MAYO CLINIC

Pathology of the late post-transplant kidney and the role of non-T cells

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Banff Conference on Allograft Pathology
March 28, 2017

Outline

- Causes of chronic injury in renal allografts
- Review of “non-T” cells in kidney transplant rejection (*late* rejection)
- Late post-transplant protocol biopsies, insights into late graft dysfunction and loss

Potential causes of late/chronic graft injury

- Donor-specific antibody/AMR
- Chronic inflammation (i-IFTA, IFTA+i)

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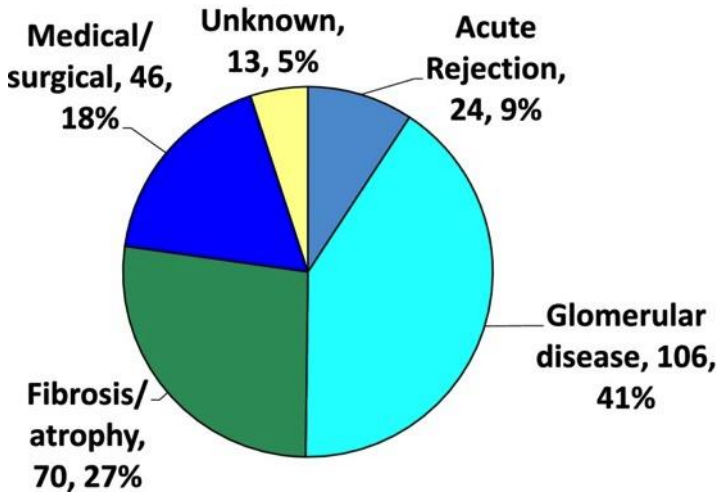
- Glomerular disease (including TG, recurrent dis)

Causes of graft loss in 153 conventional kidney transplants

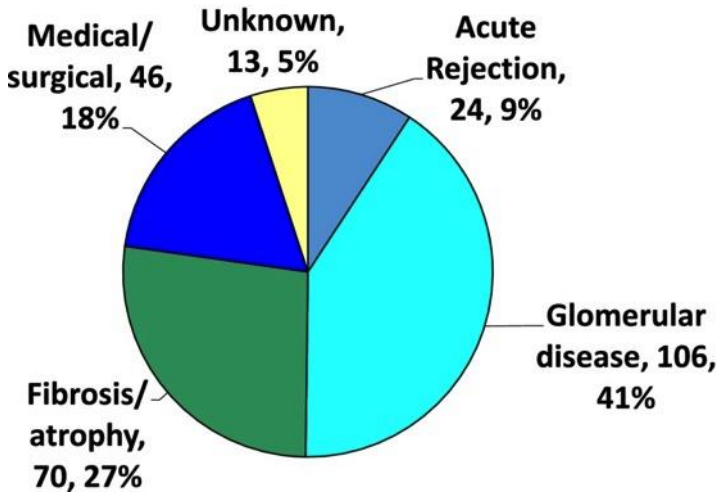


El-Zoghby et al. Identifying specific causes of kidney allograft loss. Am J Transplant 2009 Mar;9(3):527-35
Stegall MD, Gaston RS, Cosio FG, Matas A. Through a glass darkly: seeking clarity in preventing late kidney transplant failure. J Am Soc Nephrol. 2015 Jan

Potential causes of late/chronic graft injury

- Donor-specific antibody/AMR
- Chronic inflammation (i-IFTA, IFTA+i)
- Glomerular disease (including TG, recurrent dis)
- Progressive IFTA?
- Other?

Causes of graft loss in 153 conventional kidney transplants



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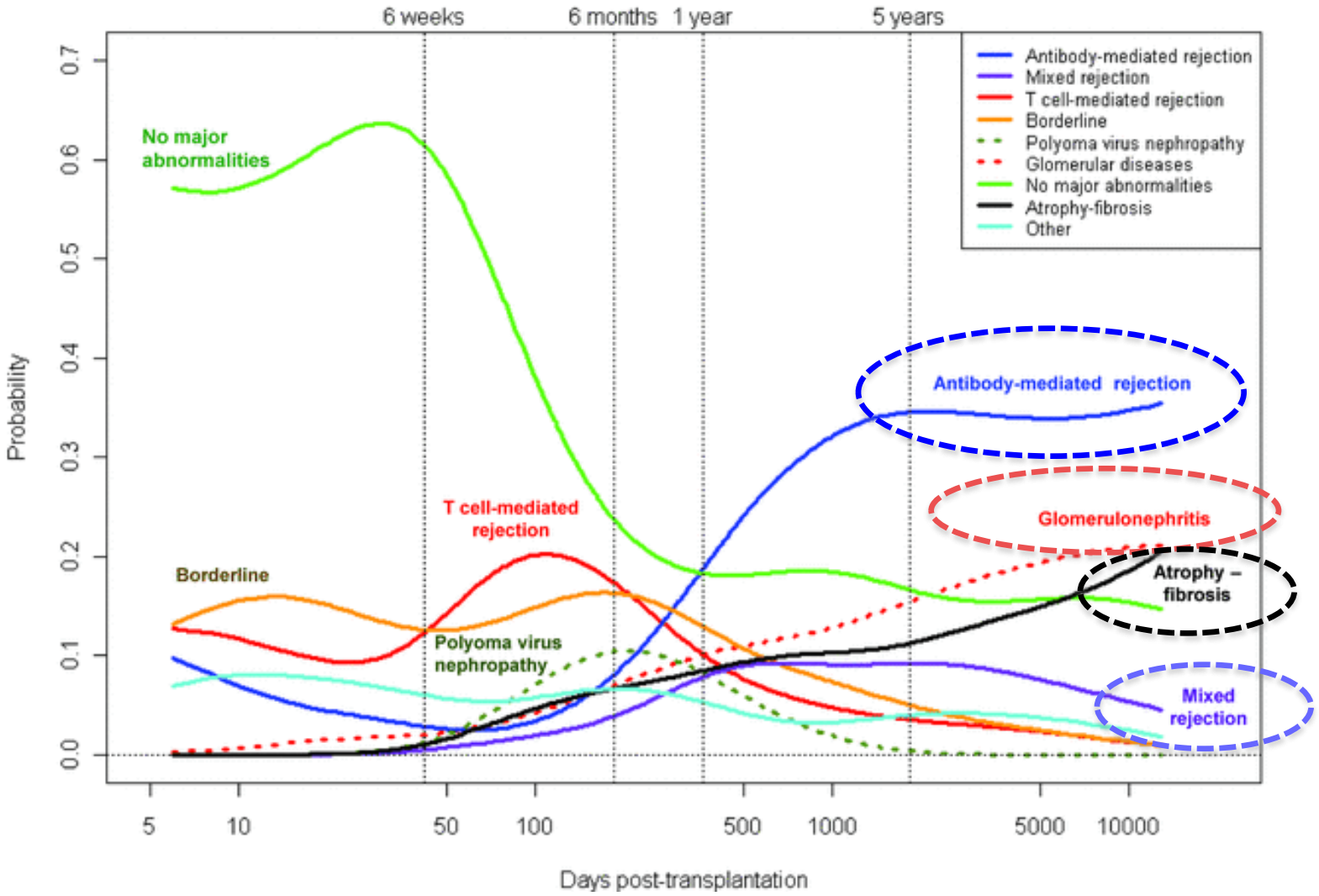
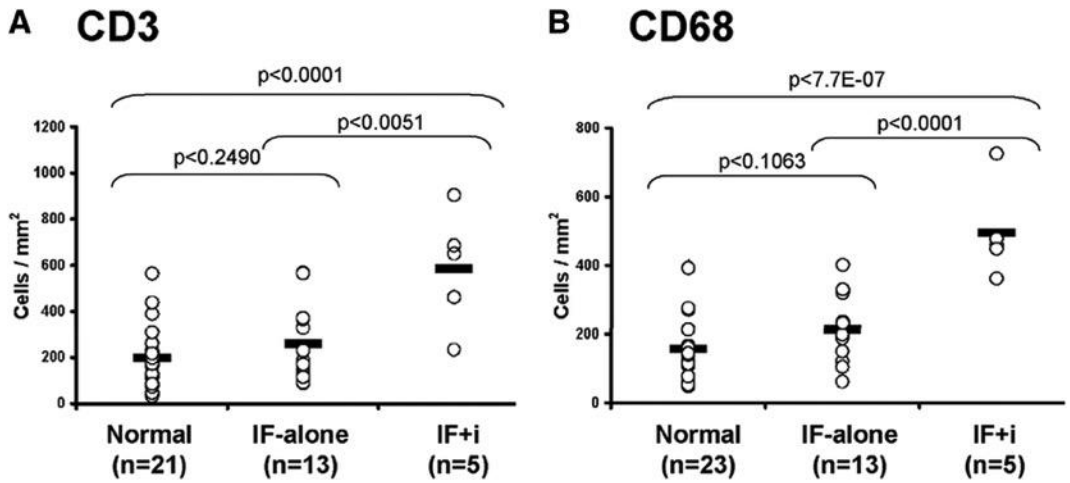
Indication biopsy diagnosis by time post-transplant



Sellarés J, de Freitas DG, Mengel M, Reeve J, Einecke G, Sis B, Hidalgo LG, Famulski K, Matas A, Halloran PF. Understanding the causes of kidney transplant failure: the dominant role of antibody-mediated rejection and nonadherence. Am J Transplant. 2012 Feb;12(2):388-99

Subclinical graft inflammation

- "IFTA+i" present in ~15% of 1 year protocol biopsies
- Increased staining for CD3, CD68 in IFTA+i



Increased immunostaining for T cells and macrophages/DCs in 1 year protocol biopsies with IF+i by Banff '97 criteria.

Park WD, Griffin MD, Cornell LD, Cosio FG, Stegall MD. Fibrosis with inflammation at one year predicts transplant functional decline. J Am Soc Nephrol. 2010 Nov;21(11):1987-97

Subclinical graft inflammation

Association with later development of IFTA

- ? Form of cell-mediated rejection

RT–PCR arrays: IFTA+i associated with increased activity of innate immune pathways including IFN-γ and Toll-like receptor responses, and T cell immunity

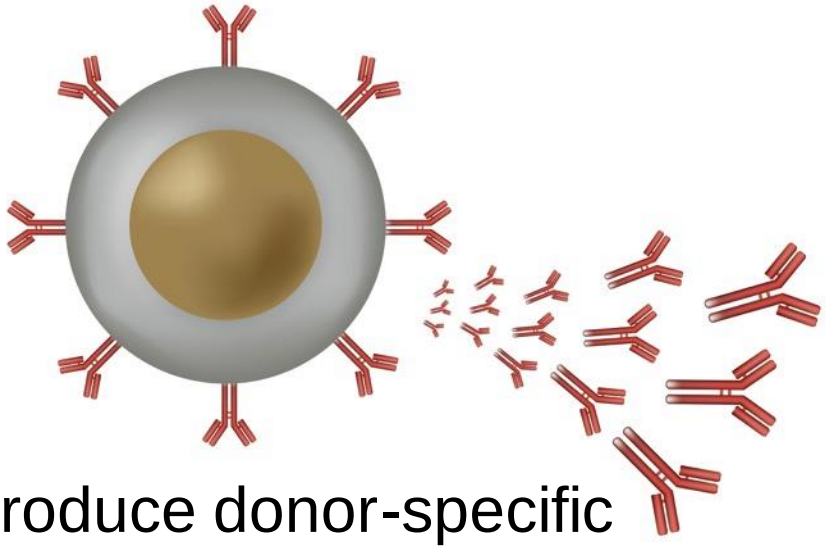
Whole-genome microarrays: cytotoxic T lymphocytes, IFN-γ response, B cells, and acute rejection signatures in IFTA+i compared with normal and IF-alone groups

Park WD, Griffin MD, Cornell LD, Cosio FG, Stegall MD. Fibrosis with inflammation at one year predicts transplant functional decline. J Am Soc Nephrol. 2010 Nov;21(11):1987-97

“Non-T cells” in kidney transplants

- B cells and plasma cells
- NK cells
- Monocytes/macrophages
- Mast cells

B cells



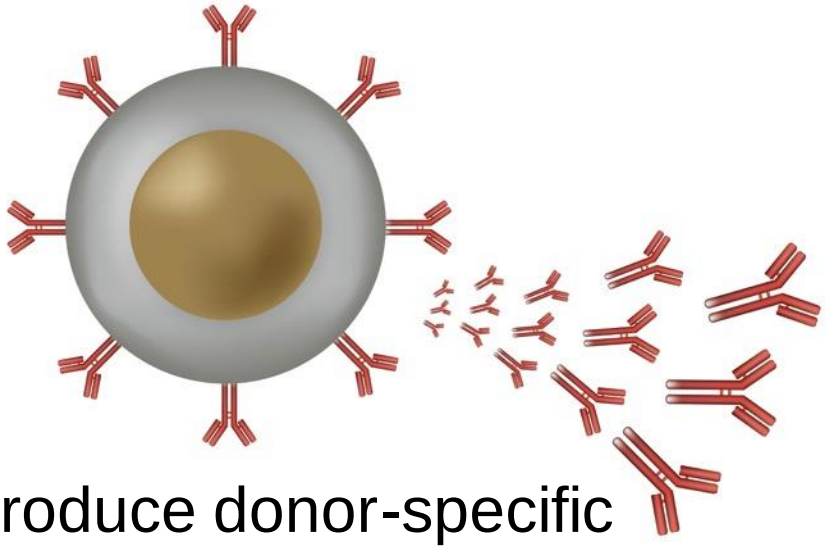
Several modes of graft injury:

Antibody-mediated rejection:

- B cells and plasma cells produce donor-specific antibody (DSA)
- B cell differentiation to plasma cells

Karahan GE, Claas FH, Heidt S. B Cell Immunity in Solid Organ Transplantation. Front Immunol. 2017 Jan 10
Li XC. The significance of non-T-cell pathways in graft rejection: implications for transplant tolerance. Transplantation. 2010 Nov 27

B cells



Several modes of graft injury:

Antibody-mediated rejection:

- B cells and plasma cells produce donor-specific antibody (DSA)
- B cell differentiation to plasma cells

Serve as antigen-presenting cells (APCs) to T cells

- Generation of alloreactive T cells, memory T cells

Secretion of cytokines (pro- and anti-inflammatory)

- May also be involved in tolerance

Karahan GE, Claas FH, Heidt S. B Cell Immunity in Solid Organ Transplantation. Front Immunol. 2017 Jan 10
Li XC. The significance of non-T-cell pathways in graft rejection: implications for transplant tolerance. Transplantation. 2010 Nov 27
Adams AB, Newell KA. B cells in clinical transplantation tolerance. Semin Immunol. 2012 Apr

B cells infiltrating the allograft

- Dense clusters of B cells in renal biopsies associated with severe graft rejection

Sarwal M, Chua MS, Kambham N, Hsieh SC, Satterwhite T, Masek M, Salvatierra O Jr. Molecular heterogeneity in acute renal allograft rejection identified by DNA microarray profiling. N Engl J Med. 2003 Jul 10;349(2):125-38

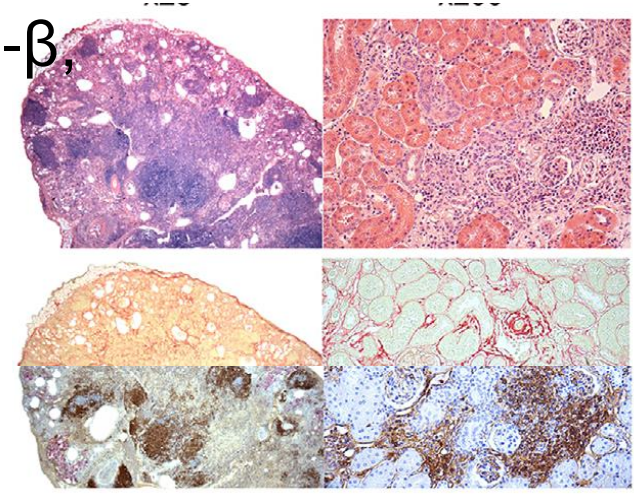
- Other studies showed conflicting results

B cells infiltrating the allograft

Mouse kidney transplant model:

- Mature B cells recruited to the kidney transplant, form tertiary lymphoid tissue; progressive IFTA
- B cell depletion attenuates these changes
- Ex vivo culture of isolated intra-allograft B cells:
  - Supernatant showed significant levels of T cell chemokines, monocyte cytokines, TGF-β, other profibrotic cytokines

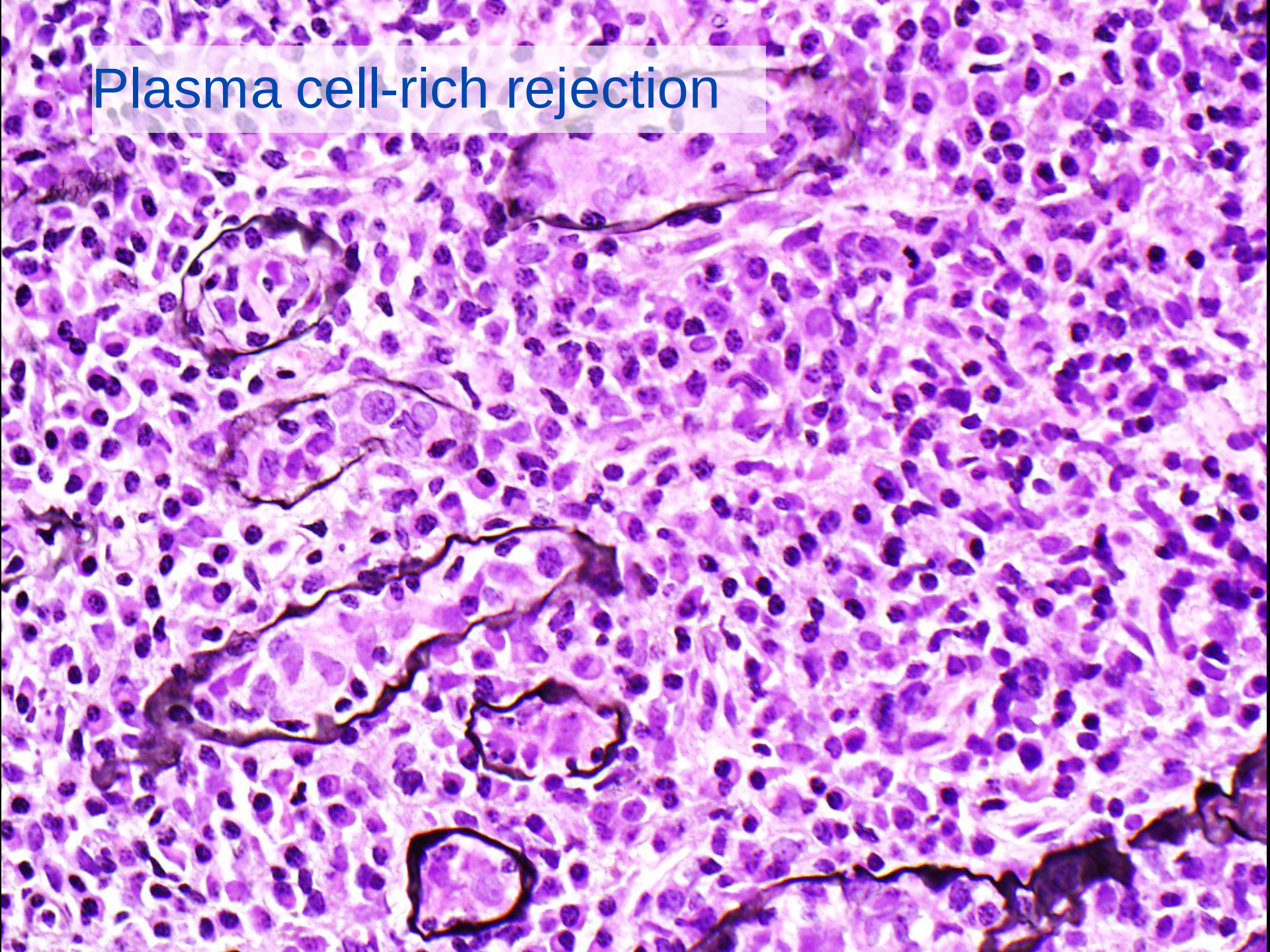
→Mechanism of B cell damage related to cytokine production



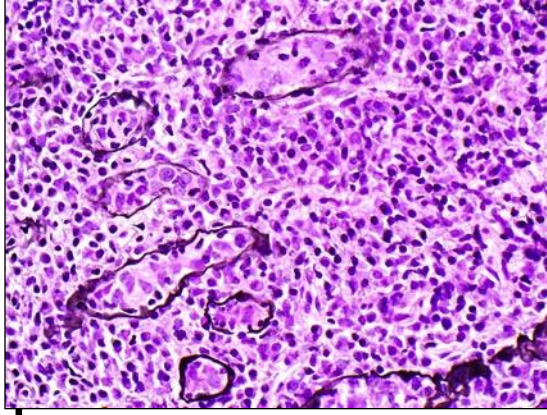
CD20

Tse GH, Johnston CJ, Kluth D, Gray M, Gray D, Hughes J, Marson LP. Intrarenal B Cell Cytokines Promote Transplant Fibrosis and Tubular Atrophy. Am J Transplant. 2015 Dec

Plasma cell-rich rejection



Plasma cell-rich rejection



Associated with:

- Resistance to rejection treatment, adverse outcome
- Medication non-adherence
- DSA, C4d deposition, capillaritis, TG
- IFTA, interstitial inflammation

Mixed AMR/TCMR

Abbas K, Mubarak M, Zafar MN, Aziz T, Abbas H, Muzaffar R, Rizvi SA. Plasma cell-rich acute rejections in living-related kidney transplantation: a clinicopathological study of 50 cases. Clin Transplant. 2015 Sep

Lerut E, Kuypers DR, Verbeken E, Cleutjens J, Vlaminck H, Vanrenterghem Y, Van Damme B. Acute rejection in non-compliant renal allograft recipients: a distinct morphology. Clin Transplant. 2007 May-Jun

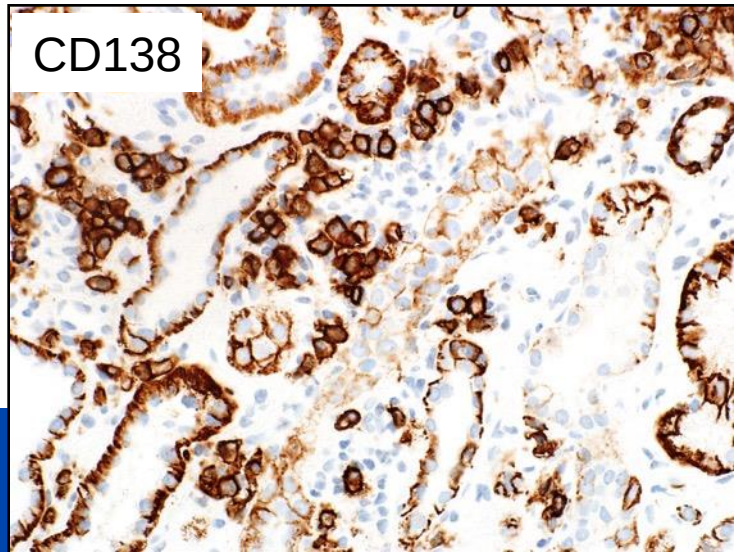
Gärtner V, Eigentler TK, Viebahn R. Plasma cell-rich rejection processes in renal transplantation: morphology and prognostic relevance. Transplantation. 2006 Apr 15

Charney DA, Nadasdy T, Lo AW, Racusen LC. Plasma cell-rich acute renal allograft rejection. Transplantation. 1999

Plasma cell-rich acute rejection with alemtuzumab induction

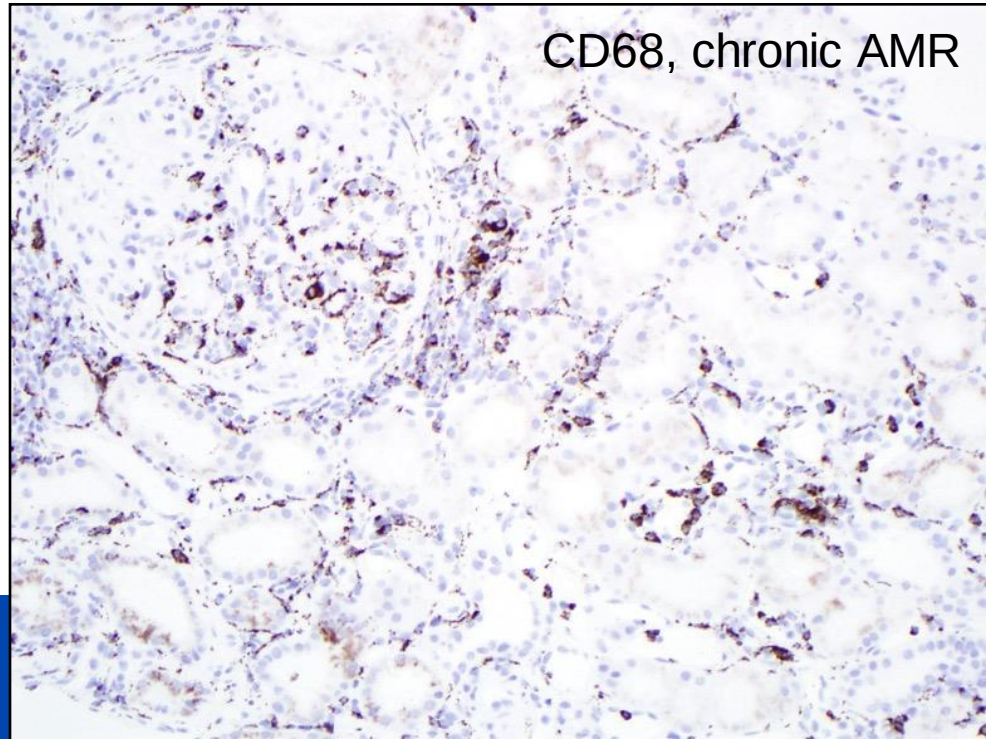
- Biopsy: Plasma cell-rich acute rejection (~10% are C4d+)
- Alemtuzumab-induced lymphocyte depletion leads to dominance of naïve B cells
- Unique phenotype may be due to a different B cell repertoire that develops
  - Potentially has a different response to conventional anti-rejection therapy, other types of plasma cell-rich rejection

P Zhang, H Amer, CA Schinstock, MP Alexander, FG Cosio, MD Stegall, LD Cornell, Acute Rejection After Alemtuzumab Induction in Kidney Transplant Recipients. ATC abstract 2017



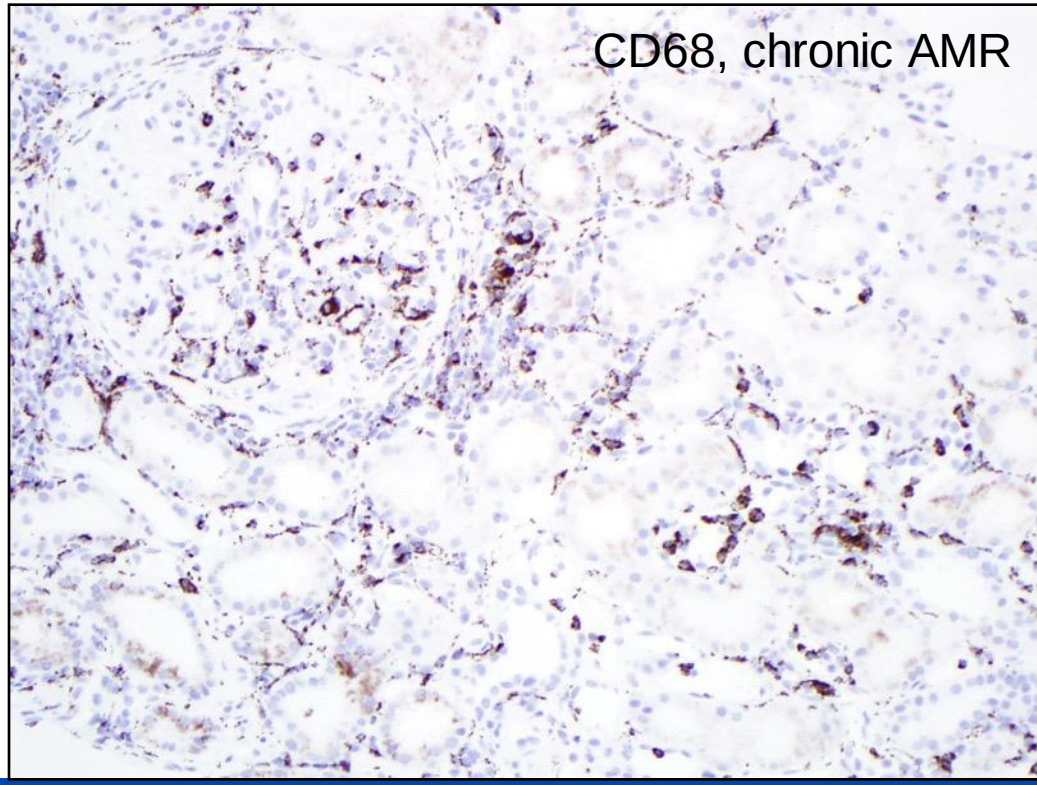
Monocytes/macrophages and rejection

- A component of inflammatory infiltrate in rejection (AMR, TCMR)
- Produce pro-inflammatory cytokines (IL-1, IL-6, TNF-α)
- Immune regulation
- Antigen presentation
- Tissue remodeling



Inflammation in +XM transplants at 5 years

- Persistent inflammation (capillaritis) and TG in +XM transplants



Bentall A, Herrera LP, Cornell LD, Gonzales MA, Dean PG, Park WD, Gandhi MJ, Winters JL, Stegall MD. Differences in chronic intragraft inflammation between positive crossmatch and ABO-incompatible kidney transplantation. Transplantation. 2014 Nov 27;98(10):1089-96.

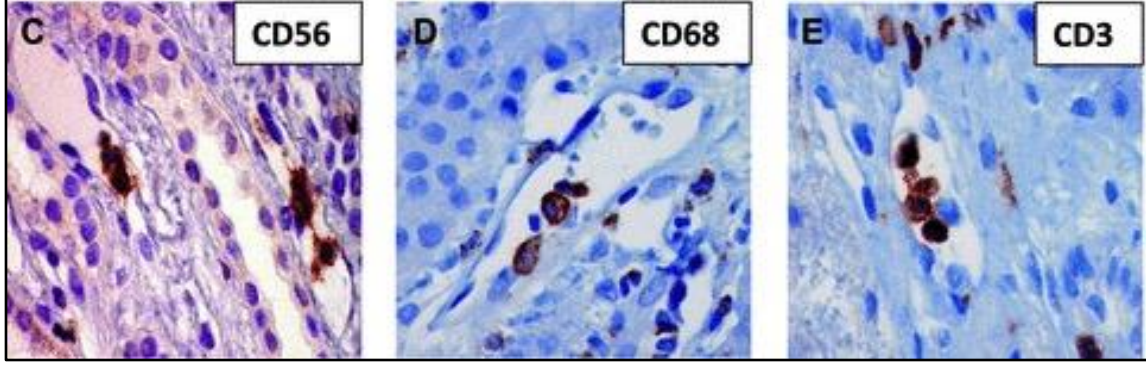
Chronic humoral rejection may occur in the absence of complement deposition in the tissue

Injury may be due to persistent inflammatory infiltrate

	Conventional	ABOi	+XM	Conv/ ABOi	Conv/ + XM	p +XM/ ABOi
cg	7.6%	12%	59.5%	0.457	<0.001	<0.001
ptc	8.9%	7.7%	66.6%	0.863	<0.001	0.001
C4d+	6.7%	77.8%	8.9%	<0.001	0.02	<0.001

Bentall A, Herrera LP, Cornell LD, Gonzales MA, Dean PG, Park WD, Gandhi MJ, Winters JL, Stegall MD. Differences in chronic intragraft inflammation between positive crossmatch and ABO-incompatible kidney transplantation. Transplantation. 2014 Nov 27;98(10):1089-96.

NK cells



- CD56+ and CD68+ cells in peritubular capillaries increased in AMR compared to T cell-mediated rejection
  - Effector role for NK cells and macrophages in AMR
- CD16 (FcγR)-inducible NK cell-selective transcripts (CD160 and XCL1) associated with AMR
  - Evidence for Ab-mediated NK cell activation via FcγR (CD16)
  - Raises the possibility of other CD16-triggered effects, including NK localization and cytotoxicity.

Hidalgo LG, Sis B, Sellares J, Campbell PM, Mengel M, Einecke G, Chang J, Halloran PF. NK cell transcripts and NK cells in kidney biopsies from patients with donor-specific antibodies: evidence for NK cell involvement in antibody-mediated rejection. Am J Transplant. 2010 Aug

Parkes MD, Halloran PF, Hidalgo LG. Evidence for CD16a-Mediated NK Cell Stimulation in Antibody-Mediated Kidney Transplant Rejection. Transplantation. 2017 Apr

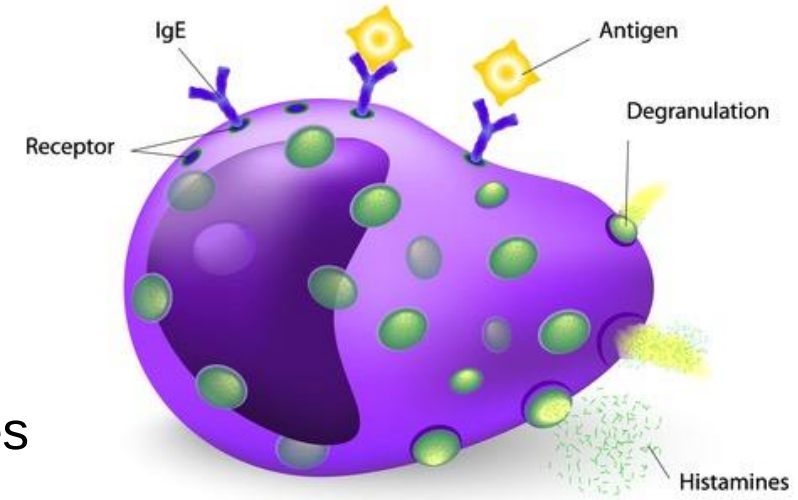
NK cells

- Differences in circulating NK cell subsets in transplant patients with DSA, non-DSA alloAb, and no DSA
- Role of CMV?
  - CMV infection promotes an adaptive differentiation and expansion of a subset of mature NK cells

Crespo M, Yelamos J, Redondo D, Muntasell A, Perez-Saéz MJ, López-Montañés M, García C, Torio A, Mir M, Hernández JJ, López-Botet M, Pascual J. Circulating NK-cell subsets in renal allograft recipients with anti-HLA donor-specific antibodies. Am J Transplant. 2015 Mar;15(3):806-14

López-Botet M, Vilches C, Redondo-Pachón D, Muntasell A, Pupuleku A, Yélamos J, Pascual J, Crespo M. Dual Role of Natural Killer Cells on Graft Rejection and Control of Cytomegalovirus Infection in Renal Transplantation. Front Immunol. 2017 Feb 16;8:166

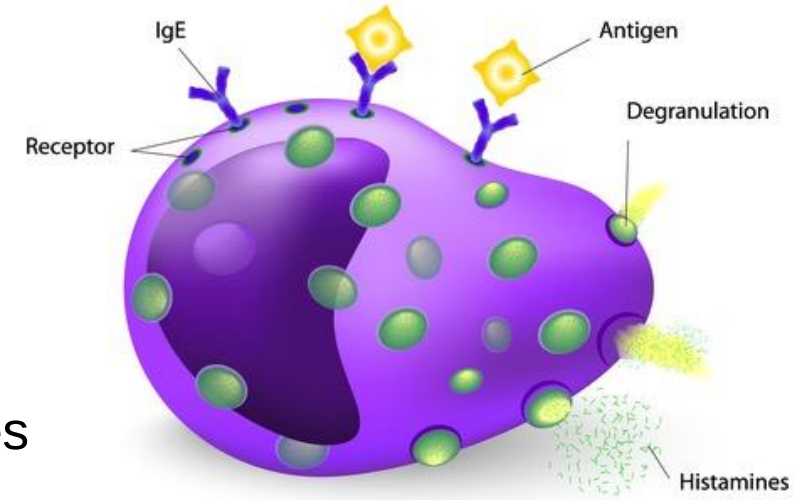
Mast cells



- Non-transplant roles:
  - Fighting parasitic infections
  - Role in IgE-mediated allergic responses
- Mast cells degranulate to release preformed mediators
  - Histamine, heparin, serotonin, and serine proteases

Modena BD, Dazy K, White AA. Emerging concepts: mast cell involvement in allergic diseases. Transl Res. 2016 Aug;174:98-121.

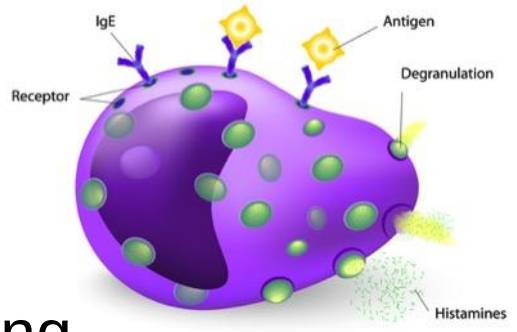
Mast cells



- Non-transplant roles:
  - Fighting parasitic infections
  - Role in IgE-mediated allergic responses
- Mast cells degranulate to release preformed mediators
  - Histamine, heparin, serotonin, and serine proteases
- Mast cells have actions even without degranulating
  - Secrete pro-inflammatory factors (leukotrienes, prostanoids, cytokines)
  - Activate nearby inflammatory cells
  - Recruit other immune cells such as eosinophils, neutrophils

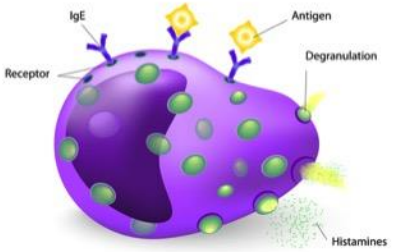
Modena BD, Dazy K, White AA. Emerging concepts: mast cell involvement in allergic diseases. Transl Res. 2016 Aug;174:98-121.

Mast cells



- Seen at sites of chronic inflammation, including nonallergic inflammation
- Mast cells help tissues heal and repair from damage
  - Produce immune-inhibitory cytokine (IL-10) and degrade proinflammatory cytokines with granule proteases
  - Release cytokines (eg, TGF-β) and other factors that change tissue morphology

Modena BD, Dazy K, White AA. Emerging concepts: mast cell involvement in allergic diseases. Transl Res. 2016 Aug;174:98-121.



Mast cells

- Mast cells present in the interstitium; occasional mast cell tubulitis (bx 5 months-5 years, graft dysfunction)
  - Giemsa-stained sections, Epon-embedded

Colvin RB, Dvorak HF. Letter: Basophils and mast cells in renal allograft rejection. Lancet. 1974 Feb 9;1(7850):212-4

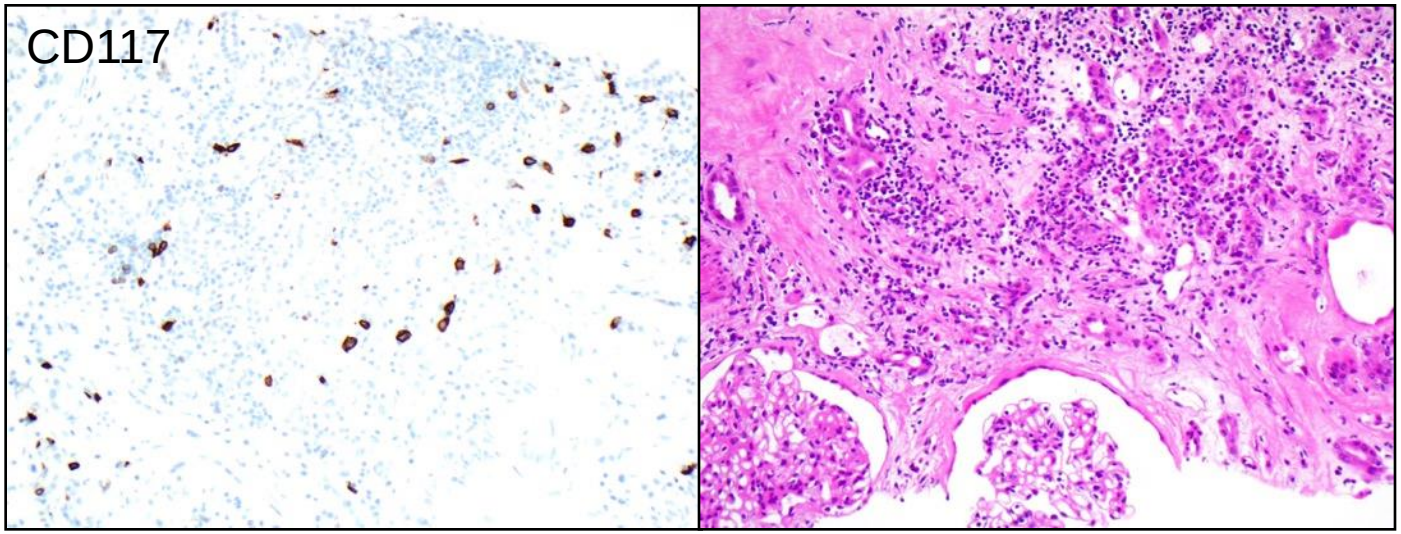
- Mast cell semi-quantitative study in acute rejection
  - Mast cell tryptase-specific monoclonal antibodies, paraffin sections
  - Correlation between number of mast cells and:
    - Time post-transplant
    - Severity of interstitial fibrosis

Lajoie G, Nadasdy T, Laszik Z, Blick KE, Silva FG. Mast cells in acute cellular rejection of human renal allografts. Mod Pathol. 1996 Dec;9(12):1118-25

Mast cells

- 461 consecutive kidney allograft biopsy specimens, CD117 immunostaining to count mast cells
  - Mast cell number correlated with:
    - Banff features of T-cell–mediated and AMR
    - Interstitial fibrosis
    - **Time post-transplant**
  - Reflective of “cumulative burden of tissue injury”

Papadimitriou JC, Drachenberg CB, Ramos E, Ugarte R, Haririan A. Mast cell quantitation in renal transplant biopsy specimens as a potential marker for the cumulative burden of tissue injury. Transplant Proc. 2013 May;45(4):1469-71



Summary-
Non-T cells in the late post-transplant kidney

Cell type in kidney	Rejection type
B cells	T cell mediated rejection, combined AMR/TCMR
Plasma cells	Late combined AMR/ T cell mediated rejection
Monocytes/macrophages	Late AMR (capillaritis), IFTA+i
NK cells	Late AMR
Mast cells	Associated with fibrosis, late T cell mediated rejection

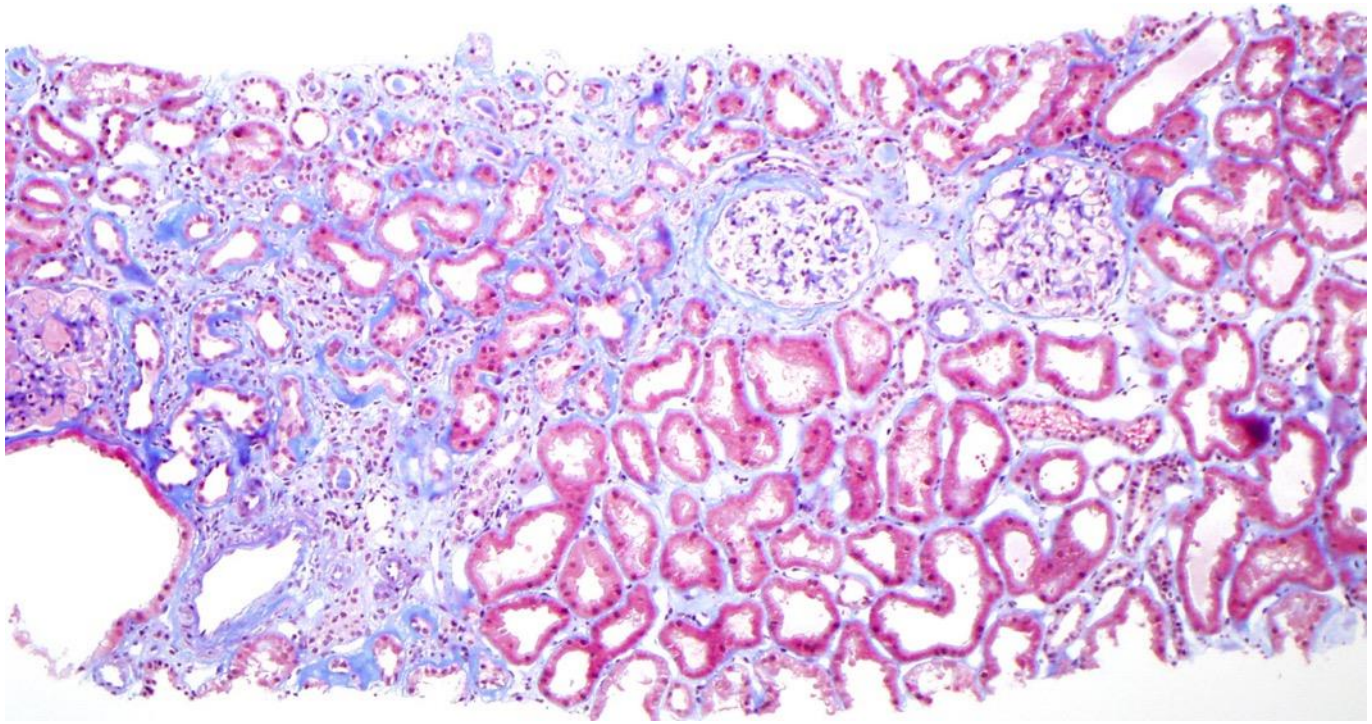
What causes late graft injury and loss?

Conventional view of late allografts

"CAN", chronic allograft nephropathy

Progressive interstitial fibrosis & tubular atrophy (IFTA), ?due to calcineurin inhibitor (CSA) toxicity

Nankivell BJ, Borrows RJ, Fung CL, O'Connell PJ, Allen RD, Chapman JR. The natural history of chronic allograft nephropathy. N Engl J Med. 2003 Dec 11;349(24):2326-33.

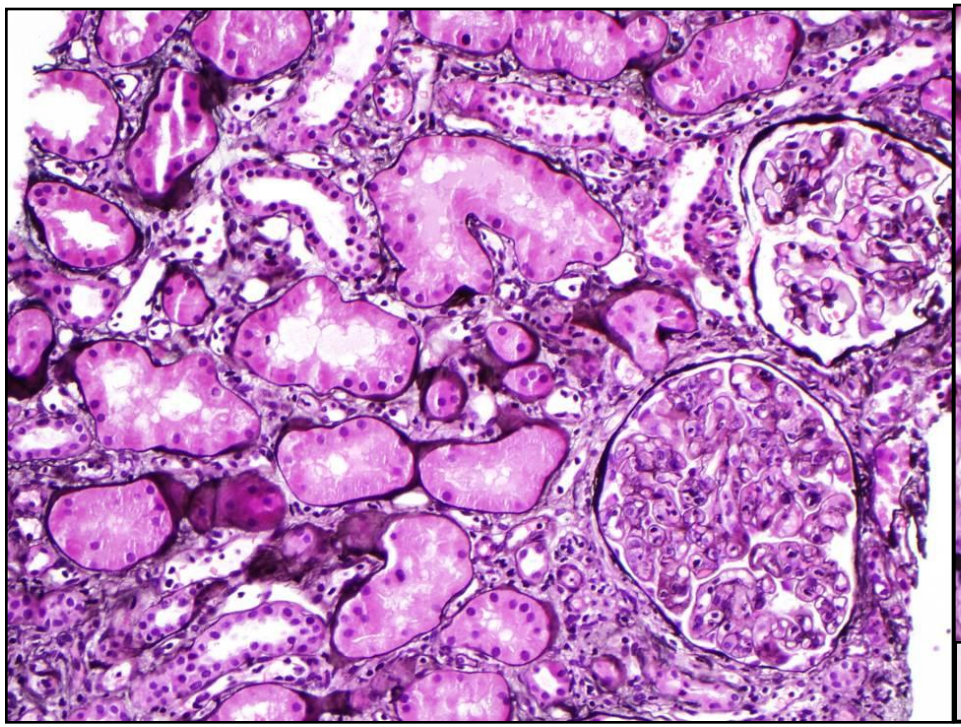


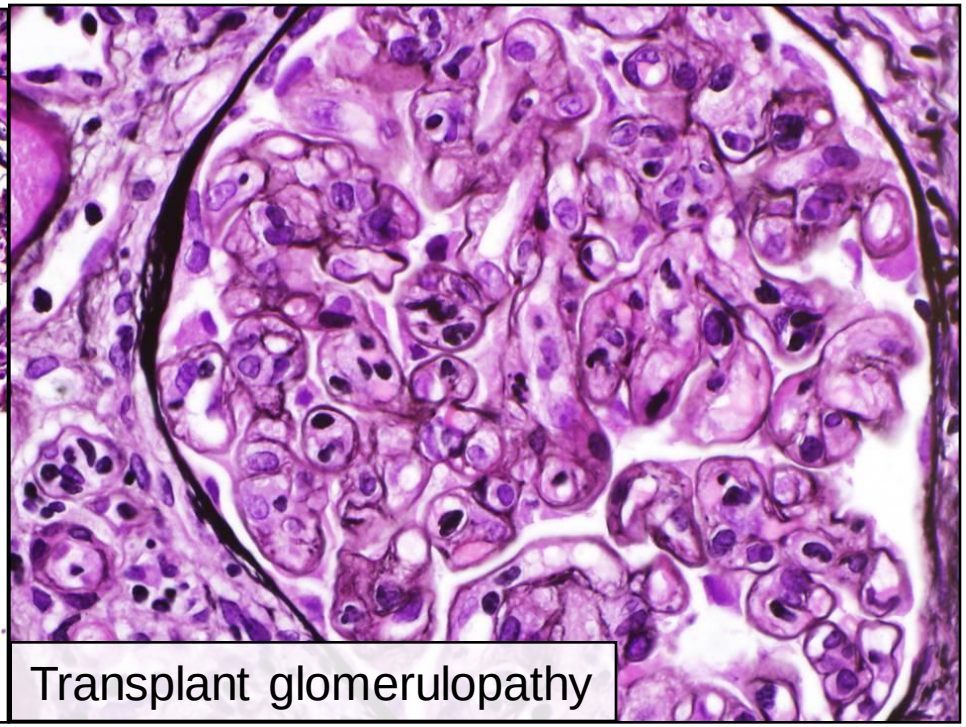
Conventional view of late allografts

Or: progressive antibody-mediated rejection

Terasaki PI. A personal perspective: 100-year history of the humoral theory of transplantation. Transplantation. 2012 Apr 27;93(8):751-6.

Sis B, Campbell PM, Mueller T, Hunter C, Cockfield SM, Cruz J, Meng C, Wishart D, Solez K, Halloran PF. Transplant glomerulopathy, late antibody-mediated rejection and the ABCD tetrad in kidney allograft biopsies for cause. Am J Transplant. 2007 Jul;7(7):1743-52.





Transplant glomerulopathy

What do late (10 year) grafts look like histologically?

160 ten year protocol biopsies from conventional kidney transplants performed 2002-2005

92% on tacrolimus-based immunosuppression

Scoring performed using the Banff 2013 system and additional scoring not addressed by Banff

What do late (10 year) grafts look like histologically?

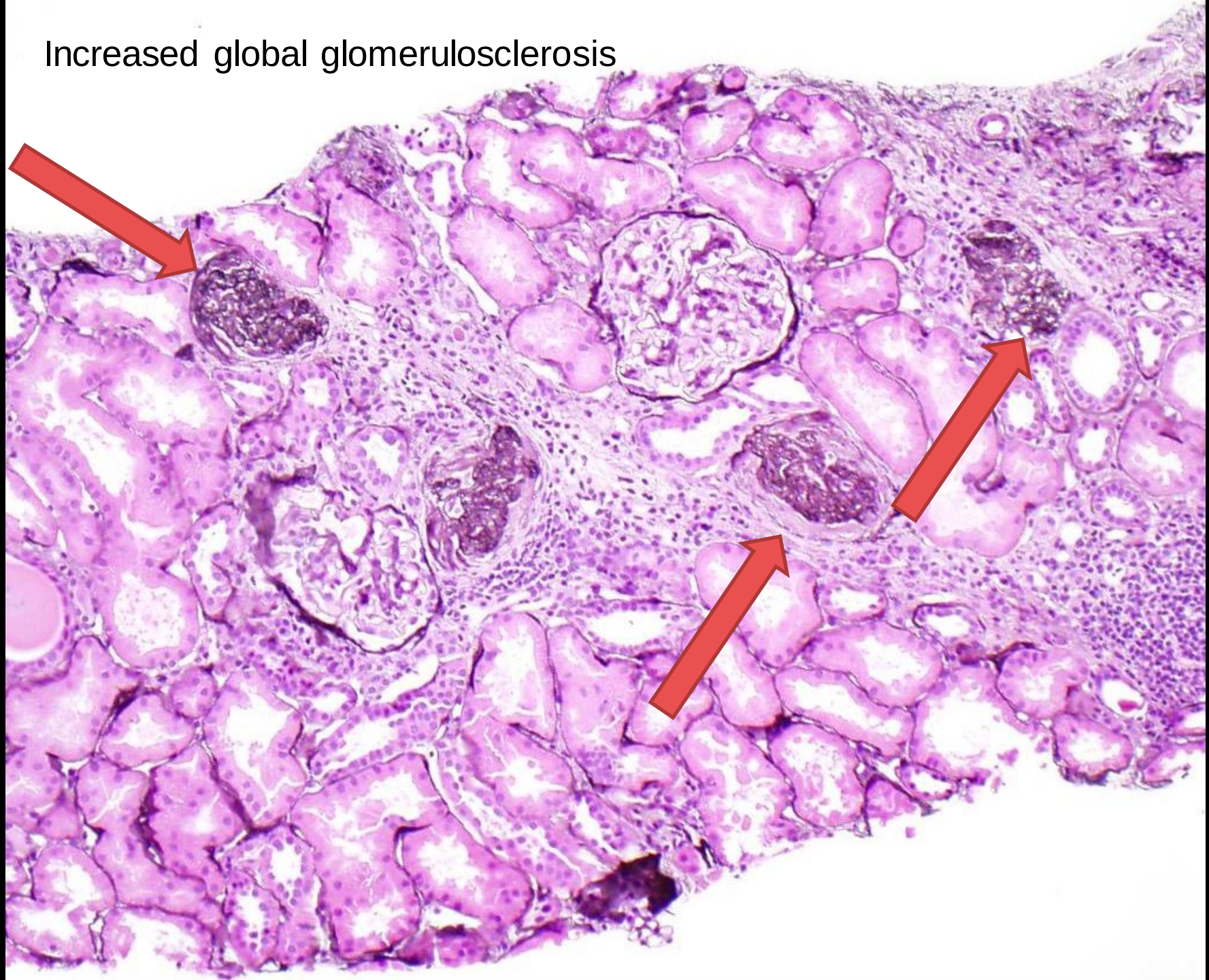
Biopsies:

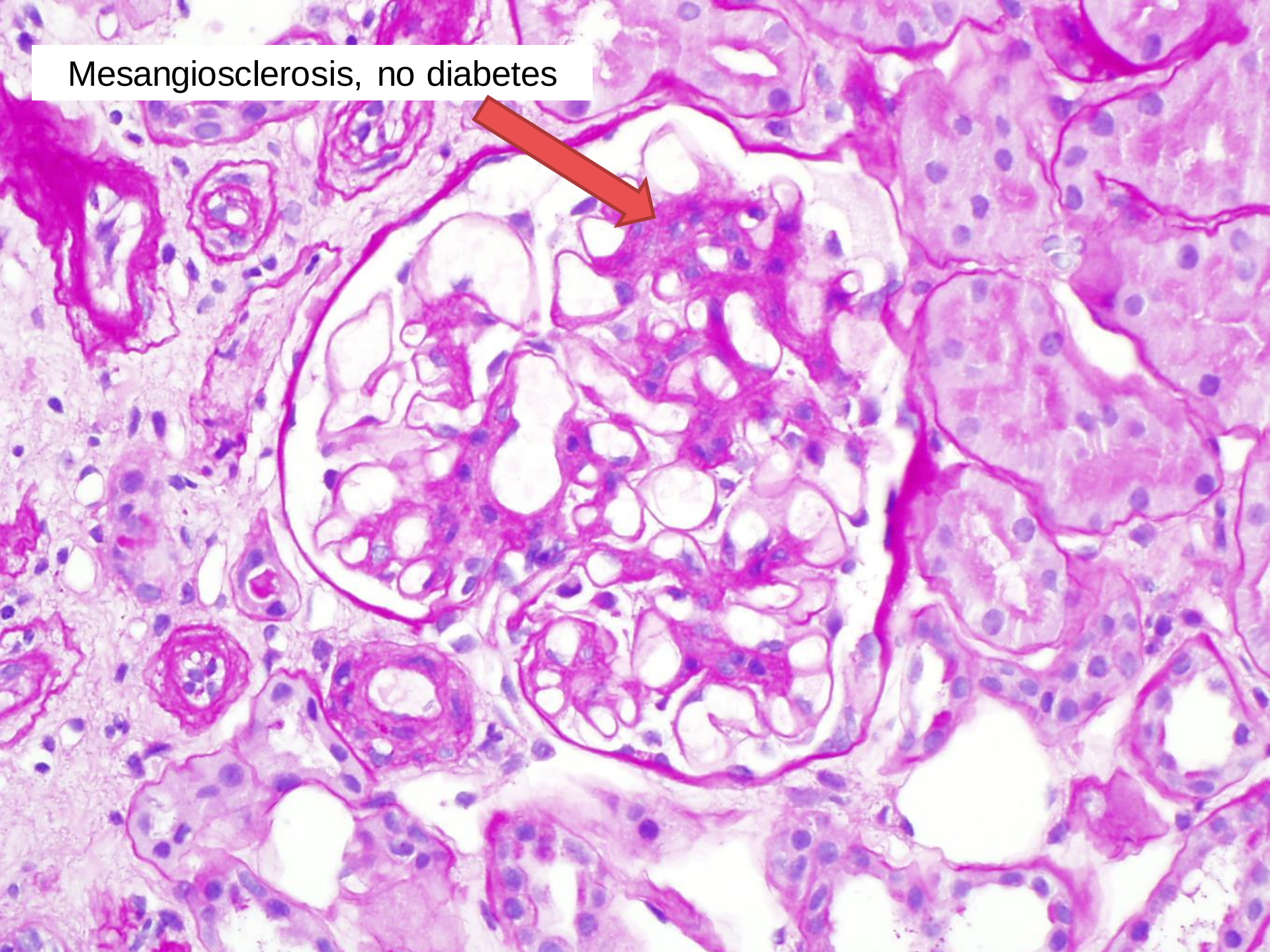
Predominantly glomerular and vascular changes

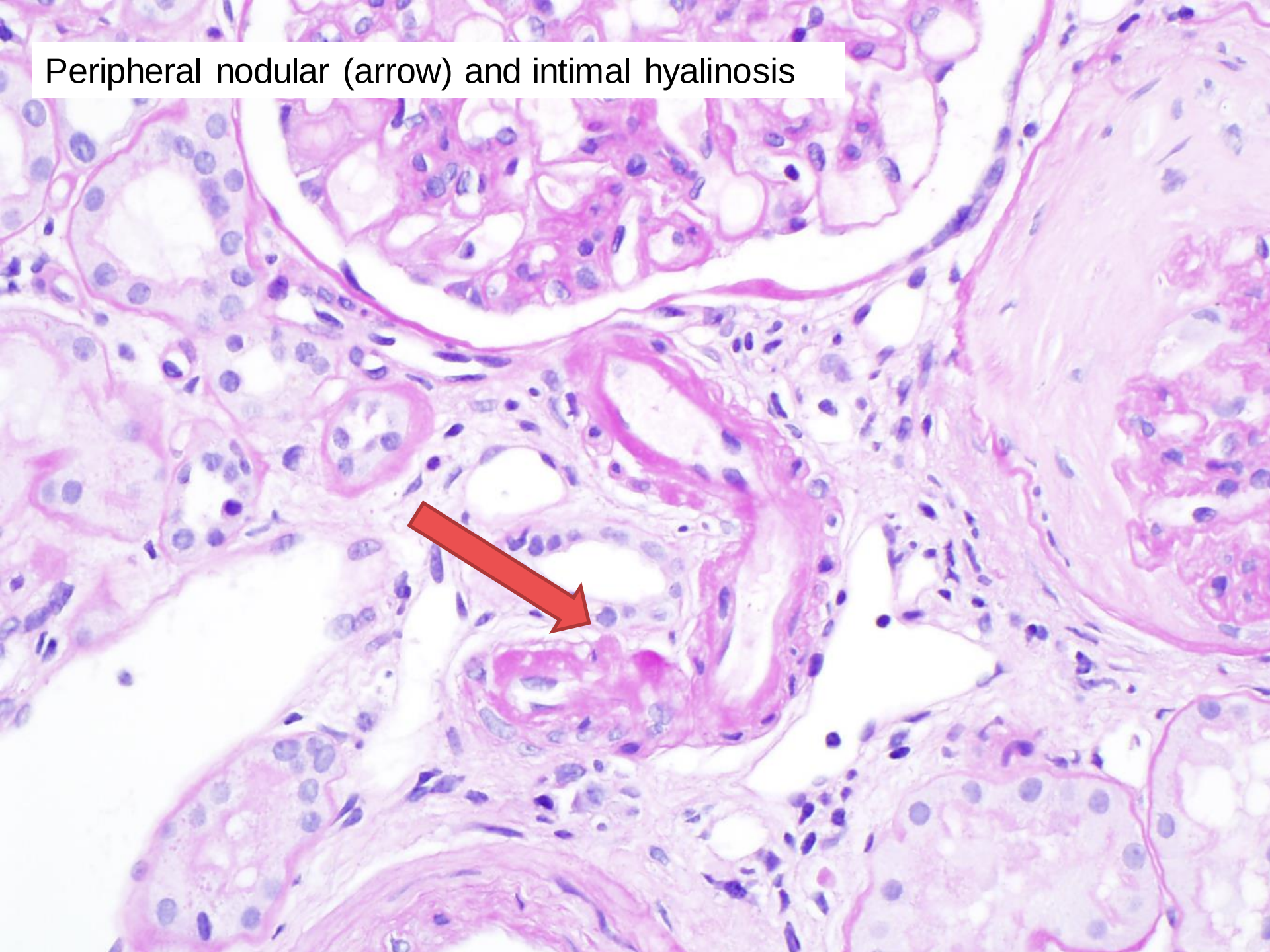
Mild/no IFTA

Low rate of chronic antibody-mediated rejection

Increased global glomerulosclerosis




Mesangiosclerosis, no diabetes


Peripheral nodular (arrow) and intimal hyalinosis

Histologic changes from 0-5-10 years

Histologic Lesion	% of biopsies, time 0 (paired, n= 150)	% of biopsies, 5 years (paired, n= 132)	% of biopsies, 10 years (n=160)	p value (5-10 year bx)
Mesangiosclerosis (MS)	<1%	31%	65%	<0.0001
Glomerulomegaly (GM)	3%	19%	34%	0.0037
Focal segmental glomerulosclerosis (FSGS)	0%	7%	17%	0.012
Increased global glomerulosclerosis (GG), >20%	0%	24%	44%	0.011
Transplant glomerulopathy (TG)	0%	7%	10%	NS (0.40)
Arteriolar hyalinosis, moderate to severe	0%	18%	65%	0.0001
Arteriosclerosis, moderate to severe	5%	17%	38%	<0.0001
IFTA, moderate to severe	<1%	9%	12%	NS (0.57)

Histologic/clinical correlates at 10 years

- Mesangial sclerosis:
  - Associated with baseline or post-transplant diabetes
  - But still present in ~20% of pts without DM
- Arteriolar hyalinosis:
  - More common in diabetics, but still prevalent in patients without DM
- IFTA: more common in diabetics

Conclusions from 10 year protocol biopsy data

- Most chronic injury appears to be due to non-immune causes
- Argues for an alternative viewpoint of the mechanism of late graft injury
- From 5 to 10+ years, the graft is confronted with new pathogenic challenges that likely we are not addressing adequately

Thank you!

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