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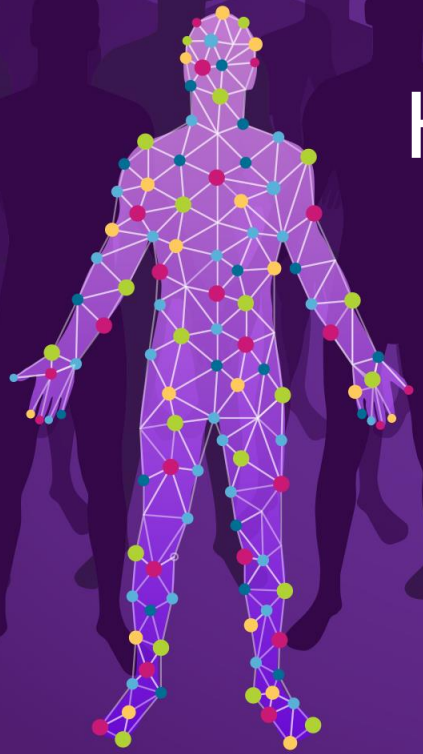

HLA Antibody Attributes

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CEOT CUTTING EDGE of TRANSPLANTATION

TRANSPLANT SUMMIT 2019

NO SIZE FITS ALL: *Uncovering the Potential of Personalized Transplantation*

FEBRUARY 21–23, 2019 • ARIZONA BILTMORE • PHOENIX, AZ

Disclosure

I have no relevant financial disclosures.

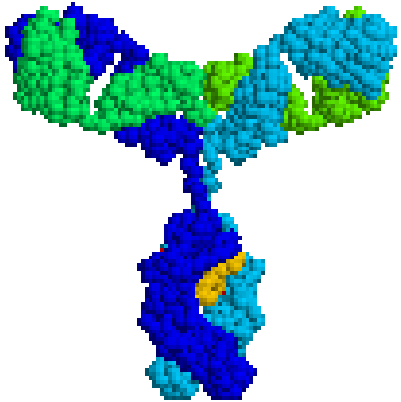
Learning Objectives

1. Understand the basic diverse effector functions of antibodies and how they contribute to AMR

2. Understand the determinants controlling antibody effector functions

3. Learn how HLA antibody effector function and characteristics may be identified and what's known to date about their clinical significance

So…there is much more to AMR and TV than complement!

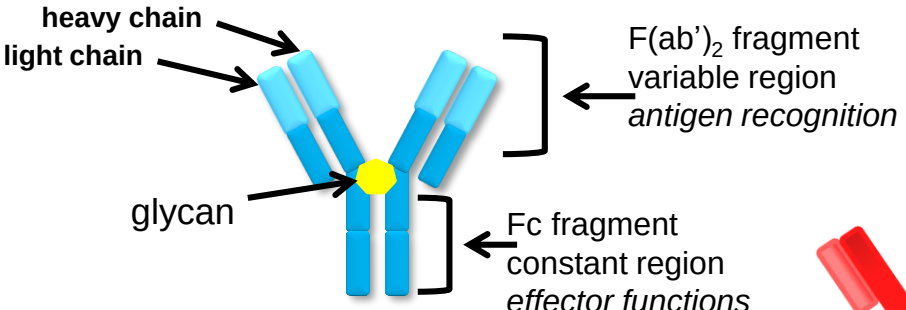


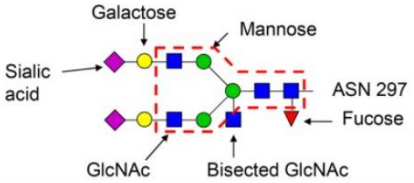
HLA DSA ≠ AMR ≠ HLA DSA

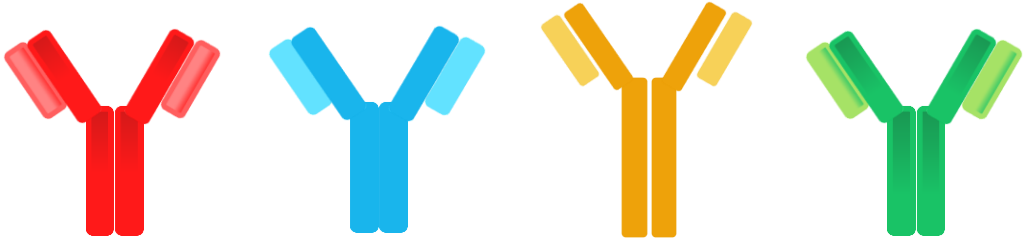
Why is there stable graft function and/or normal histology in some patients with circulating DSA?

How are HLA antibodies causing these types of graft injury?

Human Immunoglobulin System





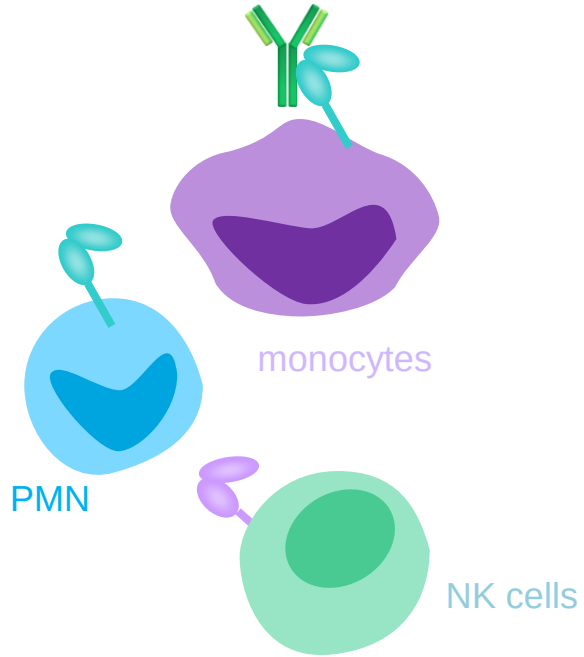


	IgG1	IgG2	IgG3	IgG4
Abundance	++++	++	+	+
Half-Life	21d	21d	7d *or 21d*	21d
Complement activating	++	+/-	+++	-

See Vidarsson *Front Immunol* 2014

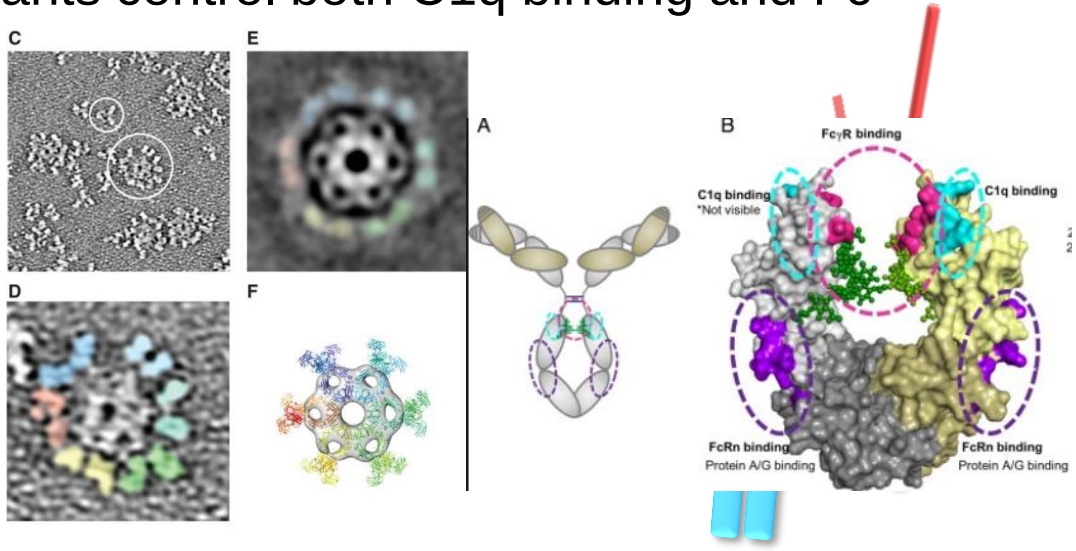
Antibody-FcγR Functions

- Many cell types have Fc gamma receptors: Monocytes, NK cells, neutrophils, DCs, B cells
- Activation of innate immune cells
  - Natural killer (NK cells)
  - Monocytes
  - Neutrophils
- Phagocytosis and opsonization
  - Cooperate with complement receptors to engulf antigen
- Antibody-dependent cell mediated cytotoxicity by NK cells, possibly monocytes
  - Important for many depleting therapeutic antibodies
  - Not definitively shown for HLA antibodies against vascular cells
- Enhancement of adhesion to endothelium
  - Send signals to firm adhesion receptors (integrins)



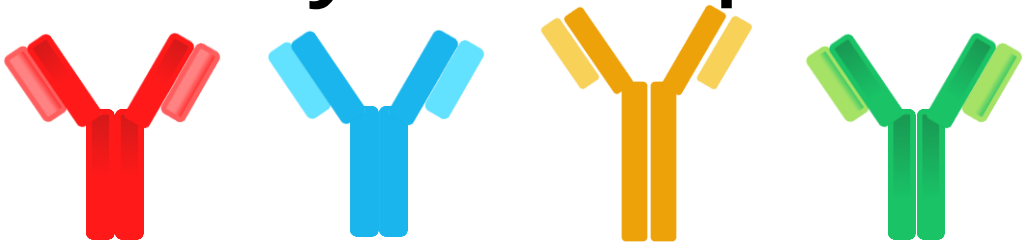
Factors controlling antibody binding to C1q and Fc receptors

- Many of the same determinants control both C1q binding and Fc receptor binding:
  - Sequence (subclass)
  - Hinge region flexibility
  - Antibody abundance and density of antigen
  - Glycosylation



Diebolder *Science* 2014

Affinity for FcγRs



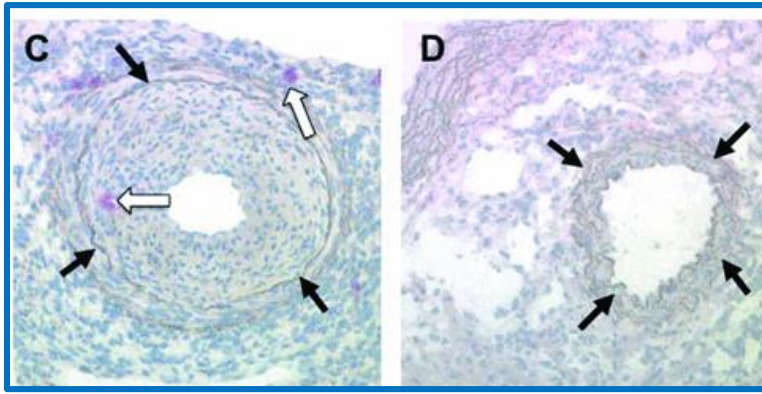
	IgG1	IgG2	IgG3	IgG4
FcγRI	+++	-	++++	++
FcγRIIa (mono/PMN)	+++	++/+*	++++	+
FcγRIIb (inhibitory)	+	-	++	+
FcγRIIIa (NK cells)	+++/++*	+/-*	++++	++/-*

*** Depends on the allele of the Fc receptor**

Adapted from Bruhns *Blood* 2009

Experimental Evidence: NK cells

- Reduced TV in NK cell-impaired mice with DSA [1, 2, 3,4]
- In murine allografts, there is rapid infiltration of NK cells and markers of activation that correlate with DSA [5]
- Depletion of NK cells attenuates rejection [6]
- In the absence of NK cells, DSA triggers an indolent and progressive injury [5]

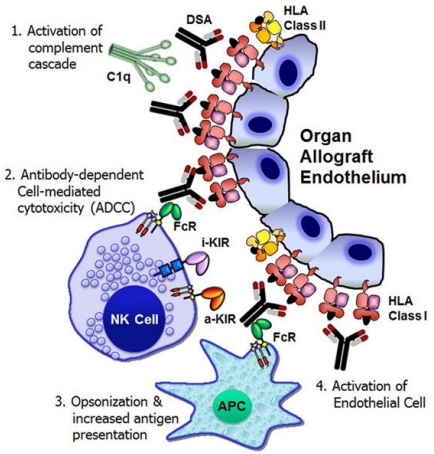


[1] Hirohashi *AJT* 2011
[2] Zhang *Transpl* 2014
[3] Uehara *JI* 2005
[4] Lin *AJT* 2016
[5] Yagisawa *Kid Int* 2019
[6] Kohei *Kid Int* 2016

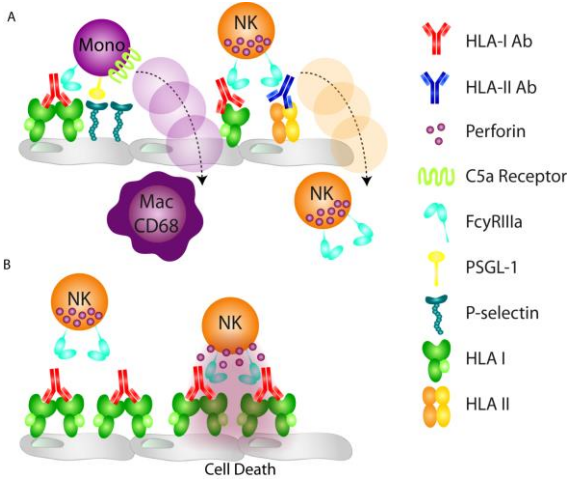
Experimental Evidence: NK cells

- Possible mechanisms:
  - Non-self recognition
  - IFNγ-dependent
  - perforin/ADCC & Fas/FasL dependent killing

- Lin AJT 2016, Kohei Kid Int 2016



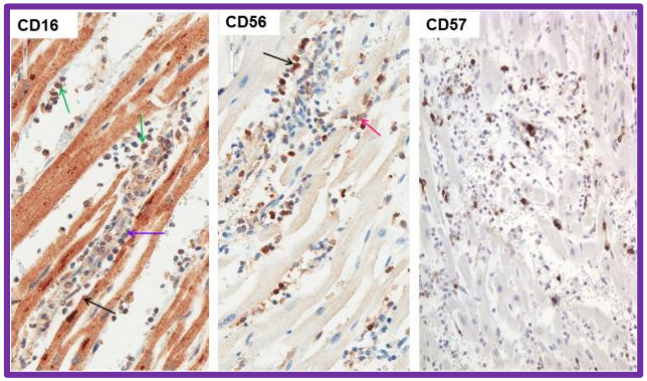
Raj *Front Immunol* 2016

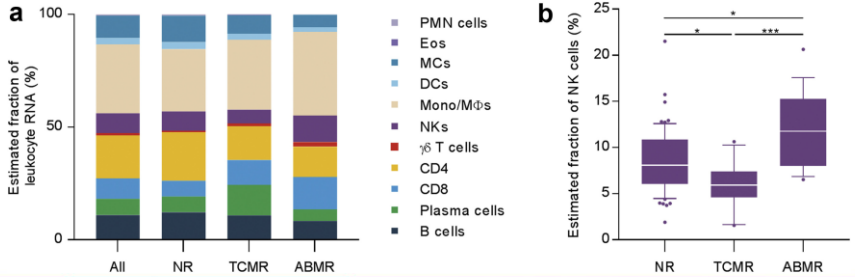


Thomas *Trends Mol Med* 2014

Clinical Evidence: NK cells

NK cell markers are observed within rejecting allografts[2, 3]





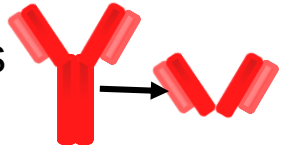
- In renal transplant biopsies, NK cell-associated transcripts are increased in grafts with AMR[4]
- In cardiac transplant recipients, FCGR3A genotype was associated with risk of CAV[1]
- Renal transplant recipients with higher "NK-CHAT" allo-reactivity associated with C4d staining[5]
  - Patients showed variability in their *in vitro* rituximab response, which has been well-described in oncology

[1] Paul *Circ* 2018
[2] Javaheri *Transpl Immunol* 2018
[3] Yazdani *Kid Int* 2019
[4] Parkes *Transpl* 2017
[5] Legris *Front Immunol* 2017

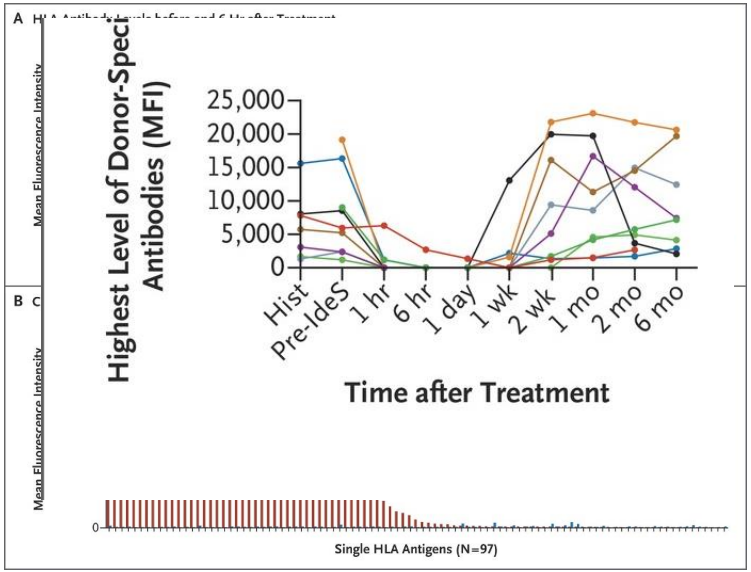
Does eliminating Fc functions (complement and FcγR) prevent graft injury?

Antibody-FcγR Functions: Therapeutic Strategies

- IgG-modifying enzymes
  - IdeS
  - EndoS



- Notable take-homes from IdeS clinical trial outcomes:
  - One hyperacute rejection (thought to be due to IgM/A or AECA)
  - Needed B cell depletion therapy
  - Antibodies reconstituted/rebounded
  - Some patients still experienced clinical and subclinical rejection (similar to Eculizumab)



Jordan *NEJM* 2017

NCT02224820, NCT02426684, NCT02475551

HLA Antibody-Induced Signaling: Experimental (*in vitro*) Evidence

agonistic signaling in vascular endothelium and smooth muscle

Analogous to reverse signaling in APCs at the immunologic synapse

Tyrosine kinase signaling cascades leading to ERK, mTOR, S6RP and S6K activation

Pro-survival signaling at low concentrations (Bcl-2, Bcl-XL)

Rapid calcium-dependent mobilization of vesicles, release of vWF and P-selectin

Increased production of chemokines and cytokines

Increased production of MMPs

Functional Consequence

Pro-growth and increased proliferation

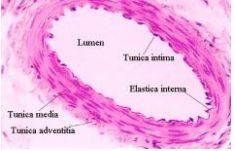
Resistance to cell death and complement-mediated injury

Increased adhesion of neutrophils, platelets and monocytes

Activation of T cells and Th17 differentiation

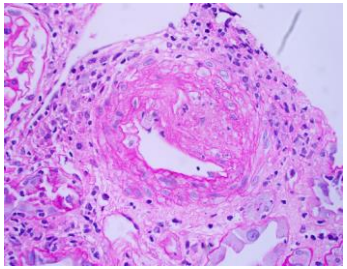
Increased tissue remodeling?

See; Thomas *Trends Mol Med* 2015 for review

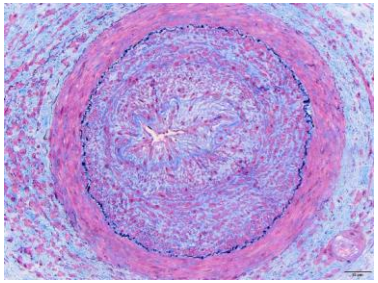


normal

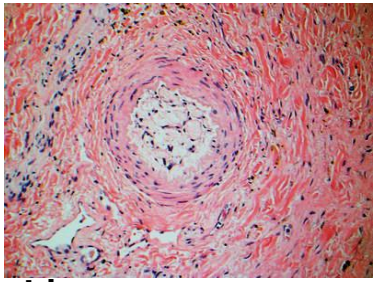
Transplant Vasculopathy



Kidney transplant arteriopathy
(J. Zuckerman)



Heart: Nearly occluded artery
(G. Fishbein)



Liver
Naini Practical Atlas of Transplant Pathology

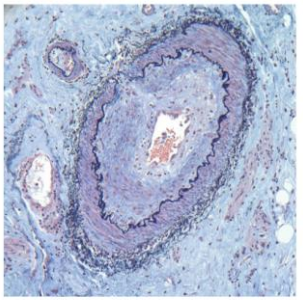
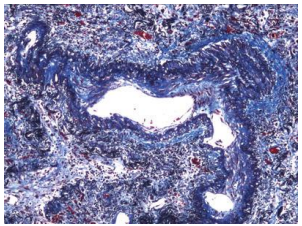
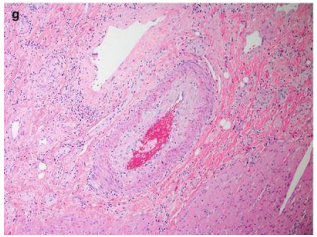


Fig. 8.9 Chronic allograft arteriopathy. Trichrome-elastin stain highlights arterial intimal fibrosis and neointima formation, with narrowing of the vessel lumen

Pancreas
Swanson Practical Atlas of Transplant Pathology



Lung
Wallace Practical Atlas of Transplant Pathology

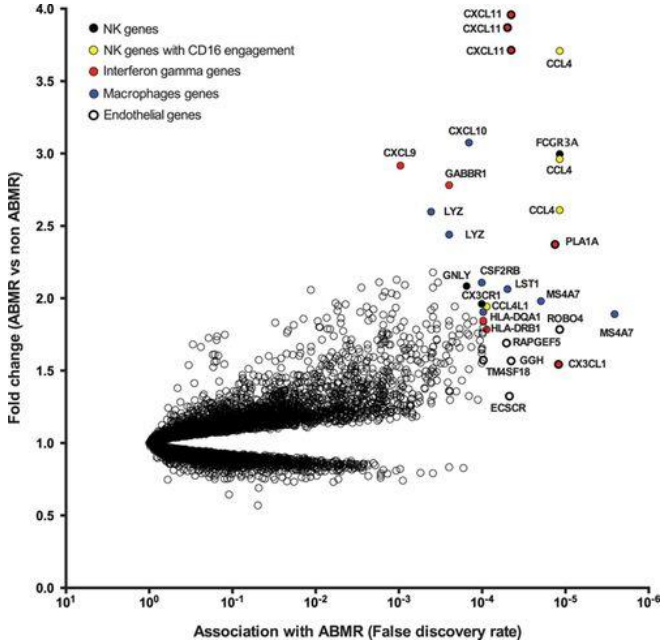


Bowel
Koo Practical Atlas of Transplant Pathology

VCA
Smart Practical Atlas of Transplant Pathology

Molecular Signatures of AMR

- Transcripts within cardiac and renal biopsies with AMR [1, 2]
  - Increased endothelial-specific signatures (ENDAT)
  - Increased NK cell-associated transcripts
  - IFNγ signatures Monocyte/macrophage also increased across rejection
  - AMR gene scores increased with pAMR severity and were more highly associated with MVI than C4d only in pAMR1 [3]



[1] Venner *AJT* 2015
[2] Loupy *Circ* 2017
[3] Afzali *AJT* 2016

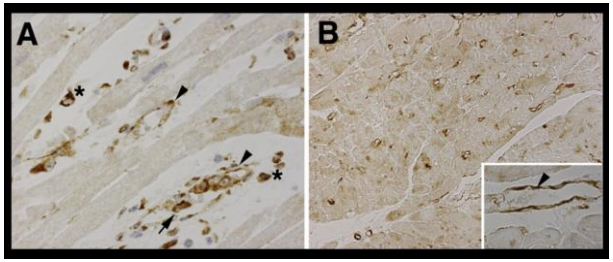
HLA Antibody-Induced Signaling: Clinical Evidence

Capillary phosphorylation of mTOR targets (S6K, S6RP) are significantly associated with AMR [1, 2, 3]

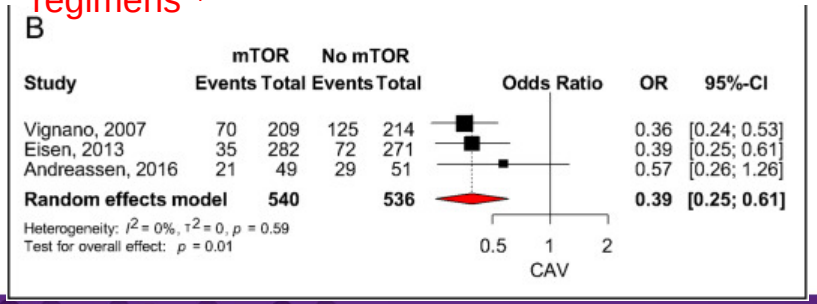
Table 3

Association between grades of staining of S6K, S6RP, ERK and pAMR

	Odds Ratio	p-value	95% CI
S6K, grade 0	Baseline	N/A	N/A
S6K, grade 1	18	0.001	3 – 100
S6K, grade 2	52	<0.001	6 – 425
S6K, grade 3+	49	0.001	5 – 521
S6RP, grade 0	Baseline	N/A	N/A
S6RP, grade 1	4	0.06	1 – 13
S6RP, grades 2, 3+	10	0.008	2 – 52
ERK, grade 0	Baseline	N/A	N/A
ERK, grade 1	5	0.2	0.4 – 53
ERK, grade 2	0.8	0.8	0.1 – 7
ERK, grade 3+	0.4	0.4	0.04 – 4



Recipients on mTORi have significantly reduced incidence of CAV compared with CNI-based regimens [4]



[1] Lepin *AJT* 2006
[2] Li *JHLT* 2016
[3] Tible *JHLT* 2013
[4] Jennings *Int J Cardiol* 2018

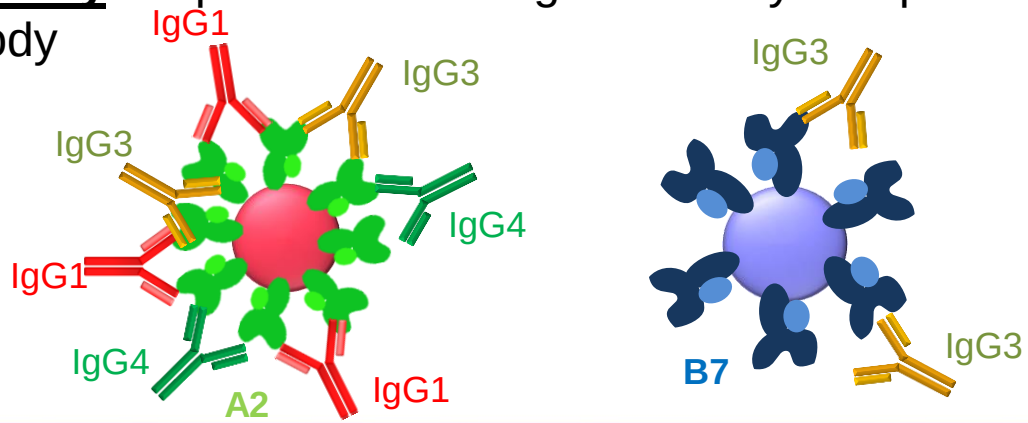
HLA Antibody Attributes

	IgG1	IgG2	IgG3	IgG4
Activating FcR on mono/PMN/NK cells	better	worst	best	good
Inhibitory FcR	better	worst	best	good
Complement	better	good	best	worst
Agonistic Signaling	?	?	?	?

Can we assess biological function [potential] with an *in vitro* diagnostic assay?

Subclass Assay: Purpose and Method

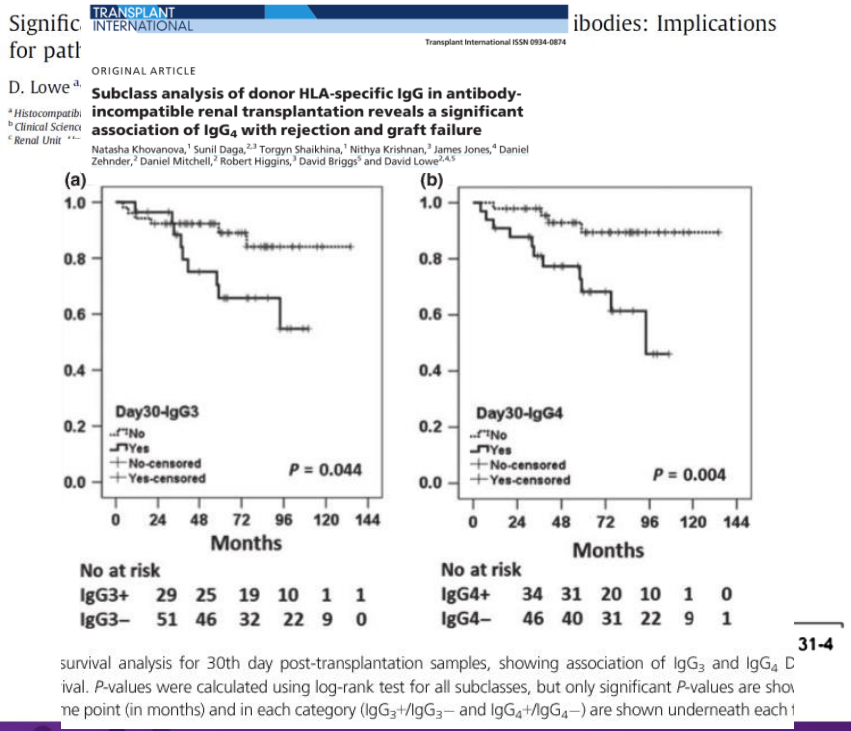
- **Purpose**: to characterize the IgG subclass (IgG1-4) of anti-HLA antibodies
- **Method**: Modified single antigen assay with swapped out secondary detection reagents against each subclass
- **Potential Utility**: To predict the biological activity and pathological potential of an antibody



IgG Subclasses of HLA antibodies

- Pre-transplant, anti-HLA antibodies are predominantly a mix of subclasses
- Pregnancy and transplantation stimulate a mix, while transfusion stimulates predominantly IgG1 only

- Pre-formed IgG1 or IgG4 DSA independently predictive of rejection in the first 30 days
- Only 1 patient showed IgG4 without IgG1
  - Reiterates that usually a mix of subclasses



Signific TRANSPLANT INTERNATIONAL ibodies: Implications for patl

Transplant International ISSN 0934-0874

D. Lowe [a]

[a] Histocompatib
[b] Clinical Scienc
[c] Renal Unit

ORIGINAL ARTICLE

Subclass analysis of donor HLA-specific IgG in antibody-incompatible renal transplantation reveals a significant association of IgG_4 with rejection and graft failure

Natasha Khovanova,[1] Sunil Daga,[2,3] Torgyn Shaikhina,[1] Nithya Krishnan,[3] James Jones,[4] Daniel Zehnder,[2] Daniel Mitchell,[2] Robert Higgins,[3] David Briggs[5] and David Lowe[2,4,5]

31-4

survival analysis for 30th day post-transplantation samples, showing association of IgG_3 and IgG_4 D
ival. *P*-values were calculated using log-rank test for all subclasses, but only significant *P*-values are sho
ne point (in months) and in each category (IgG_3+/IgG_3– and IgG_4+/IgG_4–) are shown underneath each

IgG4 DSA is not associating with no rejection!

IgG Donor-Specific Anti-Human HLA Antibody Subclasses and Kidney Allograft Antibody-Mediated Injury

Carmen Lefaucheur,*† Denis Viglietti,*† Carol Bentlejewski,‡ Jean-Paul Duong van Huyen,†§ Dewi Vernerey,‖ Olivier Aubert,† Jérôme Verine,¶ Xavier Jouven,† Christophe Legendre,** Denis Glotz,* Alexandre Loupy,†** and Adriana Zeevi‡

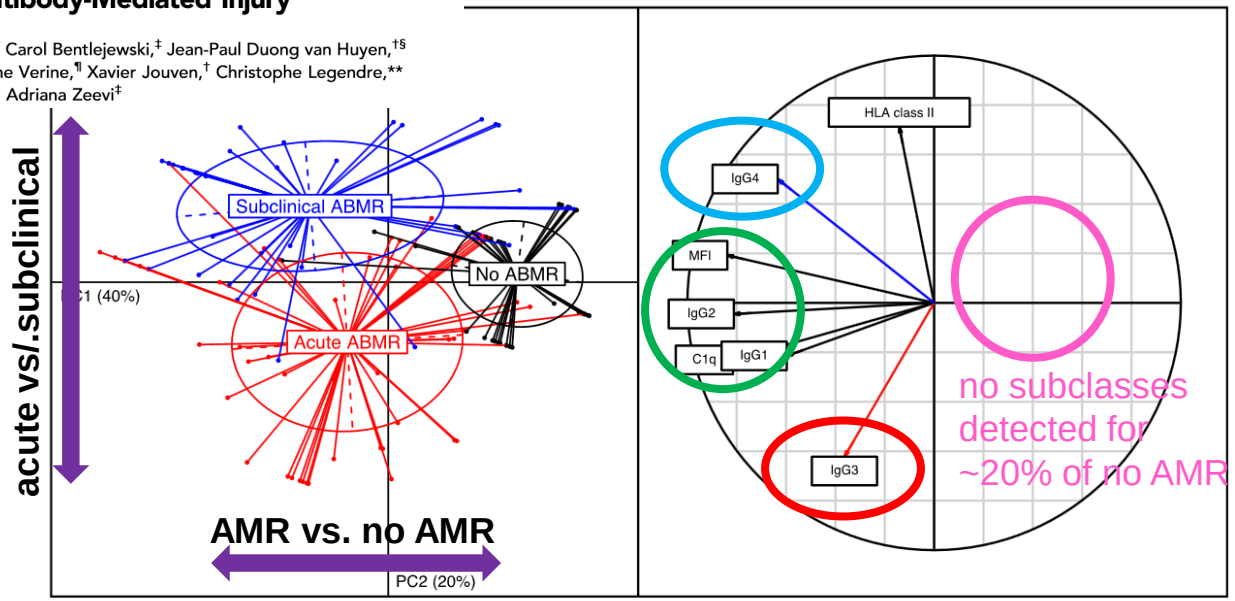


Figure 2. Identification of the three distinct rejection phenotypes according to the characteristics of the dominant donor-specific anti-HLA antibody (MFI, HLA class specificity, C1q-binding capacity, and IgG1–4).

IgG1, IgG2 C1q binding distinguish AMR vs. no AMR

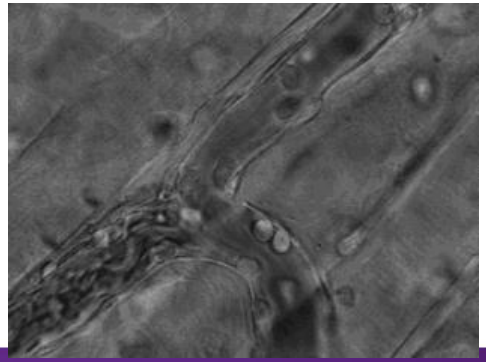
IgG3 distinguishes acute vs. subclinical

IgG4 distinguishes acute vs. subclinical

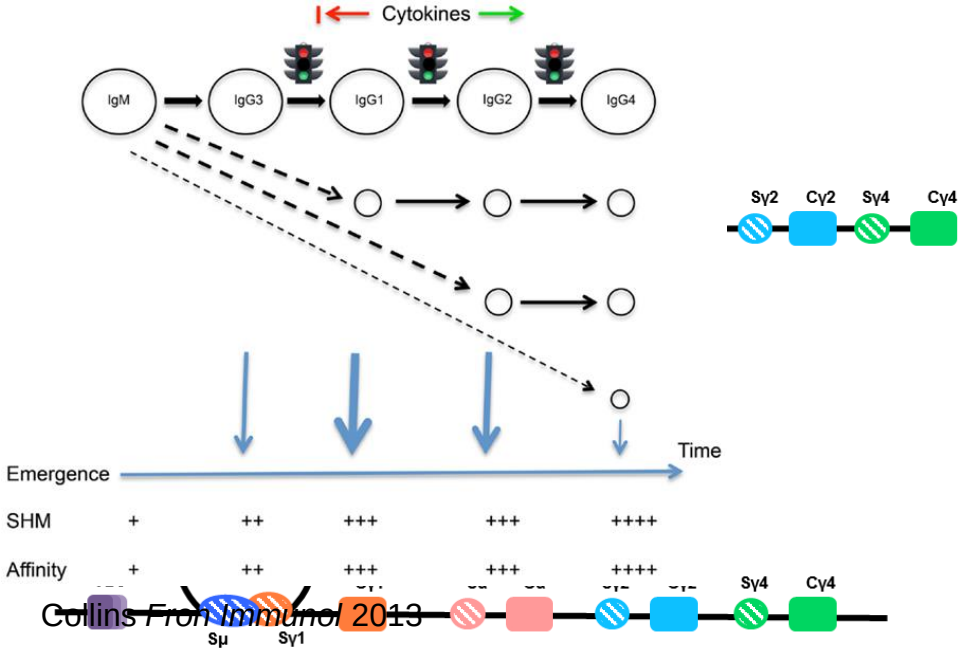
Subclass Assay: Pros and Cons

Pros
- More information than C1q or C3d
- Can infer more than complement!
  - Informs on other effector functions such as Fc receptor binding

Cons
- Not commercial/not well-validated
- Requires 5 total single antigen tests (expensive and laborious)
- Cannot directly compare the relative signals for each subclass
  - We don't know the actual quantities and relative abundance of subclasses against HLA
- Some cross-reactivity of the secondaries (not a clean test)
- Some inability (~15%) to detect any subclasses even when total IgG signal was strong
  - Sensitivity?
- Recent trend in the literature to measure only IgG3 associations with outcome
  - Not enough comprehensive, reliable assessments have been reported to neglect the other subclasses



Time-dependent IgG subclass production

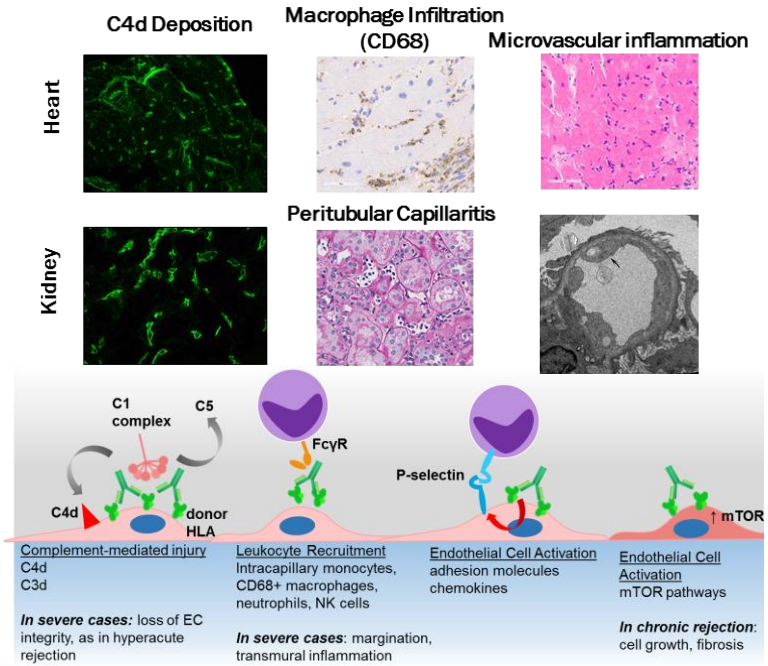


Collins *Front Immunol* 2013

- Antigen properties and immune context shape the CSR response
- B cells that class switch cannot switch back
- Production of downstream constant regions suggests repeated antigen stimulation and germinal center reactions
- IgG3 or IgG1 suggests an early, recent immune response
- IgG4 points to prolonged antigen exposure

Implications for AMR Mechanistic Understanding and Therapy

Images courtesy of D. Wallace, J. Zuckerman & G. Fishbein (UCLA)



Valenzuela and Reed *JCI* 2017

- Understanding the "attributes" of an antibody might predict pathogenic functions
  - Remains to be demonstrated experimentally
- Antibody subclasses are generated under different conditions and times
  - Not only important for effector functions;
  - implications for biology of the immune response
    - 'imbalances' in IgG subclasses are pathogenic in or markers of many diseases
  - And types of B cells producing those antibodies
- May have utility describing the individual's alloimmune status (memory, newly activated, chronically stimulated)
  - Could it also point to potential efficacy (or inefficacy) of different B cell targeted therapies?

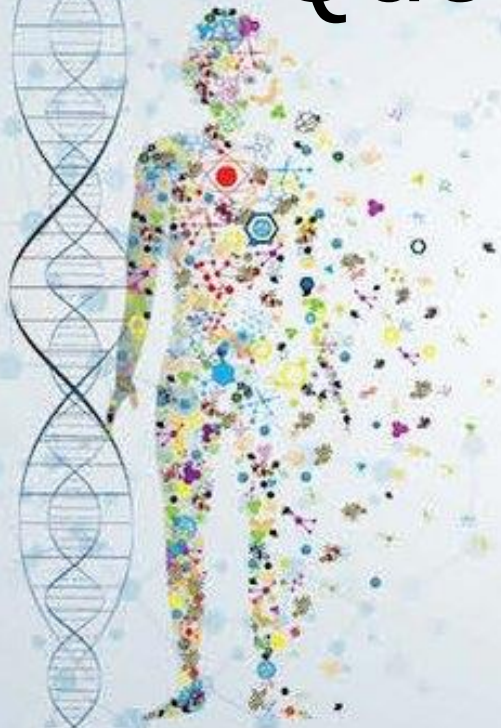
In closing…

ALL HLA antibodies that can bind donor cells, regardless of subclass, are likely to be pathogenic [until proven otherwise]

- Stratified risk:
  - cytotoxicity ~ hyperacute or accelerated rejection
  - innate immune activation ~ acute rejection, TV
  - vascular signaling ~ subclinical, smoldering, TV?

- Beyond complement and FcγRs:
  - Agonistic signaling
  - Association with chronic antigen exposure
  - Association with subclinical AMR

Questions for Discussion

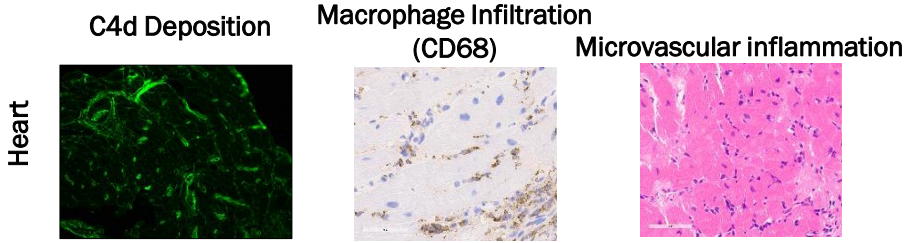


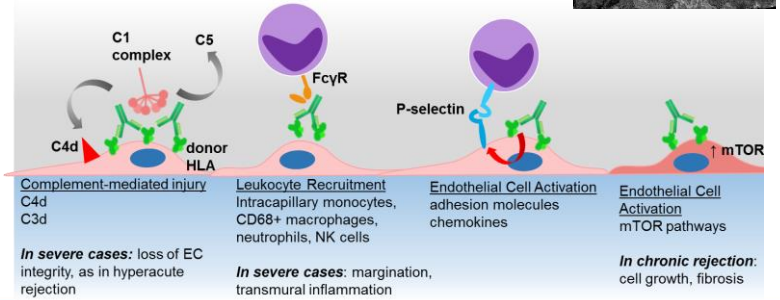
- Is there clinical utility in further characterizing HLA antibody attributes?
- Role for recipient polymorphisms?
  - Complement
  - FcγRs
  - Immunoglobulin allotypes (ex. FcRn affinities)
  - Implications for risk stratification and appropriate therapies?
- How will we integrate all the information from these myriad tests into a refined, accurate and personalized approach for our patients?

https://blogs.scientificamerican.com/guest-blog/personalized-medicine-a-faustian-bargain/

Mechanisms and histological features of (HLA) antibody mediated injury

Images courtesy of D. Wallace, J. Zuckerman & G. Fishbein (UCLA)





Valenzuela and Reed *JCI* 2017

In vitro detection of complement binding or activation by HLA antibodies (C1q, C3d, CDC-XM) is <u>not</u> directly analogous to actual human complement activation *in vivo*

IgG1 is most abundant in the serum and dominates most immune responses

- Most immune responses elicit a mixture of IgG subclasses

IgG4 is the only **true** "non-complement fixing" subclass

- But it was still associated with rejection

Determinants of C1q Binding in the Single Antigen Bead Assay

Stefan Schaub,[1] Gideon Hönger,[1] Michael T. Koller,[2] Robert Liwski,[3,4] and Patrizia Amico[1]

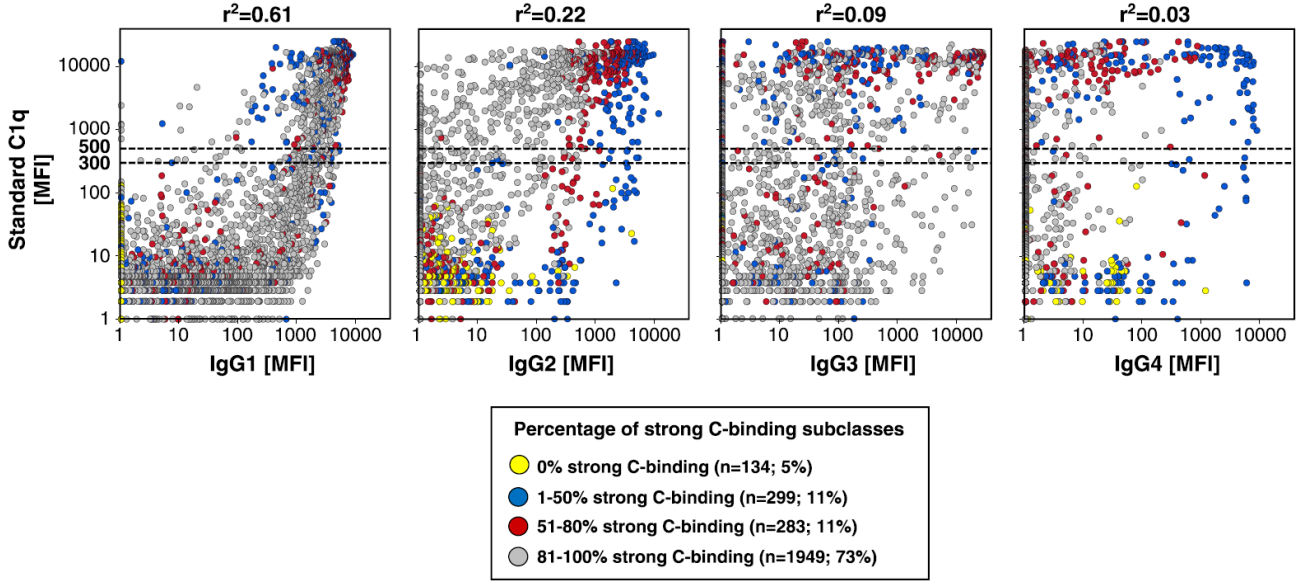
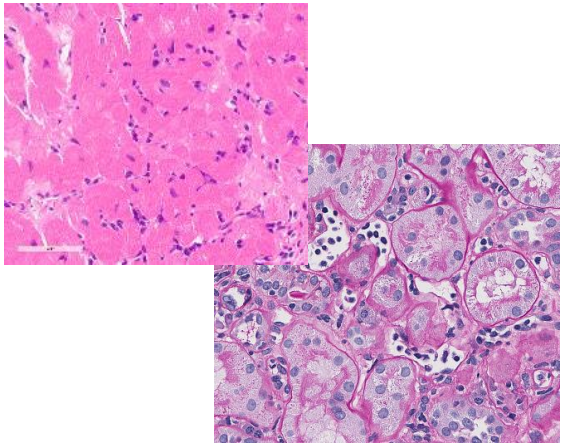


FIGURE 2. Correlation of standard C1q MFI with IgG subclass MFI. MFI values are plotted on a log scale. Only IgG_{pan}+/ $IgG_{subclass}$+ SAB were included (n=2,665). Individual SAB are color coded by the percentage of strong C-binding subclasses. The given r^2 were calculated by simple logistic regression using the standard C1q MFI greater than 300 cutoff.

Complement is not necessarily required for AMR or transplant vasculopathy

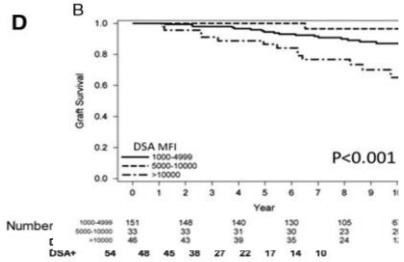


Evident both from murine studies and from clinical experience

- C3 deficient recipient mice develop transplant arteriopathy in the presence of DSA [1]
- …although it may enhance TV and adaptive alloreactivity in general [2, 3]
- C4d negative AMR recognized in renal and heart transplantation [4, 5]
  - ; Little benefit to prophylactic terminal complement inhibition in DSA+ renal transplant patients with deteriorating graft function [6]

[1] Hirohashi *AJT* 2010
[2] Jane-Wit *Circ* 2013
[3] Qin *AJT* 2016
[4] Haas *AJT* 2014
[5] Berry *JHLT* 2013
[6] Kulkarni *AJT* 2016

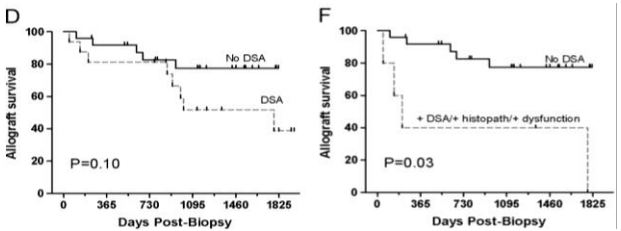
It’s the patients who experience rejection that have the worst long-term outcomes



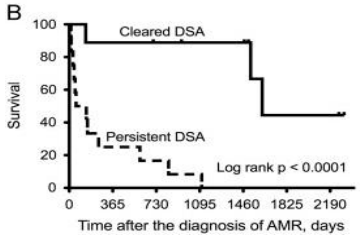
Liver: O’Leary *Transpl* 2017

Kidney: Lefaucheur *JASN* 2010

Heart: Kobashigawa *JHLT* 2015

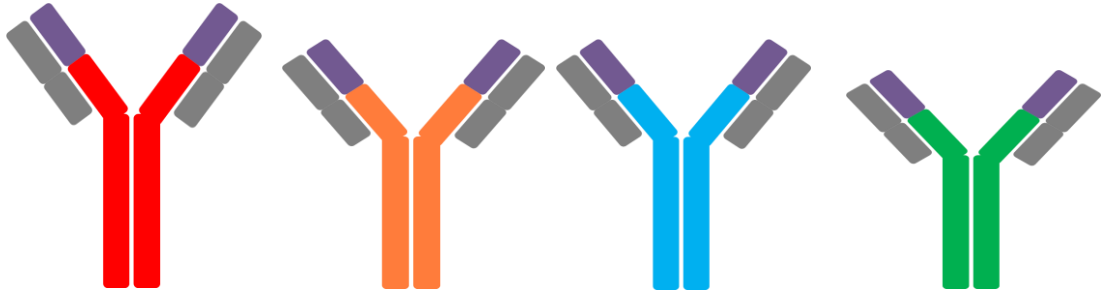


Lung: DeNicola *JHLT* 2013



Lung: Witt *JHLT* 2013

Human IgG Subclasses



	IgG3	IgG1	IgG2	IgG4
Abundance in circulation	Low ~4%	Highest ~50-60%	High ~30-50%	Low ~4%
Half-Life	7-21 days*	21 days	21 days	21 days
Affinity for Antigen	Relatively lowest	High	High	Highest
Notable for	Long hinge region	Nearly always present and dominant	Response to carbohydrate as well as protein antigens	Ability to form monovalent arms and bispecific heterodimers
Tempo	Earliest, transient	Early and memory	Later	Much later, chronic antigen exposure

Human Immunoglobulin System

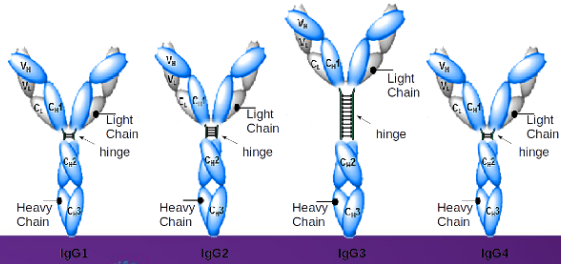
Isotypes IgD IgM IgG IgA IgE

Subclasses IgG1 IgG2 IgG3 IgG4 IgA1 IgA2

Allotypes

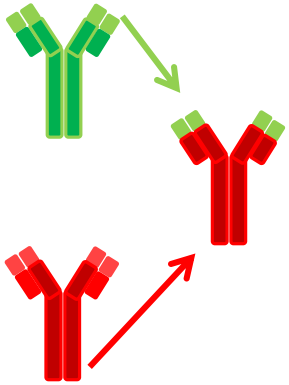
G1m	G2m	G3m	A2m
1(a)	23(n)	g1	1
2(x)		g5	2
3(f)		b0	
17(z)		b1	
		b3	
		…	
		13 alleles	

Exist in haplotypes differentially distributed among ethnic groups



Methods and Approach

Variable regions of murine anti-HLA class I (W6/32)



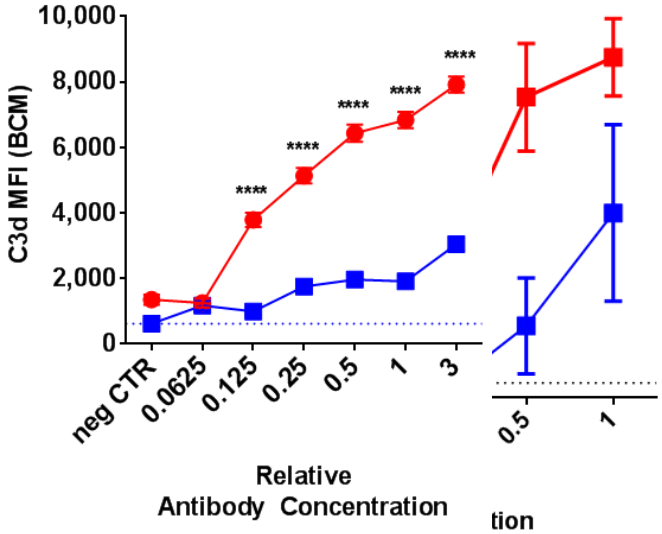
Chimeric human-mouse pan HLA I IgG

expressed in CHO cells

Constant regions of human IgG
(S Morrison)

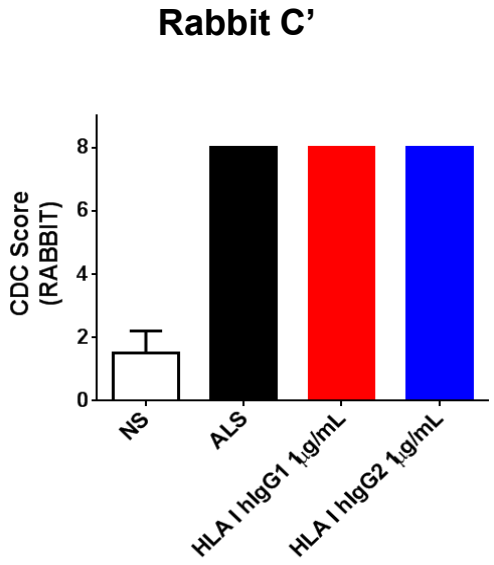
Same antigen binding region on different subclasses

Recognizes monomorphic epitope on all HLA class I molecules → recognize antigen on all beads with the same affinity



While less potent than IgG1, IgG2 was still capable of fixing C1q on single antigen beads at high concentrations

HLA I IgG1 and IgG2 both trigger cytotoxicity in the CDC assay



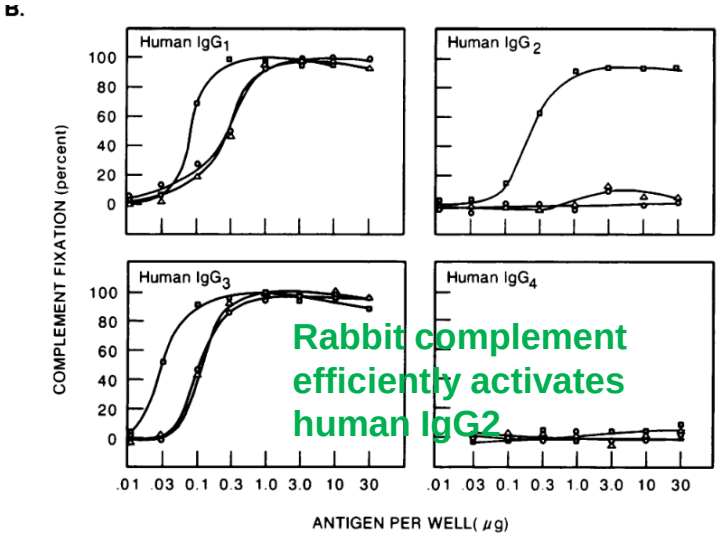
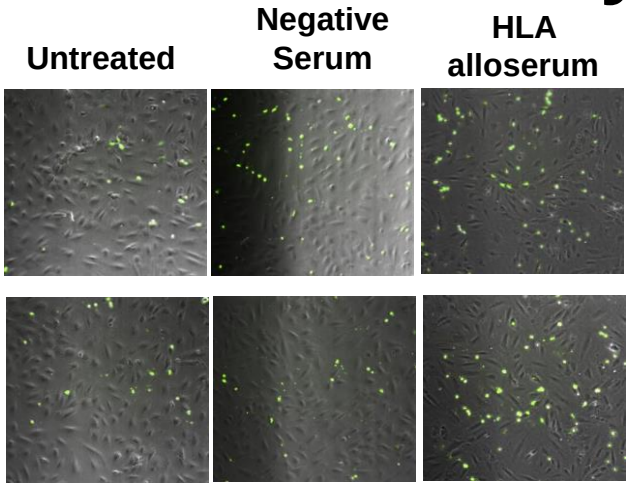
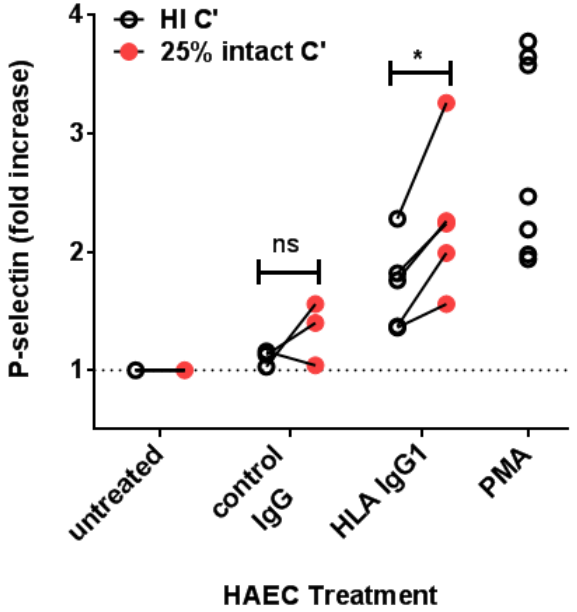


Fig. 3. Fixation of human (○), guinea pig (△), and rabbit (□) complement by chimeric anti-DNS IgG antibodies. Mouse IgG isotypes (**A**) and human isotypes (**B**) were tested in complement consumption assays (see Materials and methods). Each data point represents the mean of four to eight measurements.

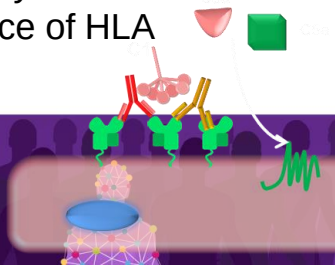
Dangl *EMBO* 1988

Monocyte adherence is enhanced by complement





Complement augments endothelial cell activation and monocyte adherence in the presence of HLA antibodies



Valenzuela *Transpl* 2017