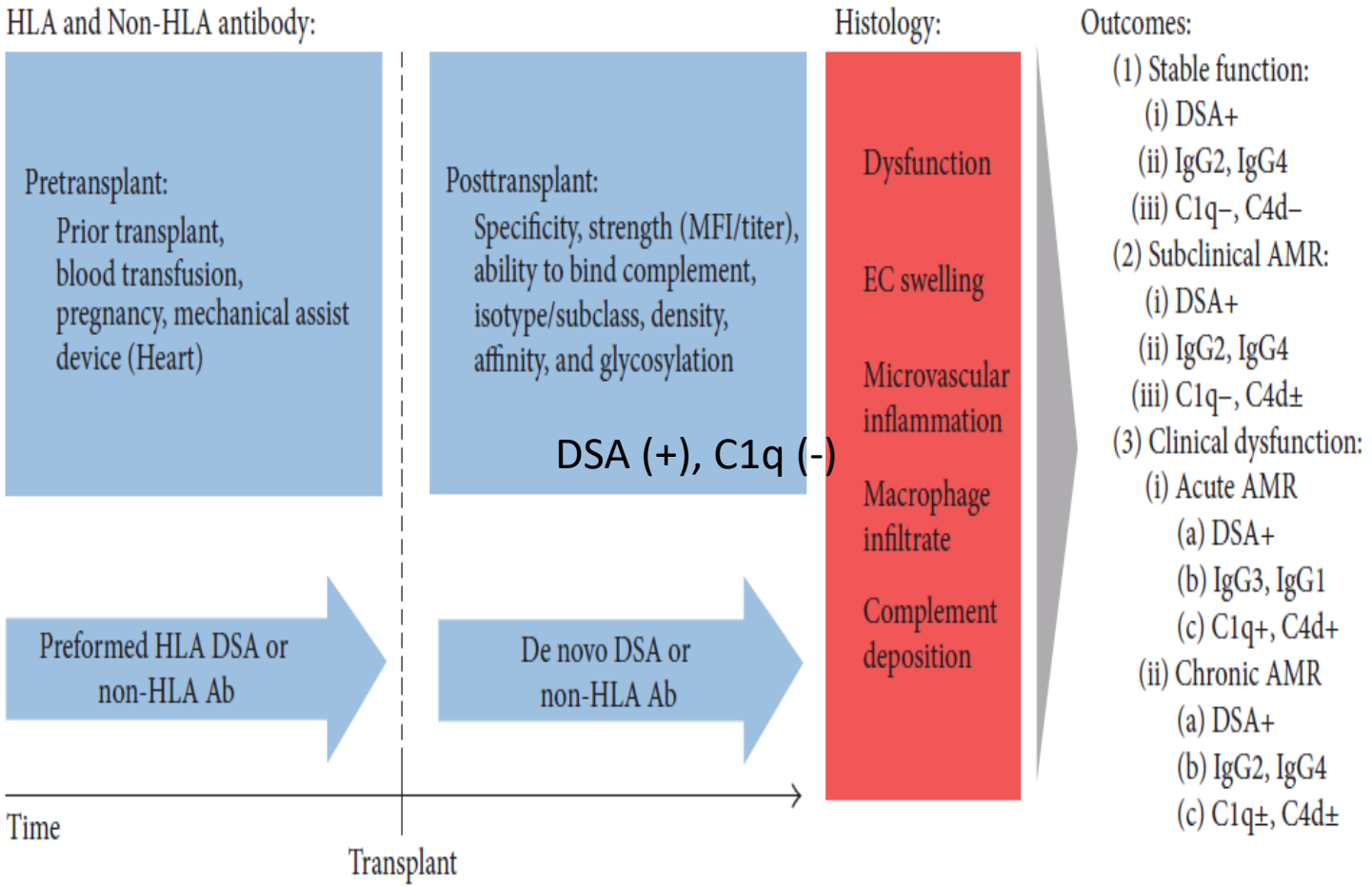
DSA and C4d

- C4d /+/ in PTC is an evidence of recent complement activation in the course of ABMR
- C4d are an independent risk factor for graft deterioration and graft loss

- C4d /+/ ABMR is complement-mediated (complement-dependent cytotoxicity) and result in more severe clinical course

- C4d /-/ ABMR is associated with non-complement dependent mechanism, subclinical chronic course, resulting in graft dysfunction and loss

DSA and clinical phenotypes



Lefaucheur C, J Am Soc Nephrol 27, 2016

Treatment of ABMR

Agents Used for Desensitization and Treatment of Antibody-Mediated Rejection			
Treatment	**Mechanism**	**Protocol (Desensitization)**	**Dose (Antibody-Mediated Rejection)**
Plasma exchange	Antibody removal	Two to four sessions or until XM acceptable, combined with IVIG	Two to five treatments, daily or every other day, combined with IVIG
IVIG	Multiple, antibody inhibition?	100-200 mg/kg after plasma exchange until acceptable XM *or* 1-2 g/kg monthly until transplant	100-200 mg/kg after plasma exchange
Rituximab	Anti-CD20 B cell inhibition	375 mg/m^2 (day 15) combined with IVIG 1-2 g/kg (days 1 and 30)	375 mg/m^2 with plasma exchange and IVIG
Bortezomib	Plasma cell inhibition	Not established	1.3 mg/m^2 × four doses over 1-2 weeks, usually combined with plasma exchange and IVIG
Eculizumab	Terminal complement C5 inhibition	Not established	For prevention in +XM transplant: 600-1200 mg weekly × 4 then biweekly until successful antibody reduction
Splenectomy	B cell removal	No longer used	N/A, for severe refractory cases only

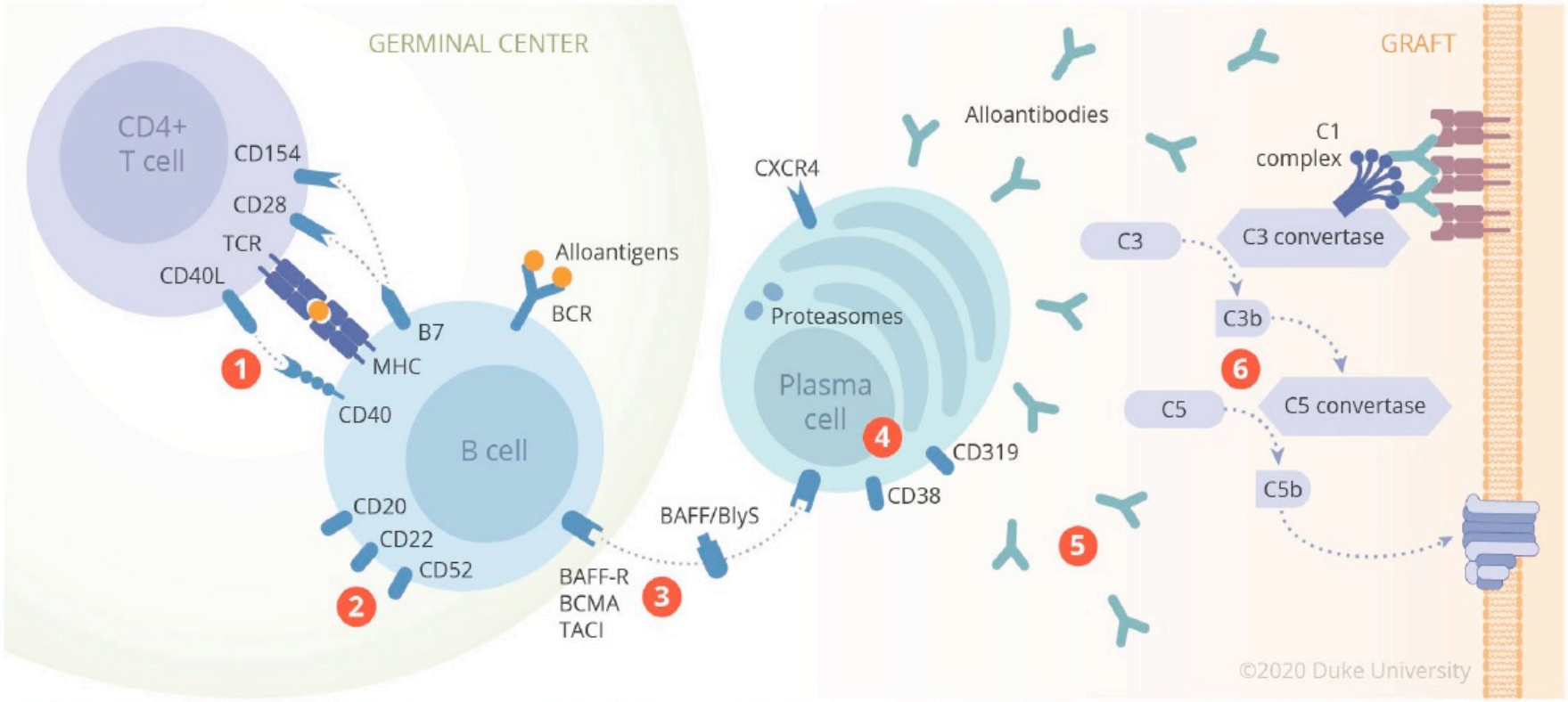


FIGURE 1 Overview of pharmacological targets of humoral response in organ transplant

Summary of 2017 FDA Public Workshop: Antibody-mediated Rejection in Kidney Transplantation

Ergun Velidedeoglu, MD,[1] Marc W. Cavaillé-Coll, MD, PhD,[1] Shukal Bala, PhD,[1] Ozlem A. Belen, MD, MPH,[1] Yan Wang, PhD,[2] and Renata Albrecht, MD[1]

Abstract. Despite major advances in understanding the pathophysiology of antibody-mediated rejection (AMR); prevention, diagnosis and treatment remain unmet medical needs. It appears that early T cell–mediated rejection, de novo donor-specific antibody (dnDSA) formation and AMR result from patient or physician initiated suboptimal immunosuppression, and represent landmarks in an ongoing process rather than separate events. On April 12 and 13, 2017, the Food and Drug Administration sponsored a public workshop on AMR in kidney transplantation to discuss new advances, importance of immunosuppressive medication nonadherence in dnDSA formation, associations between AMR, cellular rejection, changes in glomerular filtration rate, and challenges of clinical trial design for the prevention and treatment of AMR. Key messages from the workshop are included in this summary. Distinction between type 1 (due to preexisting DSA) and type 2 (due to dnDSA) phenotypes of AMR needs to be considered in patient management and clinical trial design. Standardization and more widespread adoption of routine posttransplant DSA monitoring may permit timely diagnosis and understanding of the natural course of type 2 and chronic AMR. Clinical trial design, especially as related to type 2 and chronic AMR, has specific challenges, including the high prevalence of nonadherence in the population at risk, indolent nature of the process until the appearance of graft dysfunction, and the absence of accepted surrogate endpoints. Other challenges include sample size and study duration, which could be mitigated by enrichment strategies.

(*Transplantation* 2018;102: e257–e264)

NO EFFECTIVE THERAPY AVAILABLE

Recommended Treatment for Antibody-mediated Rejection After Kidney Transplantation: The 2019 Expert Consensus From the Transplantion Society Working Group

Carrie A. Schinstock, MD,[1] Roslyn B. Mannon, MD,[2] Klemens Budde, MD,[3] Anita S. Chong, PhD,[4] Mark Haas, MD,[5] Stuart Knechtle, MD,[6] Carmen Lefaucheur, MD, PhD,[7] Robert A. Montgomery, MD,[8] Peter Nickerson, MD,[9] Stefan G. Tullius, MD, PhD,[10] Curie Ahn, MD, PhD,[11,12] Medhat Askar, MD, PhD,[13] Marta Crespo, MD, PhD,[14] Steven J. Chadban, PhD,[15] Sandy Feng, MD, PhD,[16] Stanley C. Jordan, MD,[17] Kwan Man, PhD,[18] Michael Mengel, MD,[19] Randall E. Morris, MD,[20] Inish O'Doherty, PhD,[21] Binnaz H. Ozdemir, MD, PhD,[22] Daniel Seron, MD, PhD,[23] Anat R. Tambur, PhD,[24] Kazunari Tanabe, MD, PhD,[25] Jean-Luc Taupin, PhD,[26,27] and Philip J. O'Connell, PhD[28]

Abstract. With the development of modern solid-phase assays to detect anti-HLA antibodies and a more precise histological classification, the diagnosis of antibody-mediated rejection (AMR) has become more common and is a major cause of kidney graft loss. Currently, there are no approved therapies and treatment guidelines are based on low-level evidence. The number of prospective randomized trials for the treatment of AMR is small, and the lack of an accepted common standard for care has been an impediment to the development of new therapies. To help alleviate this, The Transplantation Society convened a meeting of international experts to develop a consensus as to what is appropriate treatment for active and chronic active AMR. The aim was to reach a consensus for standard of care treatment against which new therapies could be evaluated. At the meeting, the underlying biology of AMR, the criteria for diagnosis, the clinical phenotypes, and outcomes were discussed. The evidence for different treatments was reviewed, and a consensus for what is acceptable standard of care for the treatment of active and chronic active AMR was presented. While it was agreed that the aims of treatment are to preserve renal function, reduce histological injury, and reduce the titer of donor-specific antibody, there was no conclusive evidence to support any specific therapy. As a result, the treatment recommendations are largely based on expert opinion. It is acknowledged that properly conducted and powered clinical trials of biologically plausible agents are urgently needed to improve patient outcomes.

(*Transplantation* 2020;104: 911–922).

ABMR prevention

- Adequate IS based on CNI (tac)
- On CsA 2-7x risk for dn DSA

- Tacrolimus level in 1st year >8 ng/ml
- Alternative treatment - belatacept
- HLA matching in DR/DQ
- Treatment of subclinical rejection
- Prevent from dn DSA

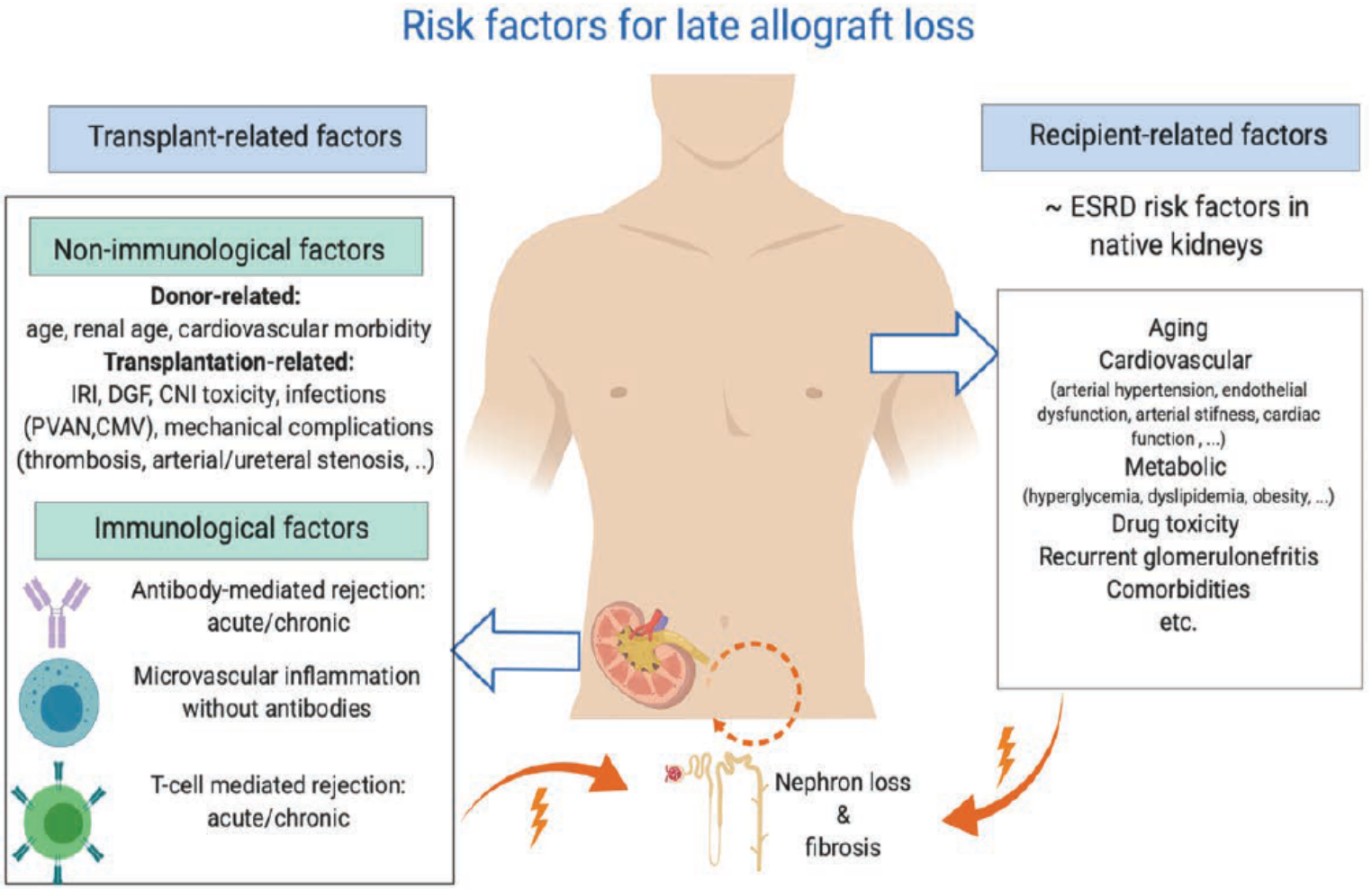


FIGURE 2. Schematic overview of risk factors for allograft loss, distinguishing recipient-related factors (analogous to risk factors in native kidneys) from transplant-related factors that can be nonimmunological and immunological. All these factors can contribute to allograft injury with nephron loss, further initiating a vicious circle of harmful hyperfiltration of the remnant nephrons, resulting in accelerated nephron loss and fibrosis. CMV, cytomegalovirus; CNI, calcineurin inhibitor; DGF, delayed graft function; ESRD, end-stage renal disease; IRI, ischemia-reperfusion injury; PVAN, polyomavirus-associated nephropathy.

(*Transplantation* 2020;104:e46–e56).

Liver graft rejection

- ABMR

  - hyperacute – liver graft deterioration/primary non-function within 2 weeks of engraftment

  - humoral rejection C4d/+/ and DSA…

- Acute cellular rejection (ACR)- immune response directed against biliary epithelial cells and endothelium (diagnostic Snover`s triad, 2 out of 3)

  - portal inflammation with mixed infiltration of T cells, plasma cells, neutrophils, macrophages, eosinophiles

  - bile duct damage (ductitis)

  - central or portal vein endothelial inflammation (venulitis, endothelitis)

- rejection activity index (RAI)

RAI 4-5- mild, RAI 6-7 – moderate, RAI 8-9 – severe

Liver graft rejection

- Late ACR (atypical) >6 months

interface activity, central perivenulitis, necrosis, lobular hepatitis

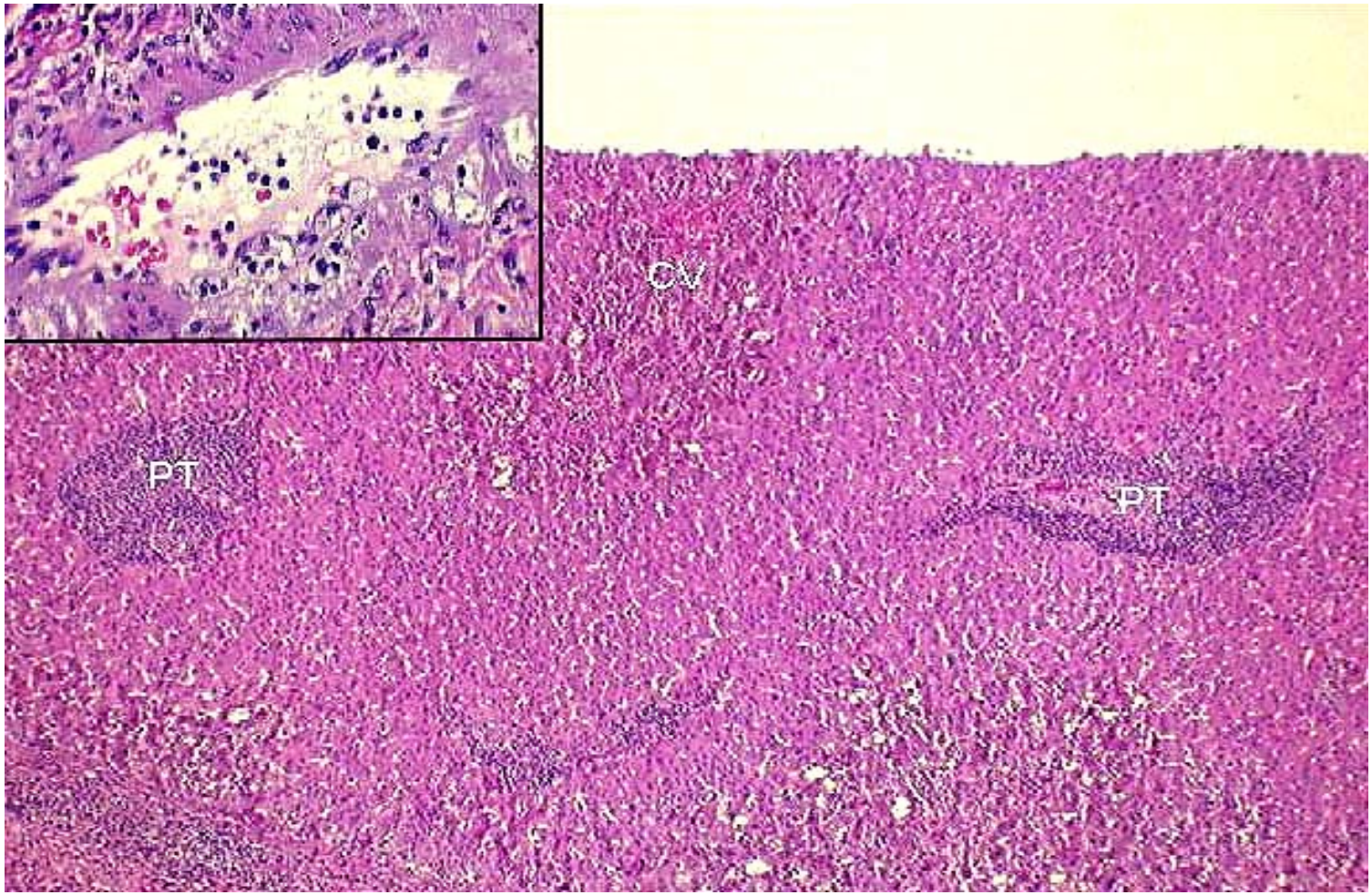
Monomorphic, mononuclear inflammation

Pathologic findings similar to chronic hepatitis or AIH-like

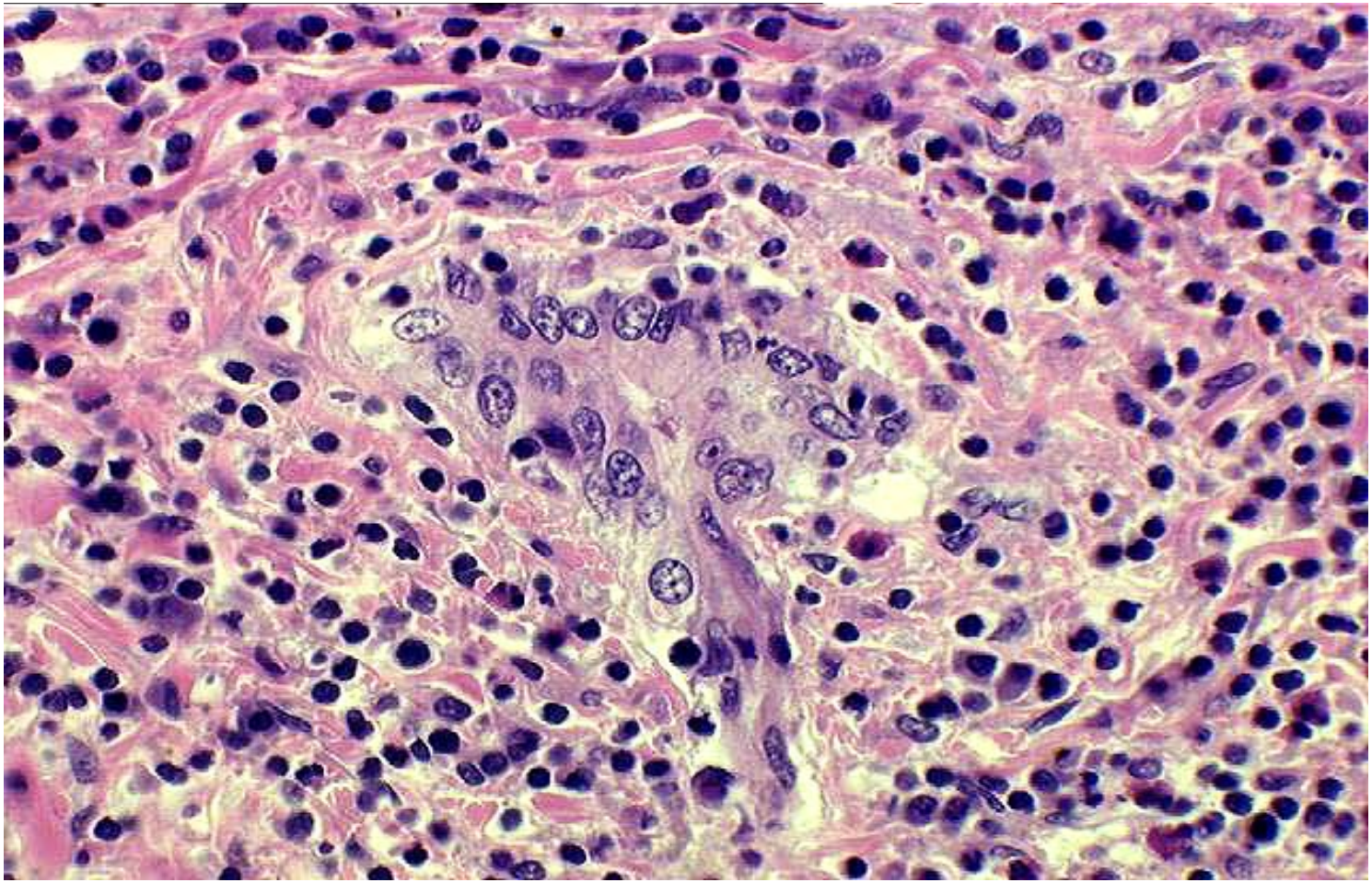
- Chronic rejection CR

Immune response is directed against endothelium of hepatic artery and biliary epithelial cells

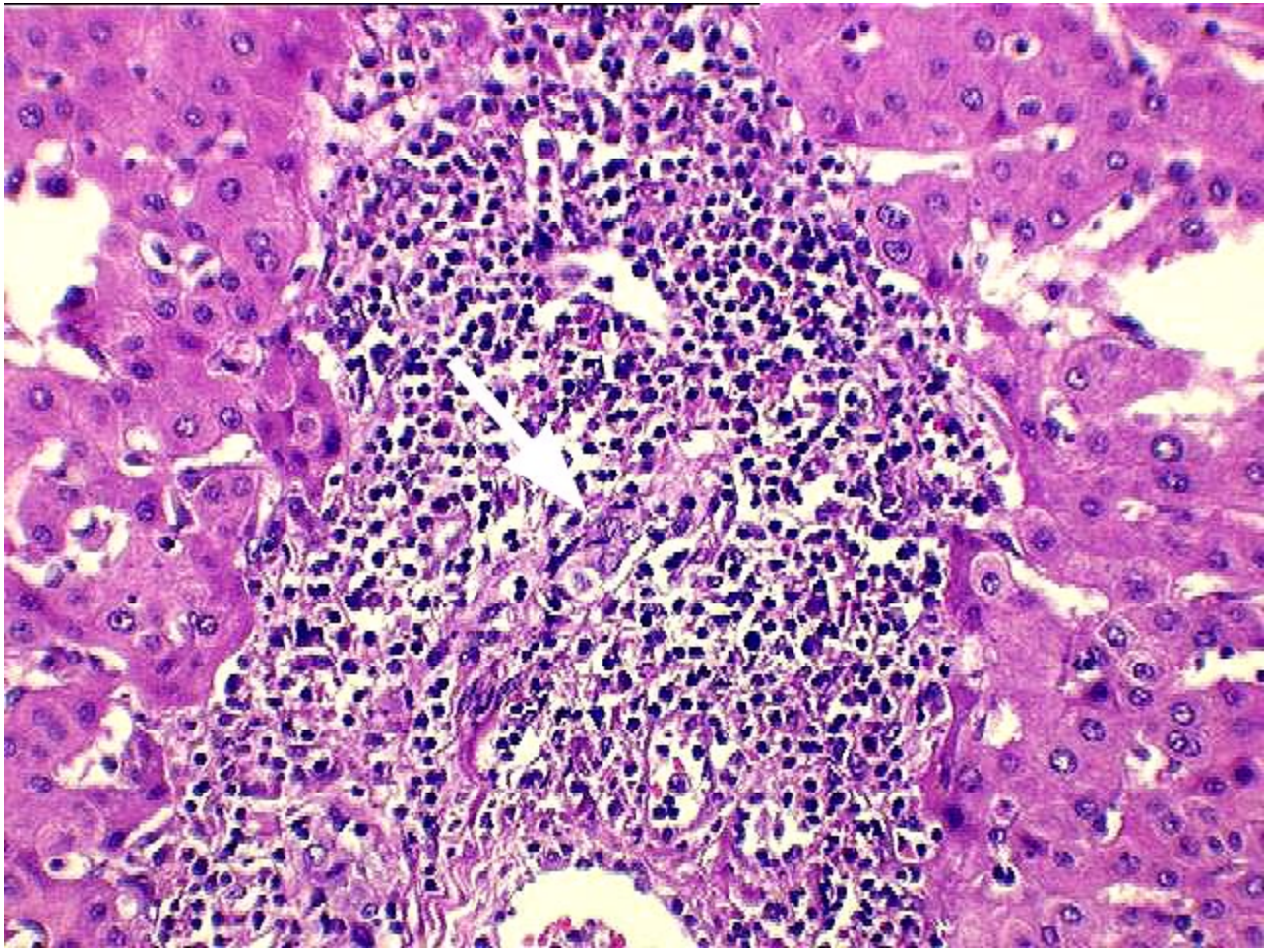
- Ductopenic rejection (vanishing bile duct syndrome)
- Vascular rejection



This example of severe acute rejection was taken from a failed allograft, removed several weeks after transplantation. Note the liver hilum and rarely sampled with a needle biopsy. marked portal tract(PT) inflammation that focally spills over into the periportal hepatic parenchyma. A similar infiltrate is seen in and around the central veins(CV), and is associated with perivenular hepatocyte necrosis and dropout.



This is an example of severe bile duct damage, a change that was present in most of the portal triads in the failed allograft shown in **Figure 1**. In this example, there are inflammatory cells inside the basement membrane, reactive changes with nuclear pleomorphism and lumenal disruption. In the **Banff schema**, this lesion would receive a score of "3" for bile duct damage.



severe or grade "3" portal inflammation in the **Banff Schema** that markedly expands the portal tracts. There is also severe or grade "3" bile duct injury(arrow).