

# Transplantation

Jonathan S. Maltzman  
FOCIS Advanced Course, San Diego  
February 2023

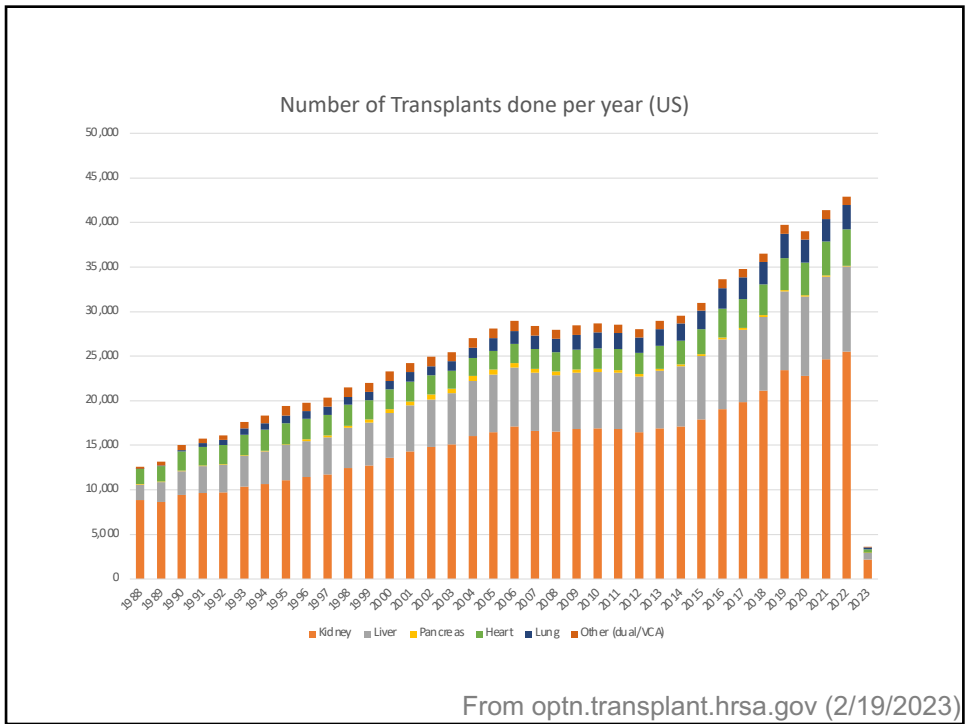


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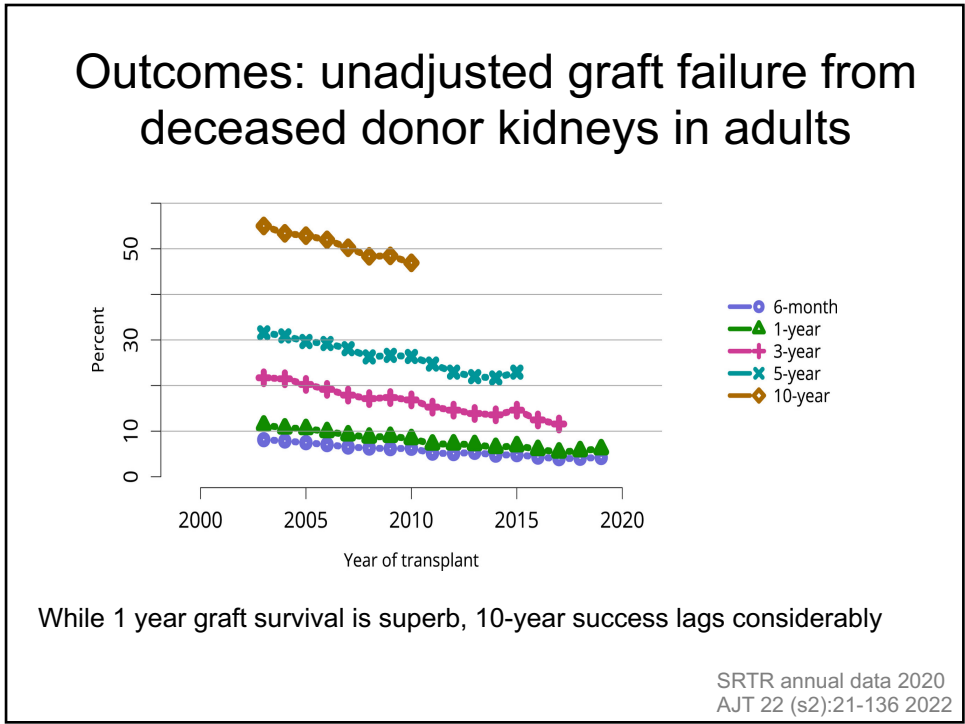
## Disclosures:

- My spouse is employed by Iconovir
- SAB - Qihan

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## Outline

- MHC / HLA
- Direct vs indirect allorecognition
- Alloreactive cells: where do they come from?
- Immunosuppression
- Xenotransplantation
- Tolerance

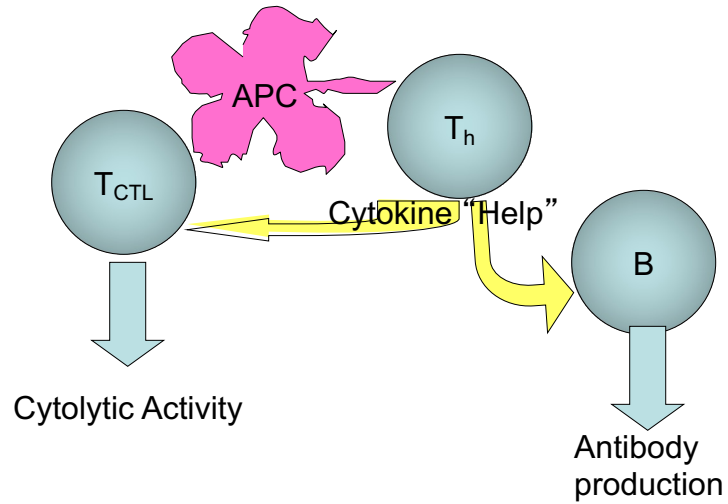
5

## A few quick definitions

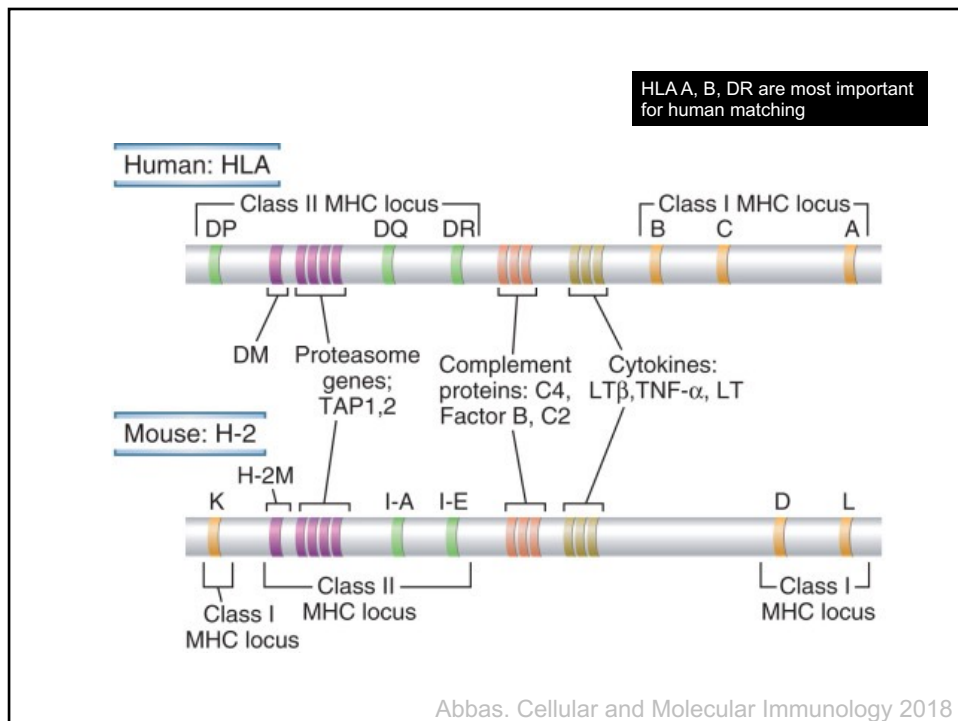
- Syngeneic: Refers transplants between genetically identical animals (i.e. identical twins, inbred strains of animals). Accept transplants from one-another without immunosuppression.
- Allogeneic: Transplants between animals mismatched for MHC or other histocompatibility genes, within the same species.
- Xenogeneic: Transplants between species.

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## Lymphocytes mediate the immune response to transplants

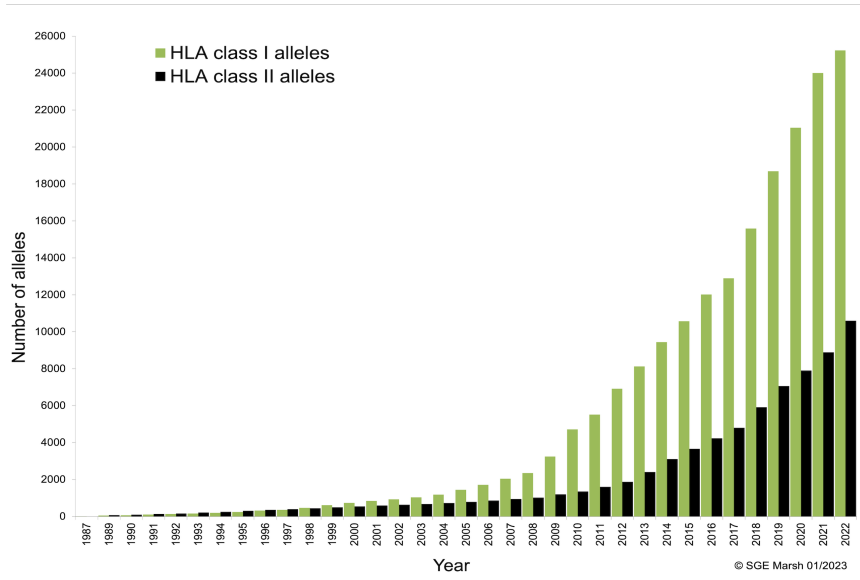


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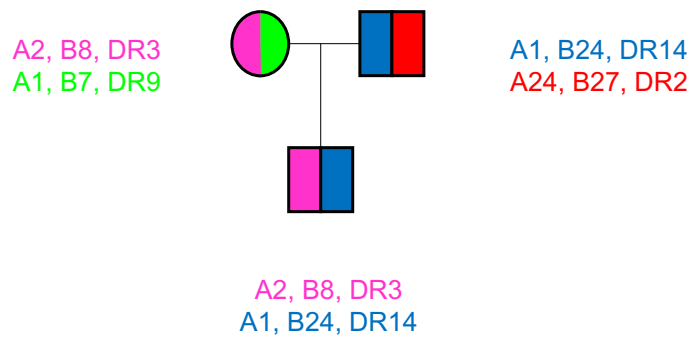
## HLA is highly polymorphic



Source: [www.hla.alleles.org](http://www.hla.alleles.org)

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## Inheritance and Expression of HLA genes



- HLA genes are inherited as a haplotype and co-dominantly expressed

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## MHC/HLA class I and class II proteins

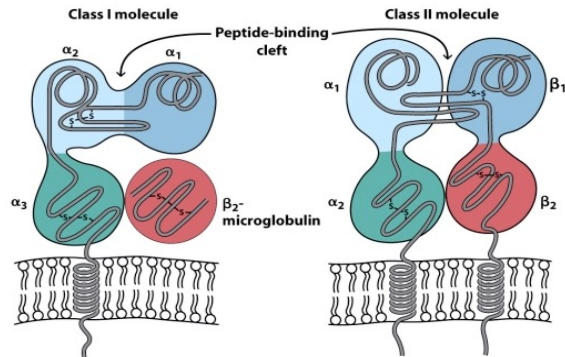


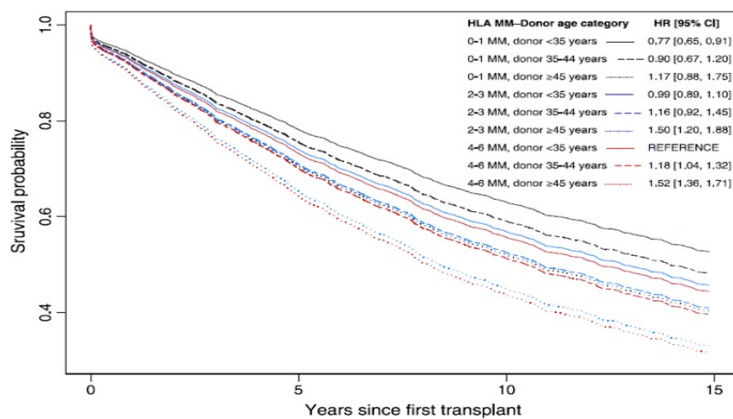
Figure 8-3  
Kuby IMMUNOLOGY, Sixth Edition  
© 2007 W.H. Freeman and Company

- Class I expressed on all nucleated cells
- Present "endogenous" peptides

- Class II expressed on APC
- Present "exogenous" peptides

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## HLA matching improves the survival of transplanted kidneys



Matching at HLA-A, -B and -DR is particularly important

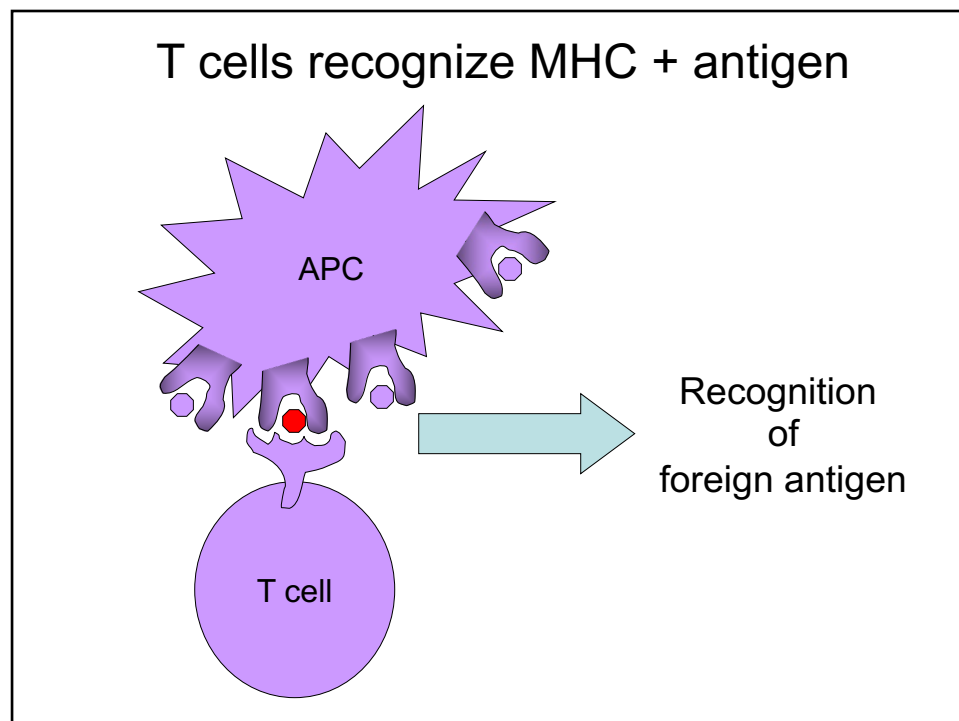
Ped Nephrol 2019

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## High Precursor Frequency

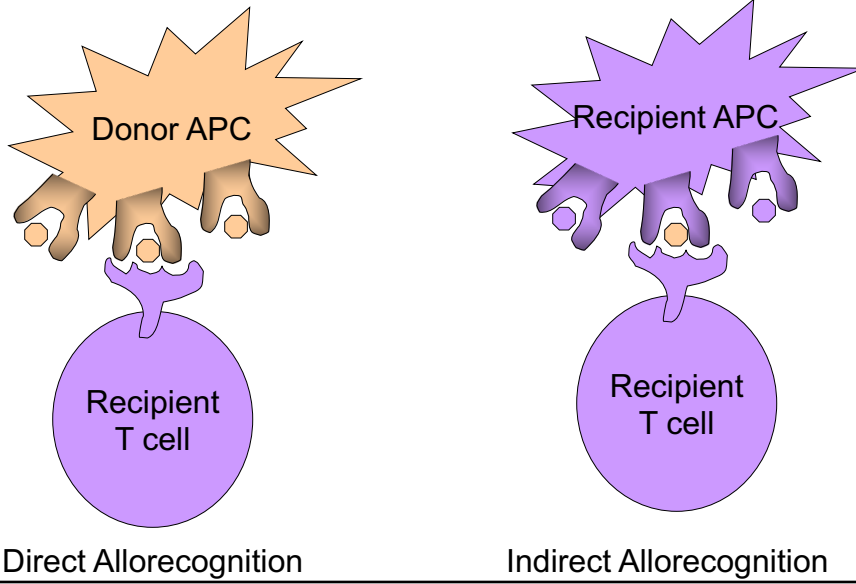
- Frequency of T cells that recognize a given peptide antigen:  
Approx. **1/100,000 - 1/1,000,000**
- Frequency of T cells that recognize an alloantigen:  
Approx. **1/20**

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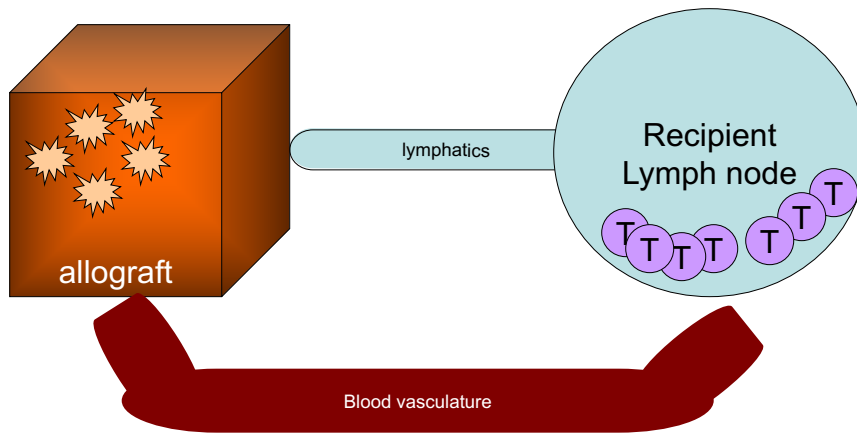
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# Alloreactive T cells recognize either allo-peptide and/or allo-MHC



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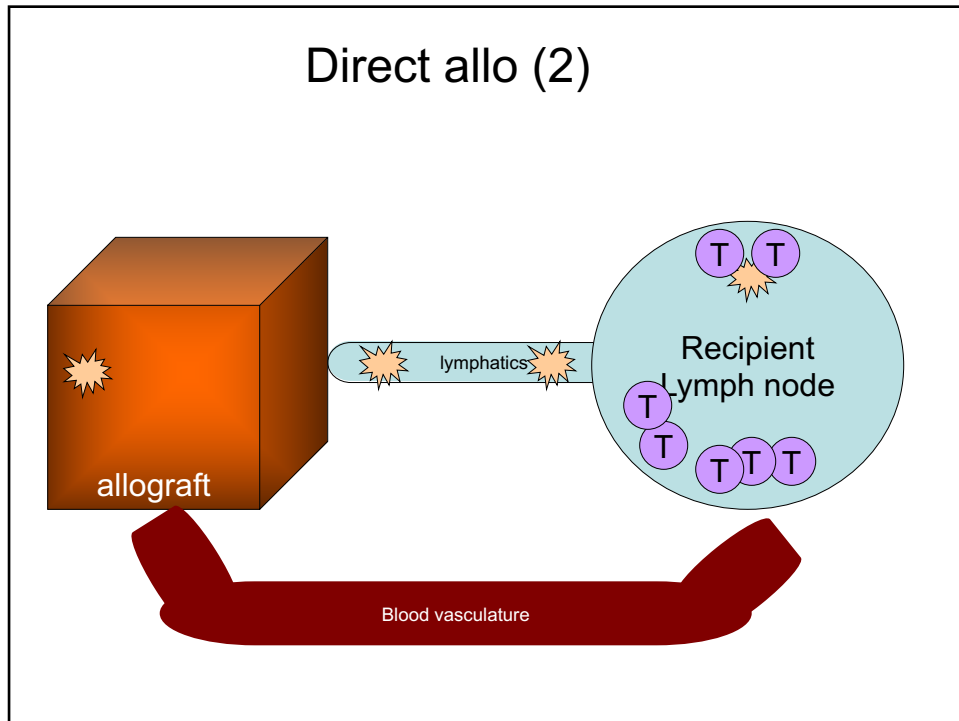
# Direct allo (1)



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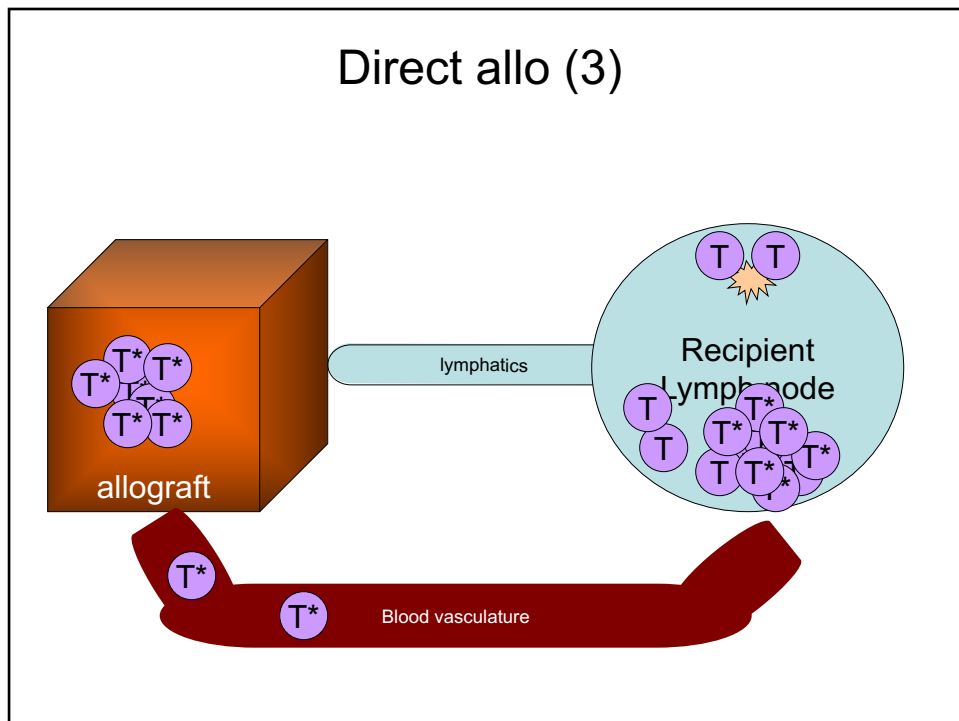


## Direct allo (2)

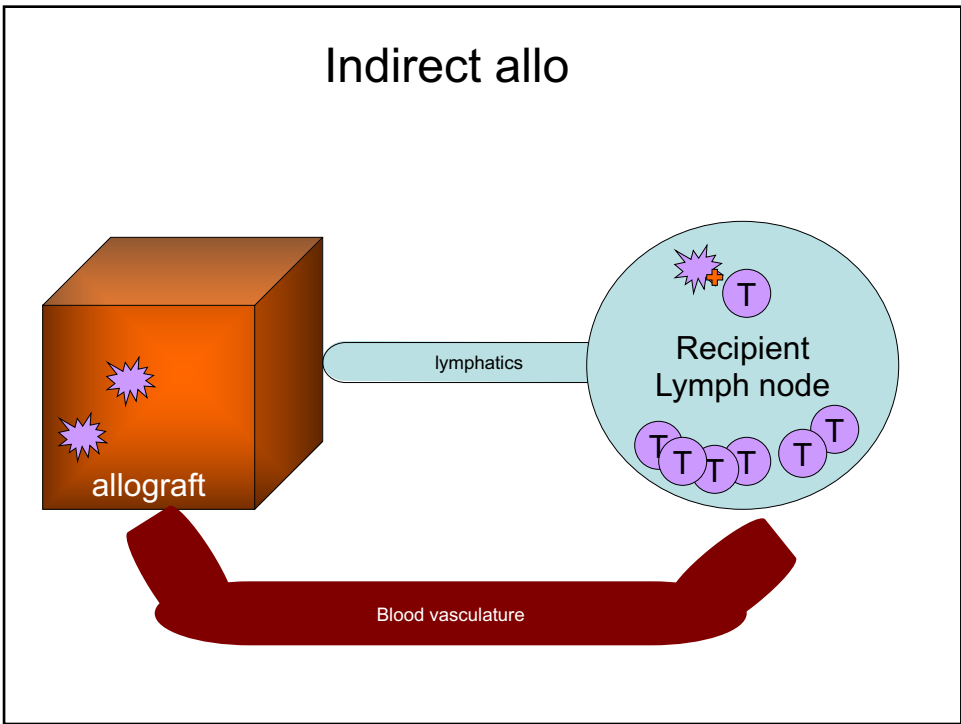


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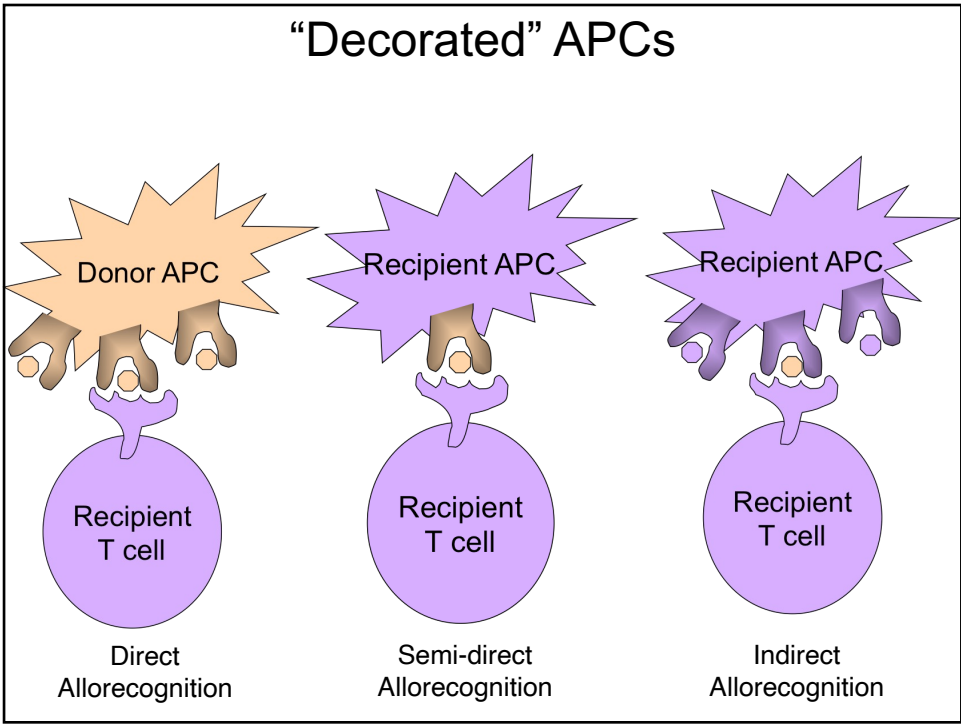
## Direct allo (3)



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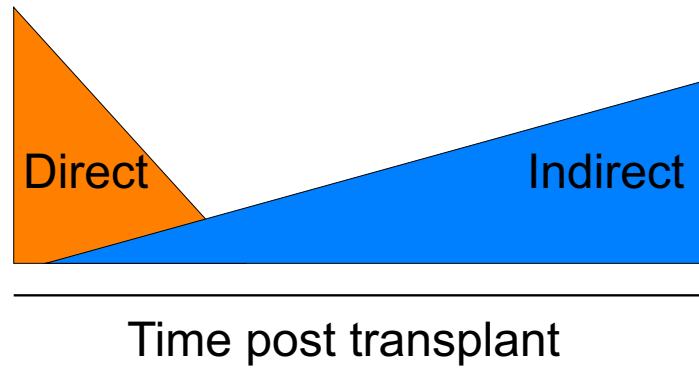


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## Relative timing of direct vs indirect alloresponse



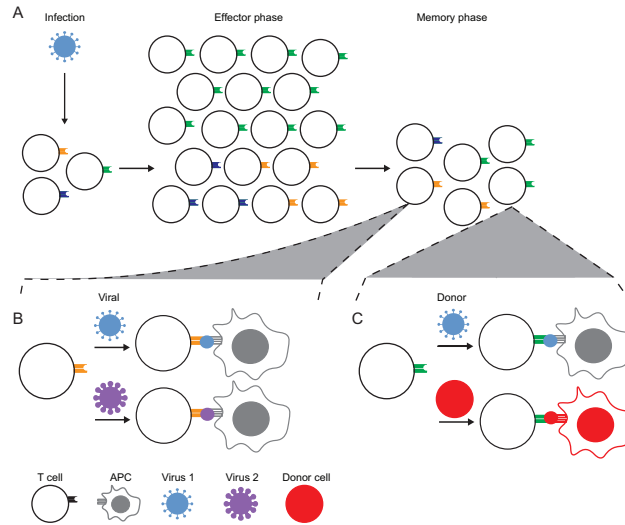
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## Where do alloreactive memory T cells come from?

- Frequency of alloreactive cells is 1-10%
- Human alloreactive T cells are 50% naïve and 50% memory  
(Lombardi et al. Int Immunol 1989; Macedo et al. Am J Transp 2009)
- Presensitization (Pregnancy, transfusion, transplant)
- Immune repertoire has intrinsic alloreactivity (naïve)
  - High percentage of pre-selection  $\alpha\beta$  TCRs react with at least one MHC haplotype (Zerrahn et al Cell 88:627, 1997)
  - Enrichment by positive selection in the thymus
- Heterologous Immunity (Burrows et al. JEM 1994; Elkington et al. Blood 2005)

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## Heterologous Immunity in infection and transplantation



Higdon et al. Transplantation 2022

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## Why are memory T cells a problem in transplantation?

- Poor response to lymphodepletion strategies
  - More difficult to deplete
  - Numbers recover faster (homeostatic mechanisms)
- Less susceptible to conventional immunosuppression
- Less dependent on co-stimulatory blockade

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## Types of Immune Rejection

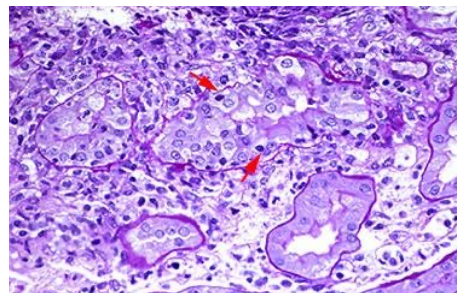
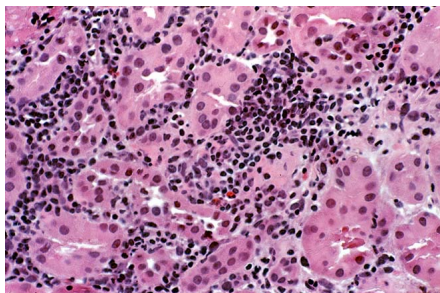
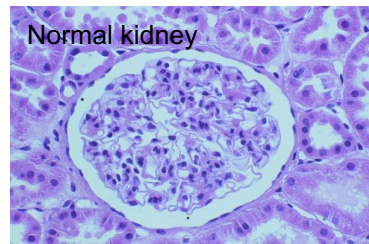
	Hyperacute	Acute Humoral	Acute Cellular	“Chronic”
Time Frame	Minutes	Days to weeks	Days to weeks	Years
Mediated by	Preformed Antibody	Antibody	T cells	Immune + Non-immune
Prevention/ treatment	Test PRA/cross-match	Immuno-suppression	Immuno-suppression	Immuno-suppression/ avoid damage

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## Acute T Cell Mediated Rejection

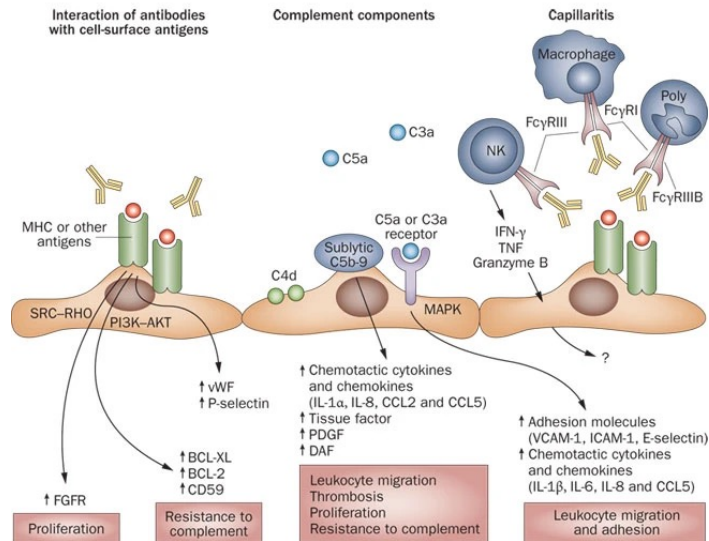
Direct alloreactivity

Mononuclear infiltrate  
tubulitis  
interstitial infiltrate



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## Antibody-mediated Rejection



Nat Rev Immun 8:255 (2012)

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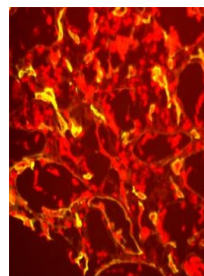
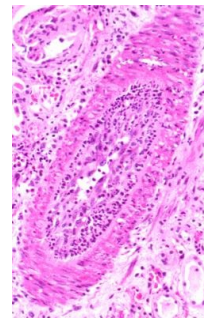
## Acute Antibody-Mediated Rejection

### Pathophysiology

Activation of both B and T cells  
 Circulating anti-donor antibodies  
 Complement activation

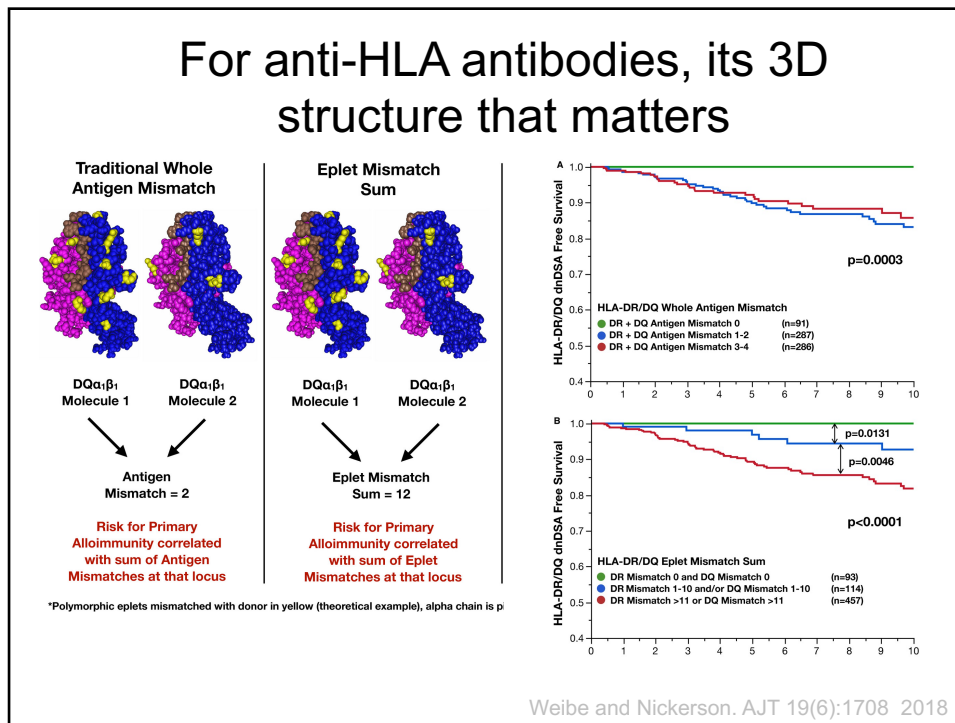
### Histopathology

C4d+ immunofluorescence  
 Donor Specific Antibody (DSA)  
 Vasculitis/peritubular capillaritis  
 Interstitial hemorrhage  
 Capillary-endothelial swelling  
 Lymphocytic infiltrate not required



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## For anti-HLA antibodies, its 3D structure that matters



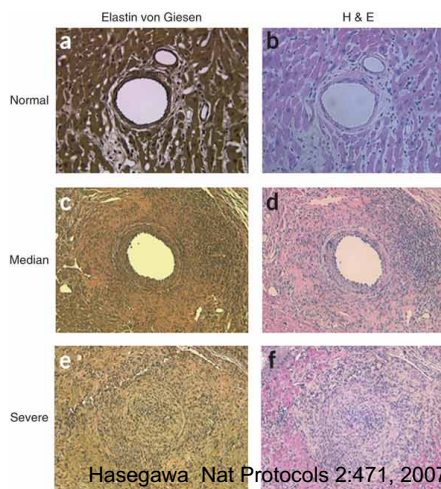
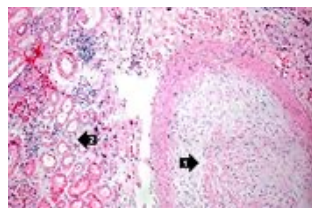
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## Chronic Rejection

### Non-immune and Immune mediated

Ischemic injury  
 Calcineurin inhibitors  
 Previous acute rejection  
 Indirect Alloreactivity with antibody production

Fibrosis  
 Neointimal thickening



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# Immunosuppression

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## Axioms of Immunosuppression

- Three effects:
  - Immunosuppressive effect
  - Immunodeficient complications
  - Non-immune toxicity
- Non-immune toxicity is dose-limiting
- Immunosuppressive effect and immunodeficient complications are inherently linked. Increasing immunosuppression always increases immunodeficiency.

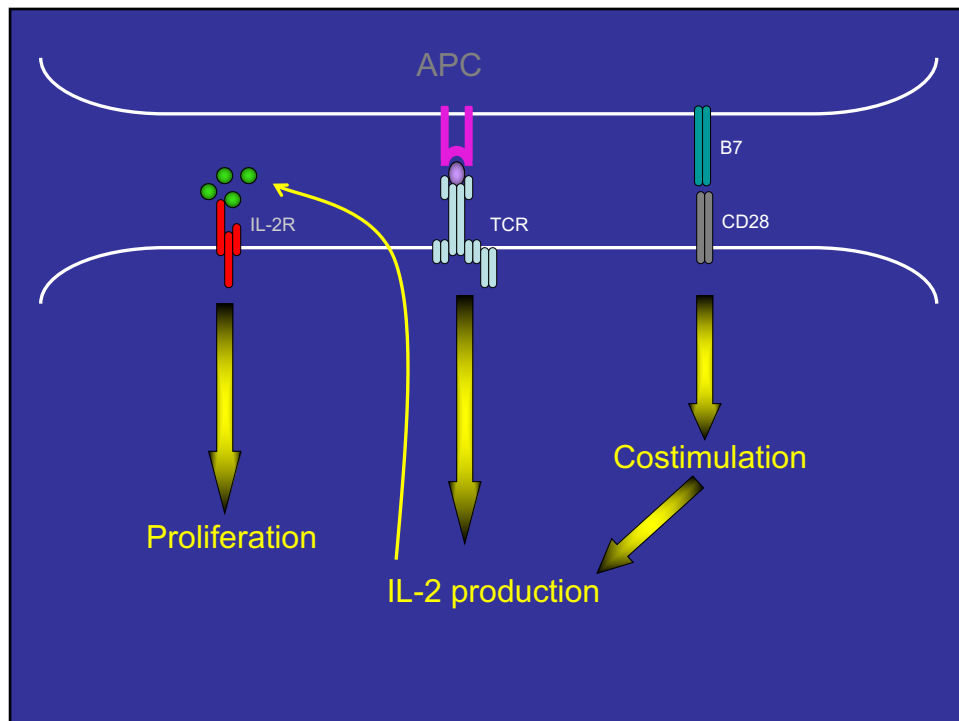
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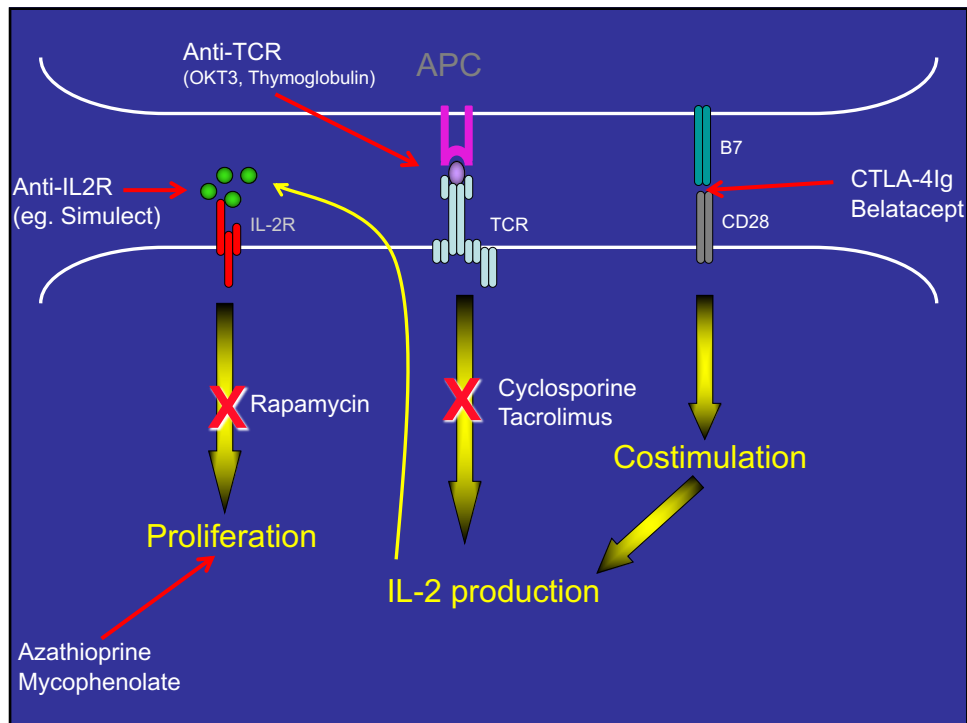
## Clinical Immunosuppression

- Induction vs maintenance
- Use many drugs at lower doses to enhance immunosuppression and decrease individual side effects
- More immunosuppression ==> increased infection, increased risk of post-transplant lymphoproliferative disease (PTLD)
- Treatment of a rejection episode

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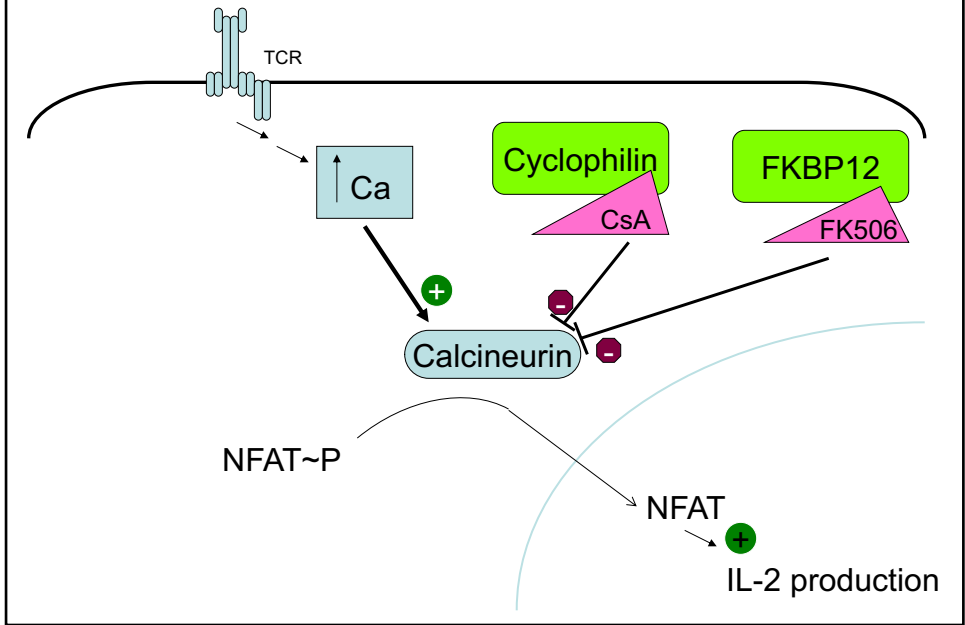
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## Drugs that affect Signal 1

- **Anti-T cell agents**
  - Thymoglobulin: rabbit anti-thymocyte antiserum
  - ATGAM: horse anti-thymocyte antiserum
  - OKT3: monoclonal anti-CD3 $\epsilon$ 
    - Cytokine storm, anti-murine antibody reaction
  - Campath/anti-CD52
- **Calcineurin Inhibitors**
  - Cyclosporin A
  - Tacrolimus/FK506/Prograf

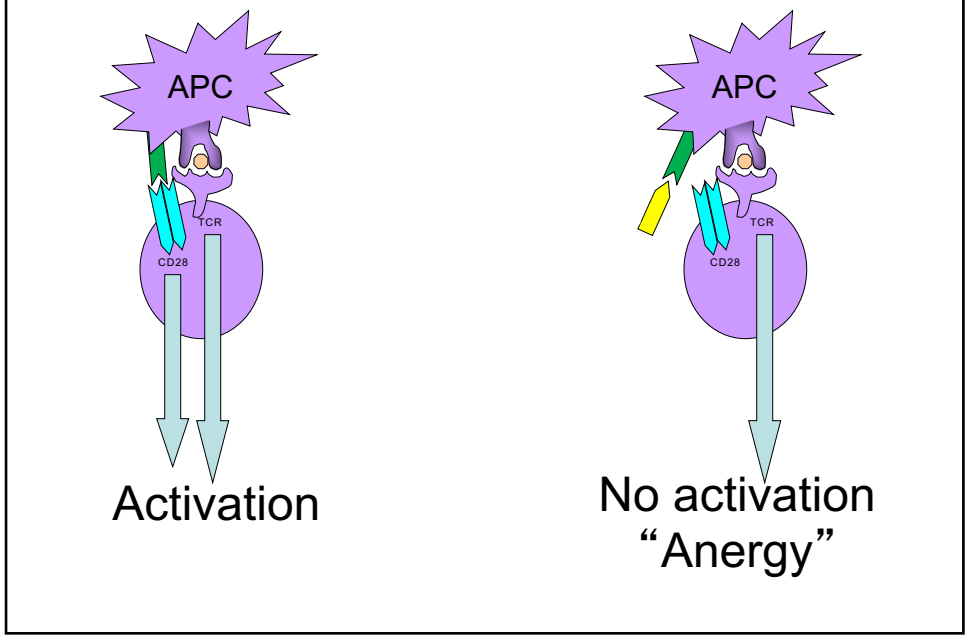
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# Calcineurin inhibitors -- mechanism of action



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# Antigen specific signals without costimulation lead to anergy



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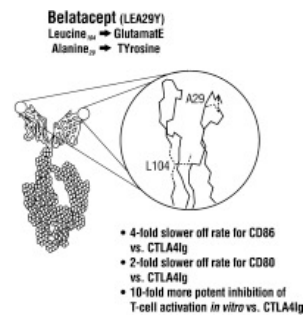
## Costimulatory Inhibition CTLA4-Ig

- Binds to B7-1 and B7-2 inhibiting stimulation of CD28
- Induces T cell anergy in mice
- Abetacept -- failed in primate transplant setting but currently used in RA
- Belatacept developed by mutagenesis and is 10x increased binding affinity

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## Development of LEA29Y

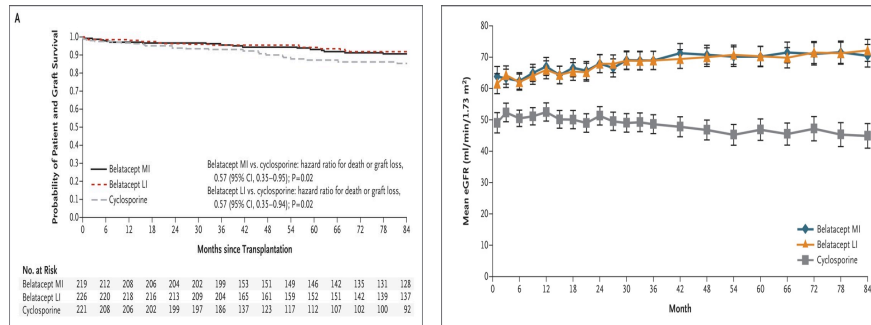
- Directed mutagenesis of CTLA4Ig
  - screened 2300 individual clones Identified L104E as decreased off rate.
- Repeated mutagenesis A29-->Y. Two mutations combined increase affinity by 10-fold
- LEA29Y prolongs allografts in cynomolgous monkeys
- Phase II clinical trial versus Cyclosporin showed “non-inferiority” (with anti-CD25/MMF/steroid regimen)
- Phase III clinical trials: BENEFIT/BENEFIT-EXT. LEA29Y treated had better renal function but slightly higher acute rejection and PTLD



AJT 5:443, AJT 6:876  
NEJM 353:770  
AJT 10:535, AJT 10:547,  
AJT 13: 2875

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## BENEFIT – Phase III belatacept vs cyclosporin



Vincenti F et al. N Engl J Med 2016;374:333-343

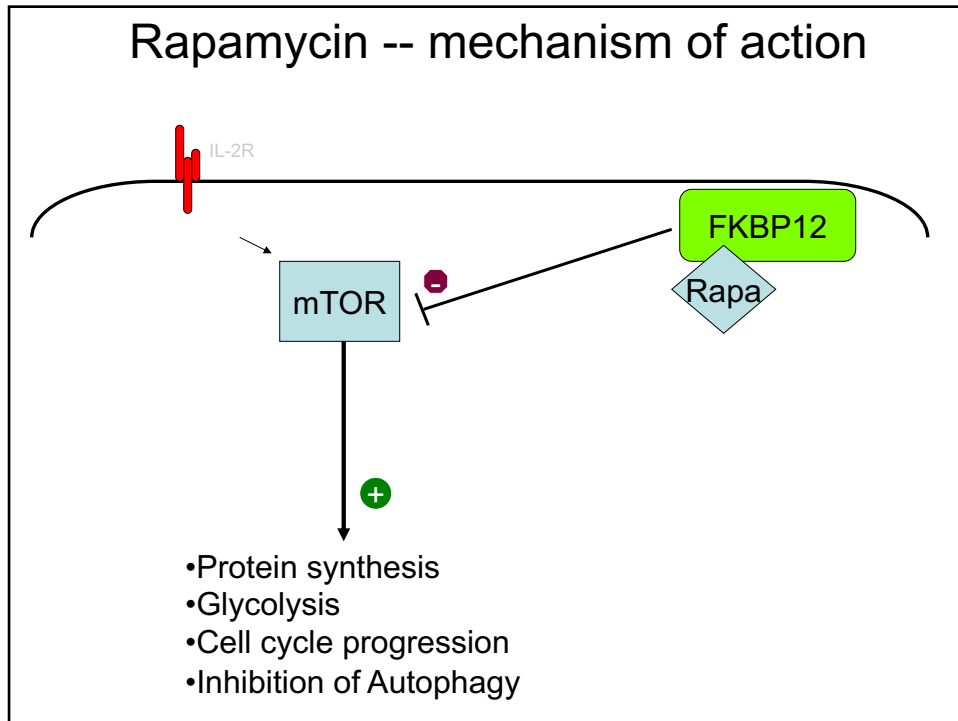
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## Cytokine Inhibition (signal 3)

- Anti-CD25: daclizumab or basiliximab
- Rapamycin/sirolimus and everolimus
  - Inhibits mTOR
  - Hyperlipidemia, poor wound healing
  - Anti-neoplastic properties/cardiac stenting
- Jak3 inhibitors (affect signals from  $\gamma c$  containing receptors for IL-2, 4, 7, 9, 15 and 21)
  - Failed in transplant due to non-superiority and increased side effects

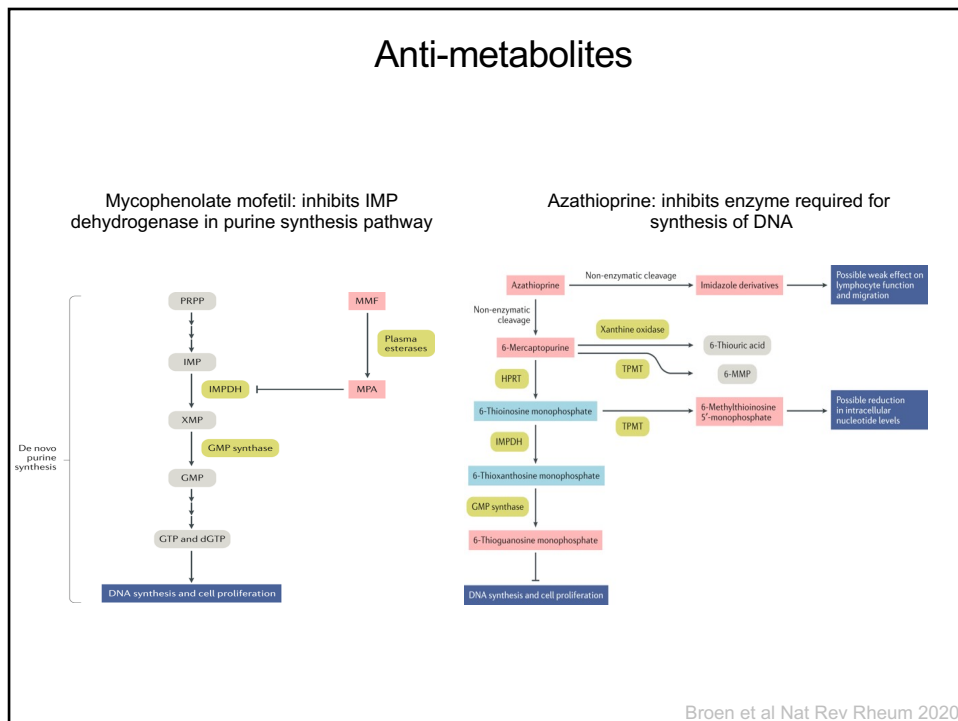
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## Rapamycin -- mechanism of action



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## Anti-metabolites



Broen et al Nat Rev Rheum 2020

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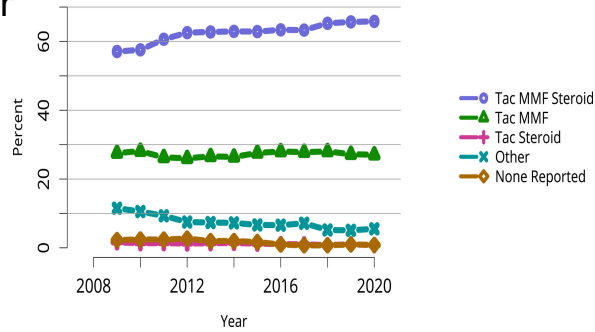
## Standard of care

### Induction

- anti-IL2R or anti-T cell depleting antibodies
- high dose steroid

### Maintenance

- calcineurin inhibitor
- low dose steroid
- anti-metabolite



AJT 22(S2): 21-136

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## Agents to target B cells and complement

### T/B cell interactions

- anti-CD40 mAb

### B cell surface molecules/receptors

- CD20\*
- CD52
- CD22
- BAFF
- TACI-Ig

### Plasma Cells

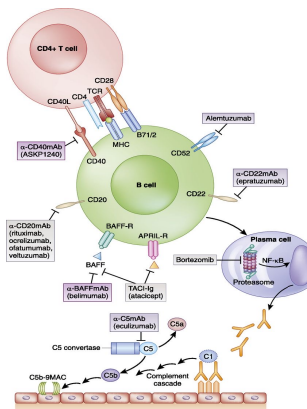
- Bortezomib\*
- Carfilzomib\*

### Immunoglobulin

- IVIG\*

### Complement Pathway

- anti-C5 mAb\*
- Anti-C1

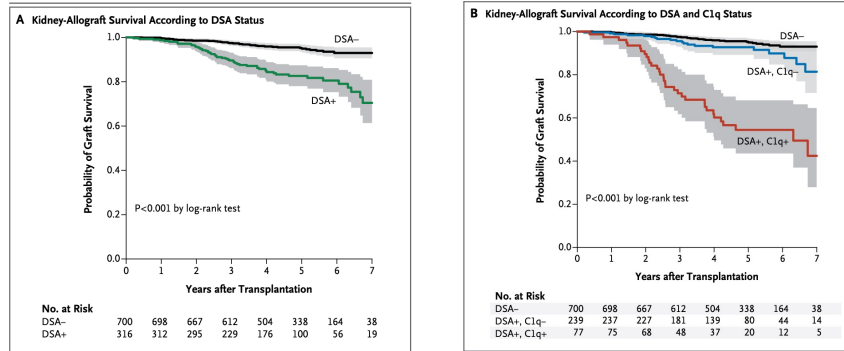


\*used clinically

Wiseman CJASN 2016

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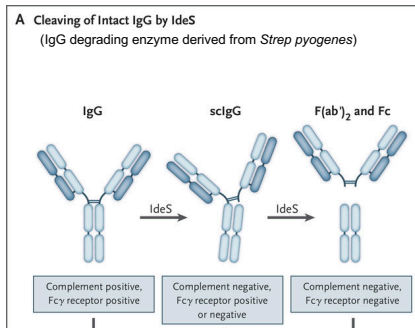
## Complement fixation by DSA matters



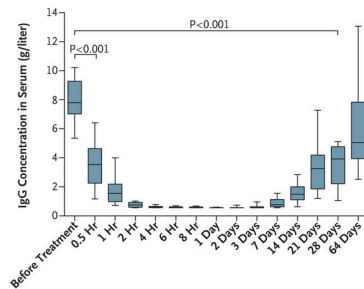
N Engl J Med 2013;369:1215-26

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## Modify pre-existing antibodies



**D Effect of IdeS on Circulating IgG Levels in Highly Sensitized Patients**



Jordan SC et al. N Engl J Med 2017;377:442-453.

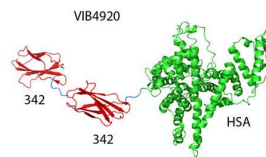
Modified from NEJM 377:442 (2017)

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# On the Horizon

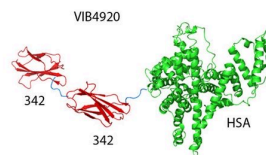
- Signal 1
  - Teplizumab: anti-CD3 engineered to lack FcR binding
  - Tol 101 – IgM anti-TCR $\alpha\beta$
- Signal 2
  - Anti-CD28
    - History of TGN1412
    - “domain antibody” or dAb, monovalent, pegylated
      - Lulizumab
      - VEL101 (formerly FR104) – FDA fasttrack status 2/2022
  - Target CD40/CD40L pathway
    - Anti-CD40L and thromboembolism
    - Anti-CD40
      - Bleselum/ASKP1240 – humanized, non-depleting
      - Iscalimab/CFZ533
    - Anti-CD40L
      - VIB-4920
- IL6/IL6R
- Antibody-mediated rejection
  - Costimulation blockade
  - Anti-CD38 to eliminate plasma cells



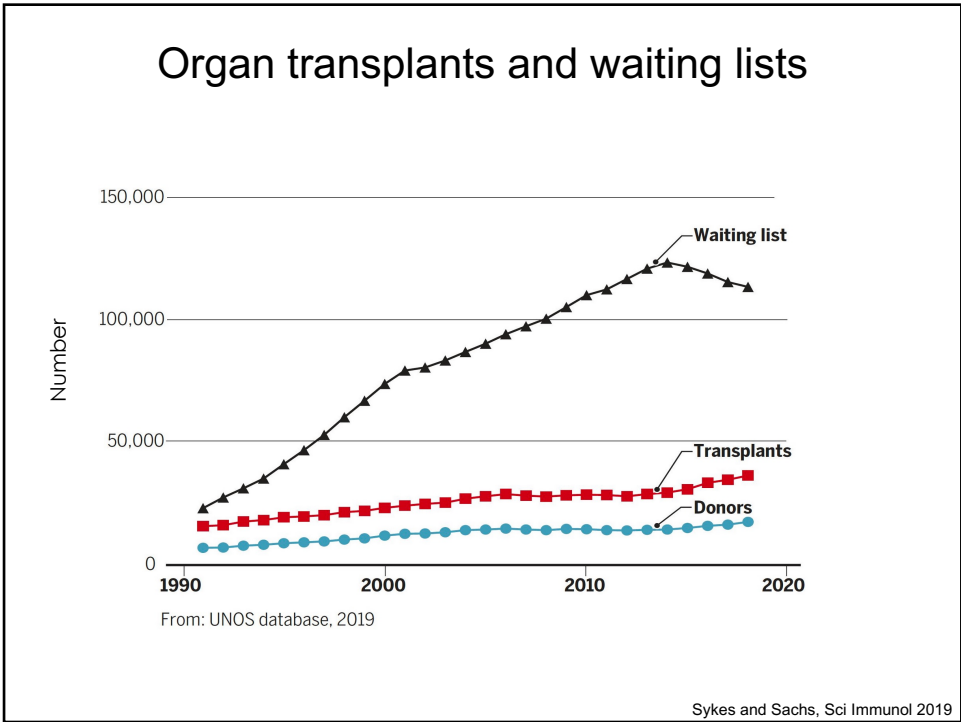
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# On the Horizon

- Signal 1
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- Signal 2
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  - Anti-CD38 to eliminate plasma cells



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# Xenografts

**BRIEF REPORT** June 22, 2022

### Genetically Modified Porcine-to-Human Cardiac Xenotransplantation

Bartley P. Griffith, M.D., Corbin E. Goerlich, M.D., Ph.D., Avneesh K. Singh, Ph.D., Martine Rothblatt, Ph.D., Christine L. Lau, M.D., Aakash Shah, M.D., Marc Lorber, M.D., Alison Grazioli, M.D., Kapil K. Saharia, M.D., Susie N. Hong, M.D., Susan M. Joseph, M.D., David Ayares, Ph.D., and Muhammad M. Mohiuddin, M.D.

**First clinical-grade porcine kidney xenotransplant using a human decedent model**

**AJT**

Porrett et al. AJT Jan 22, 2022

**ORIGINAL ARTICLE** May 19, 2022

### Results of Two Cases of Pig-to-Human Kidney Xenotransplantation

Robert A. Montgomery, M.D., D.Phil., Jeffrey M. Stern, M.D., Bonnie E. Lonze, M.D., Ph.D., Vasishta S. Tatapudi, M.D., Massimo Mangiola, Ph.D., Ming Wu, M.D., Elaina Weldon, M.S.N., A.C.N.P.-B.C., Nikki Lawson, R.N., Cecilia Deterville, M.S., Rebecca A. Dieter, Pharm.D., B.C.P.S., Brigitte Sullivan, M.B.A., Gabriella Boulton, B.A., Brendan Parent, J.D., Greta Piper, M.D., Philip Sommer, M.D., Samantha Cawthon, B.S., Erin Duggan, M.D., David Ayares, Ph.D., Amy Dandio, M.S., Ana Fazio-Kroll, Ph.D., Maria Kokkinaki, Ph.D., Lars Burdorf, M.D., Ph.D., Marc Lorber, M.D., Jef D. Boeke, Ph.D., Harvey Pass, M.D., Brendan Keating, Ph.D., Adam Griesemer, M.D., Nicole M. Ali, M.D., Sapna A. Mehta, M.D., and Zoe A. Stewart, M.D., Ph.D.

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# Barriers to Xeno

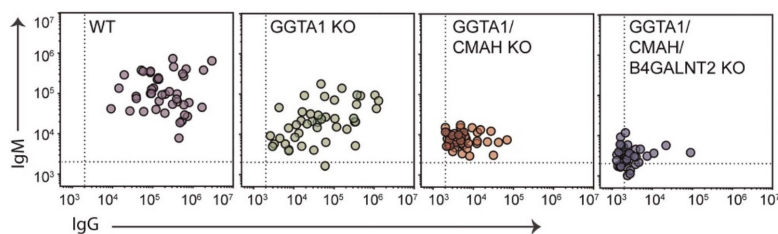
- Non-immunological
  - Ethical
  - Regulatory
  - Cross-species viral transmission
  - Size matters
- Immunological
  - Glycosylation differences
  - Innate and adaptive

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# Overcoming natural antibodies in Xeno

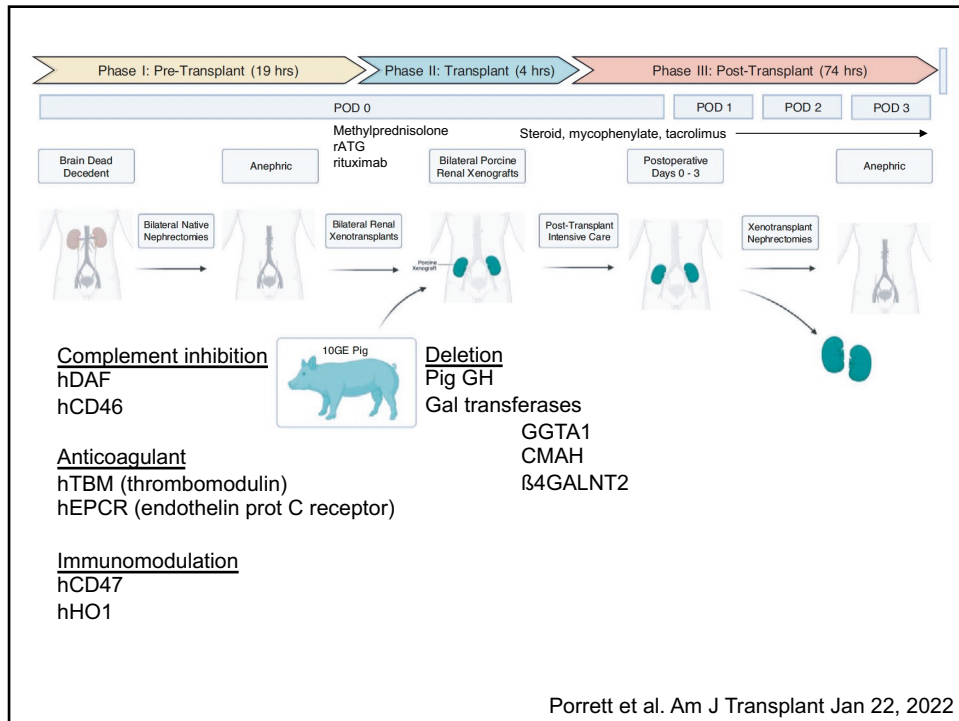


Yue et al. Nat Biomed Engin 2021



Martens et al. Transplantation 2017

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## Tolerance

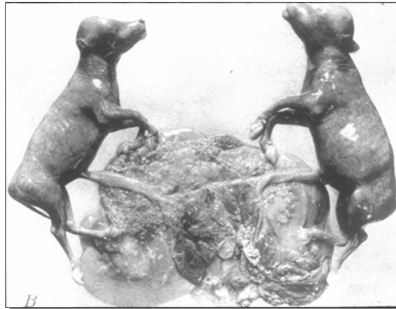
Definition: Acquired specific failure of the immunological mechanism to respond to a given antigen, induced by exposure to the antigen.

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## 1945 – Owen – natural immune tolerance

- Dizygotic bovine twins share a placenta
  - Red blood cell chimeras at birth
  - exhibit tolerance to each other's tissues

cattle of dizygotic twin



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## 1950s - Medawar – Tolerance can be acquired

No. 4379    October 3, 1953    NATURE    603

**'ACTIVELY ACQUIRED TOLERANCE' OF FOREIGN CELLS**  
 By Dr. R. E. BILLINGHAM\*, L. BRENT and Prof. P. B. MEDAWAR, F.R.S.  
 Department of Zoology, University College, University of London

“(1) Mice and chickens never develop . . . the power to react immunologically against foreign homologous tissue cells with which they have been inoculated in foetal life. . . .  
 (2) Acquired tolerance is immunologically specific...  
 (3) Acquired tolerance is due to a specific failure of the host's immunological response . . .”

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# Mechanisms of Tolerance

## “Central Tolerance”

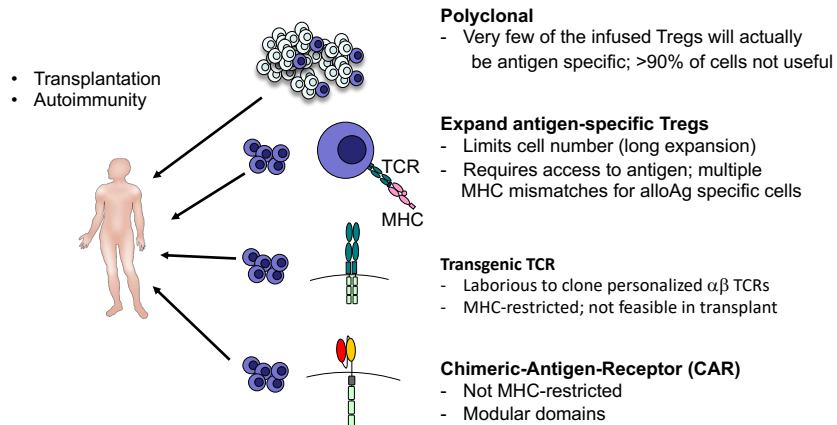
- Deletion of autoreactive cells during development

## “Peripheral Tolerance”

- Regulatory T cells - T lymphocytes that dampen immune responses

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# Antigen-specific Tregs are more potent



Courtesy of Megan Levings

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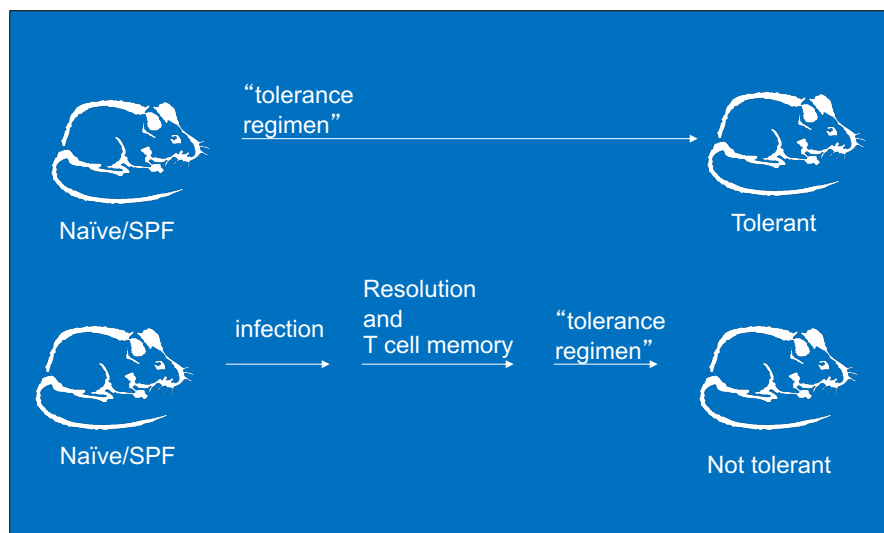
## Ongoing Treg clinical trials (2022)

Study ID	Age	Phase	Intervention	Comparator	Primary Endpoint	Secondary Endpoints	Study Status	Location	Notes
NCT02146525	18-65 (adult, older adult)	I	TRAC1 Reagent Don. Tr1	None	Safety Study of Using Regulatory T Cells Induce Liver Transplantation Tolerance		Recruiting	Cambridge University Hospitals NHS Foundation Trust, Cambridge, UK; Royal Free London NHS Foundation Trust, London, UK; King's College Hospital NHS Foundation Trust, London, UK	A multicenter, first-in-human, open-label, single-arm study of an autologous CAR T regulatory (CAR-Treg) in HLA-A2 mismatched liver transplant recipients. The aim is to see if CAR-Tregs to be introduced on recognition of HLA-A2 antigens present on the donated liver and subsequently induce and maintain immunological tolerance to the organ.
NCT02471870	18-75 (adult, older adult)	III	Cell 1 Immun In Trans	None	Liver Transplantation with Trags at UCSF		Completed	UCSF	
NCT03867617	>18 (adult, older adult)	III	Cell 1 Immun In Trans	None	Liver Transplantation with Trags at UCSF		Completed	UCSF	
NCT02089891	18-50 (adult)	I	TR1 Chimeric Trans Inj. CD4+CD137	None	Liver Transplantation with Trags at MGH		Recruiting	MGH	
NCT01446484	1-18 (child)	III	TR1 Chimeric Trans Inj. CD4+CD137	None	Liver Transplantation with Trags at MGH		Completed	MGH	
NCT02091232	>18 (adult, older adult)	I	TR1 Chimeric Trans Inj. CD4+CD137	None	Liver Transplantation with Trags at MGH		Completed	MGH	
NCT02371454	18-65 (adult, older adult)	III	The C m1 (ON)	None	Donor Alloantigen Reactive Trags (darTrags) for Calcineurin Inhibitor (CNI) Reduction (ARTEMIS)		Completed	University of California at San Francisco, San Francisco, CA, US; Northwestern University, Chicago, IL, US; Mayo Clinic in Rochester, Rochester, NY, US	Safety of donor-alloantigen-reactive Trags to facilitate minimization and/or discontinuation of immunosuppression in adult liver transplant recipients. Results: Problems with Trag product manufacturing; discontinuation of immunosuppression not possible.
NCT04817774	18-70 (adult, older adult)	III	Donor-Reactive T Cell Transpl ON	None	Donor-Alloantigen-Reactive Regulatory T Cell (darTrags) in Liver Transplantation (darLT)		Completed	University of California at San Francisco, San Francisco, CA, US; Northwestern University, Chicago, IL, US; Mayo Clinic in Rochester, Rochester, NY, US	Safety of receiving one or three different doses of donor-alloantigen-reactive Trags (darTrags) while receiving a specific drug combination. Results: Issues regarding donor-specific Trag manufacturing using donor B-cells led to termination.
NCT03943238	18-65 (adult, older adult)	I	Donor-Reactive T Cell Transpl ON	None	Donor-Alloantigen-Reactive Regulatory T Cell (darTrags) in Liver Transplantation (darLT)		Completed	University of California at San Francisco, San Francisco, CA, US; Northwestern University, Chicago, IL, US; Mayo Clinic in Rochester, Rochester, NY, US	Safety of receiving one or three different doses of donor-alloantigen-reactive Trags (darTrags) while receiving a specific drug combination. Results: Issues regarding donor-specific Trag manufacturing using donor B-cells led to termination.
NCT03284242	18-65 (adult, older adult)	III	The OP Tr	None	Safety and Efficacy Study of Regulatory T Cell Therapy in Liver Transplant Patients (TRIL)		Completed	King's College Hospital, London, UK	Results: Safety of Trag transfer was confirmed. Transient increase of the pool of circulating Trags and reduced anti-donor T-cell responses were detected. Low applicability of earlier Trag dose (3 months post-transplant). [25]
NCT0271826	>18 (adult, older adult)	III	ISRCTN 11038572	UMIN-000015789	Tolerance induction by a regulatory T cell-based cell therapy in living donor liver	Donor-reactive Treg-enriched cell product	Recruiting	Hokkaido University Graduate School of Medicine, Japan	Results: 7 of 10 patients are immunosuppression-free for >48 years. [13]

Pilat, Steiner and Sprent. Intl j Mol Sciences 24(2). 2023

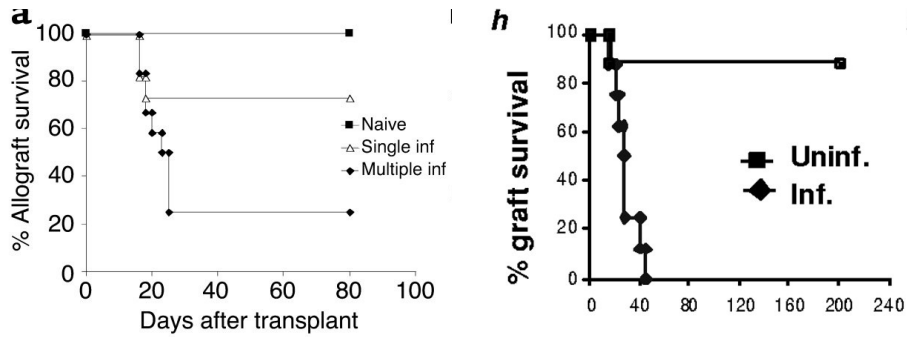
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## Heterologous Immunity as a barrier to co-stimulation blockade induced tolerance



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## Both acute and chronic infection result in heterologus immunity



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## Chimerism



### Classical definition

*"a thing of immortal make, not human, lion fronted and snake behind, a goat in the middle and snorting out the breath of the terrible flame of bright fire"*

--Homer's Iliad

### Hematopoietic Chimerism

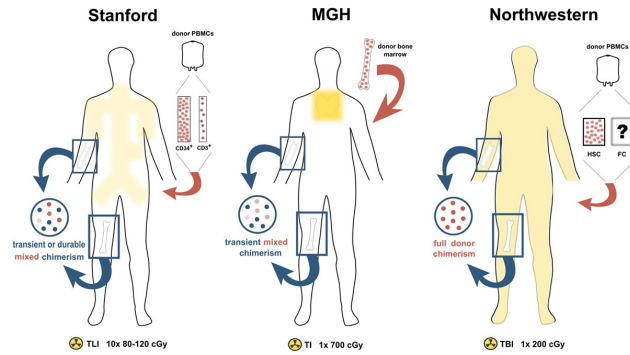
Presence of donor hematopoietic cells

- Complete: 100% of hematopoietic cells are of donor origin
- Mixed: Variable number of donor cells of multiple lineages
- Micro: Donor cells below the level of detection by flow cytometry (<1%)

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## Experience with human “chimeras”

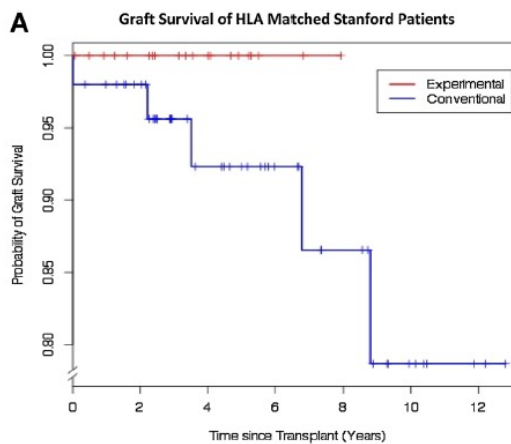


Mahr et al. Front in Immun 2017

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## Stanford Experience – HLA matched

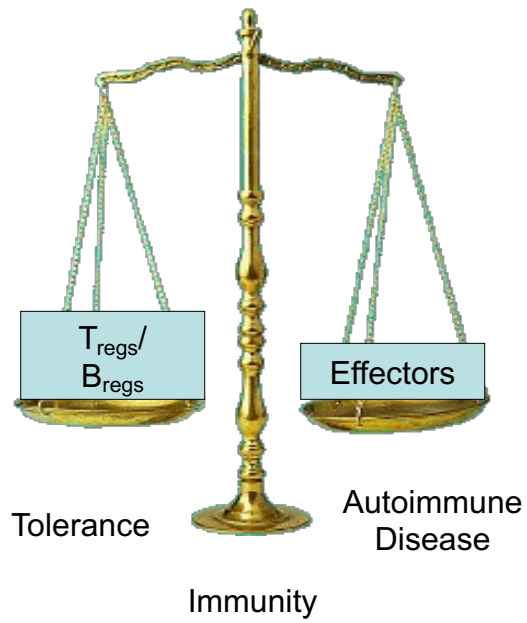
Total lymphoid irradiation  
 ATG  
 CD34+ cell infusion  
 (CD3+ cells)  
 6 month measured chimerism  
 Weaning of MMF/CsA



Scandling et al AJT (2015) 15:695

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The normal immune response is an ongoing balance



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