Crossmatching and Desensitization

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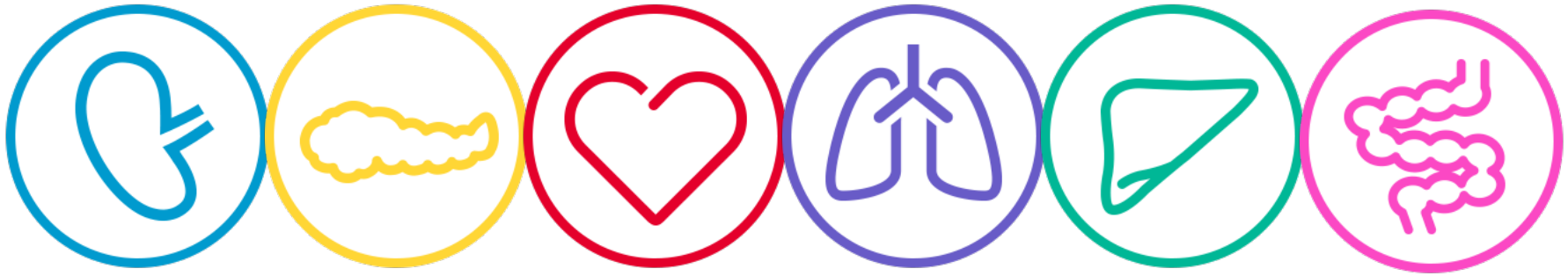

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SIGNIFICANCE OF THE POSITIVE CROSSMATCH TEST IN KIDNEY TRANSPLANTATION*

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

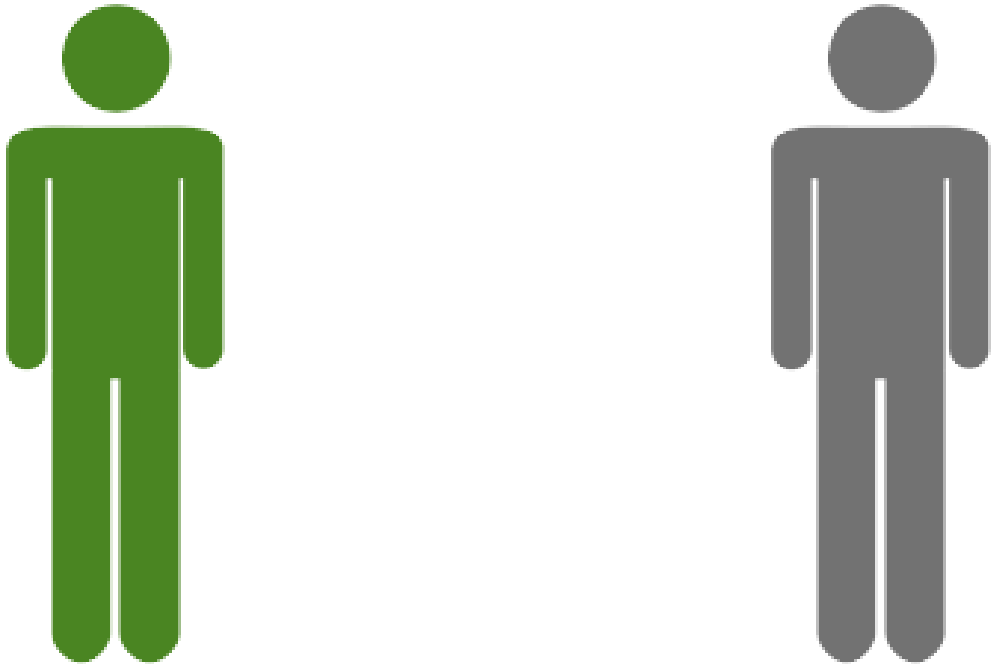
Abstract Crossmatch tests of the prospective kidney-transplant donor's lymphocytes with the serum of the prospective recipient in 225 transplants showed that eight of 195 with negative crossmatch failed to function immediately, in contrast to 24 of 30 with positive crossmatch (p less than 0.001). Immediate 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.

- 24/30 (80%) of patients with a positive crossmatch had immediate graft failure
- Multiparous females and prior transplants had higher risk
- Organs from unrelated donors failed faster

Objectives

- Review of human leukocyte antigens
- Define sensitization
- Discuss tools for donor:recipient crossmatching
- Desensitization options and considerations

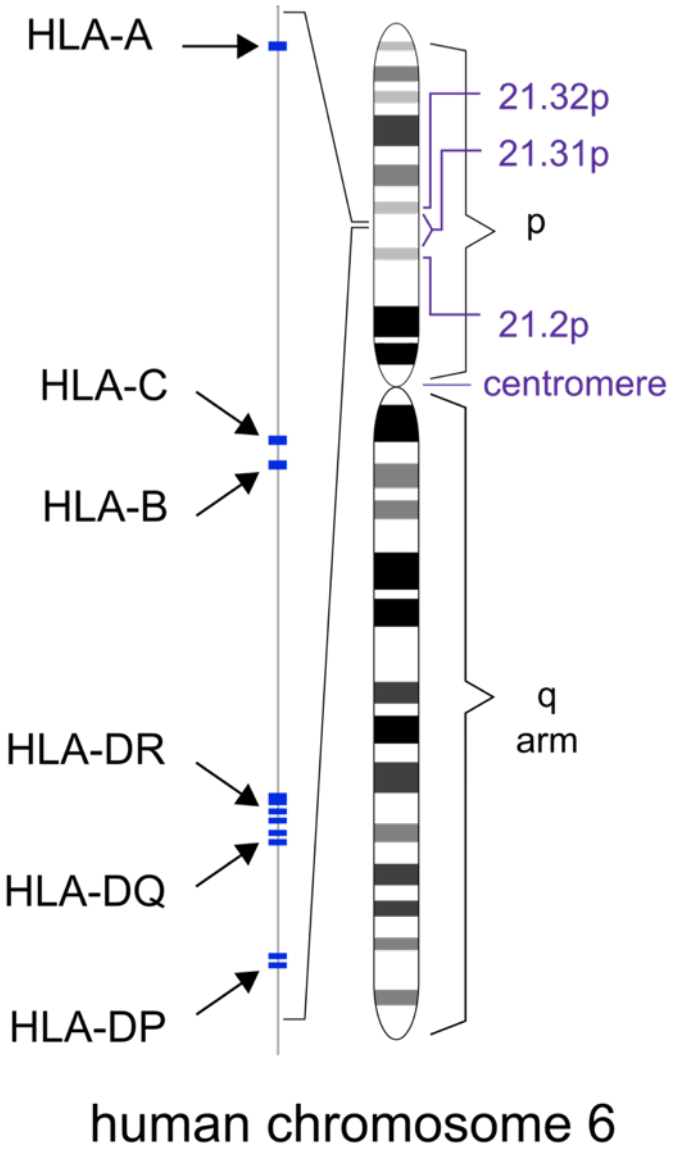
Self (recipient) vs. Non-self (donor)



Human leukocyte antigens (HLA) encode the major histocompatibility complex (MHC) proteins that identify your cells as “self”

Human leukocyte antigen (HLA)

- Each person has two haplotypes of specific combinations of class I and class II alleles
  - Class I: A, B, C
  - Class II: DR, DQ, DP
- Class I antigens are on all cells
- Class II antigens are on antigen presenting cells

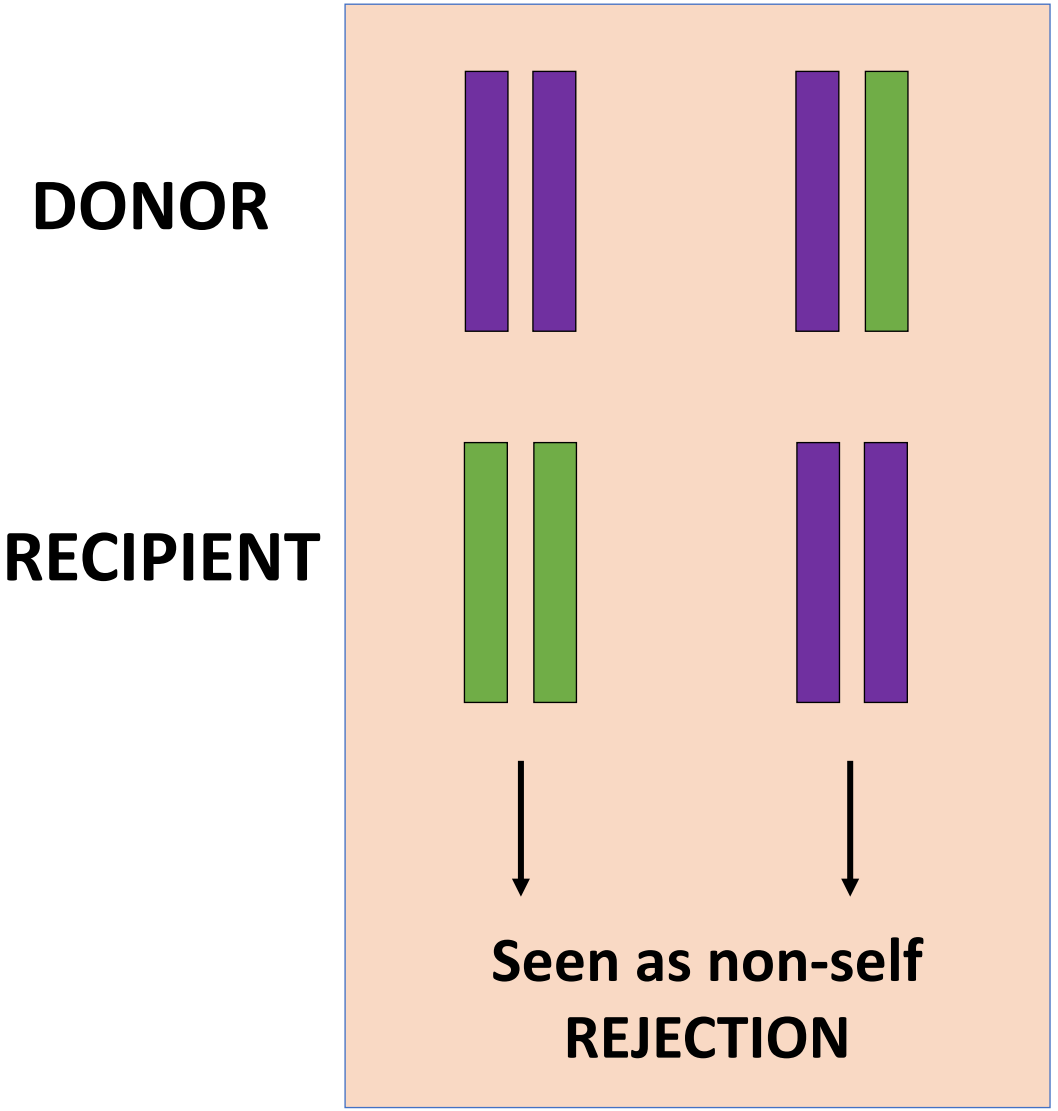


HLA matching

- Ideally, a recipient and a donor would be an identical HLA match
  - HLA variability makes perfect matching almost impossible
- HLA type is determined by genetic sequencing
- HLA type from a potential donor can be compared to the recipient

Donor:Recipient Matching

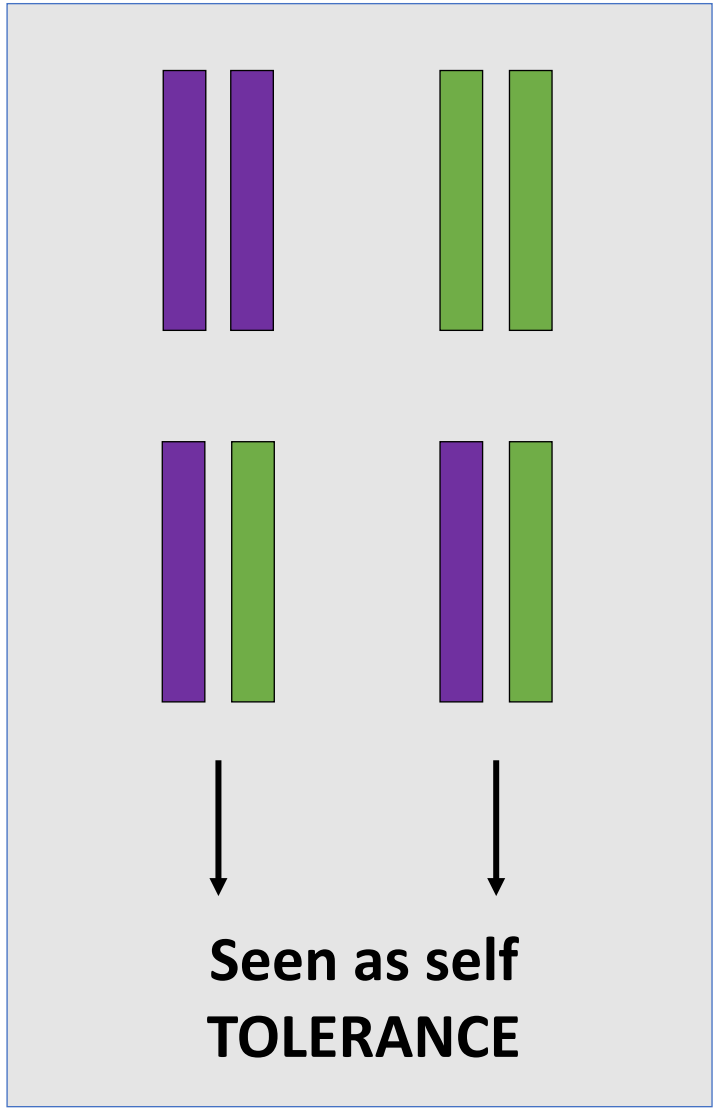
- Rejection happens when the donor has HLA antigens that the recipient has never seen
  - The recipient's T cells will recognize the graft's HLA as foreign



Donor:Recipient Matching

- Rejection happens when the donor has HLA antigens that the recipient has never seen

- Tolerance happens when the donor's HLA antigens are already part of the recipient

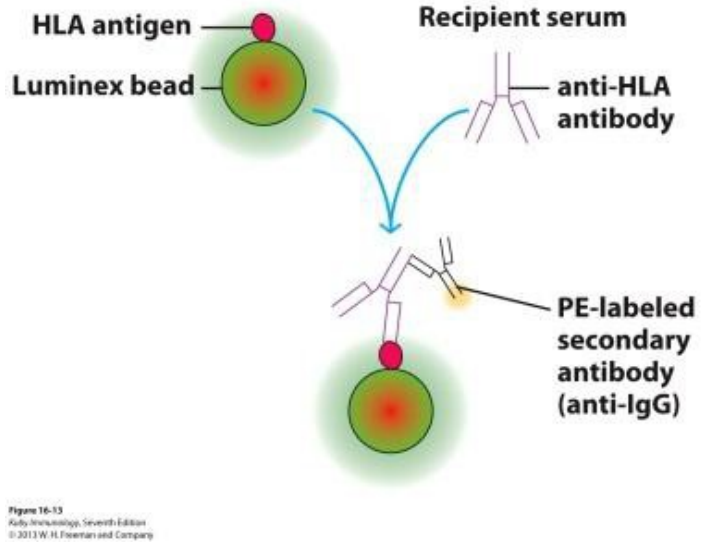


Anti-HLA antibodies and Sensitization

- Patients may develop antibodies against non-self HLA antigens
- Risk factors
  - Pregnancy
  - Transfusion
  - Prior transplant
- If a potential recipient has pre-formed anti-HLA antibody, that patient is called “sensitized” against donors with that HLA

How do I know if my patients is sensitized?

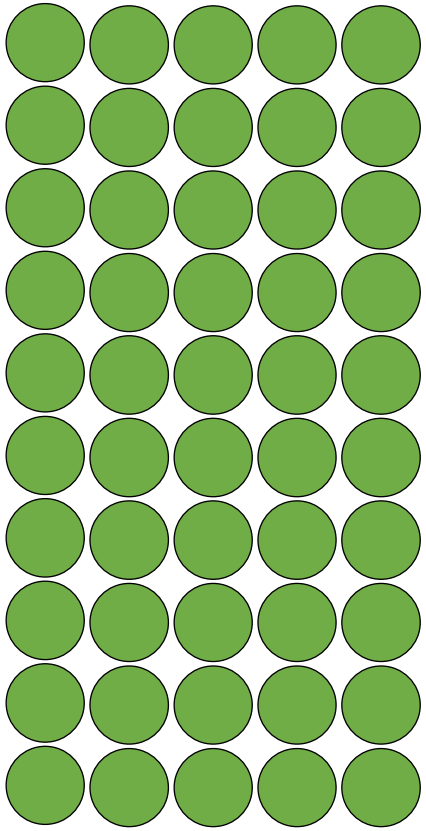
- Test for anti-HLA antibodies in the serum of a transplant candidate
  - If antibody is present, this test will identify which HLA it targets
- Mean fluorescence intensity (MFI)
- Titer based on serial serum dilutions
- How many potential donors will be excluded because of an anti-HLA antibody?



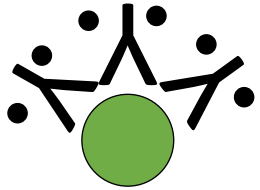
Each bead has a different HLA antigen

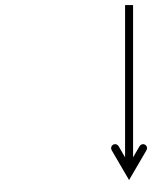
Panel reactive antibodies (PRA)

Panel of HLA-typed lymphocytes
from 50 individuals
+
Fluorescent Dye



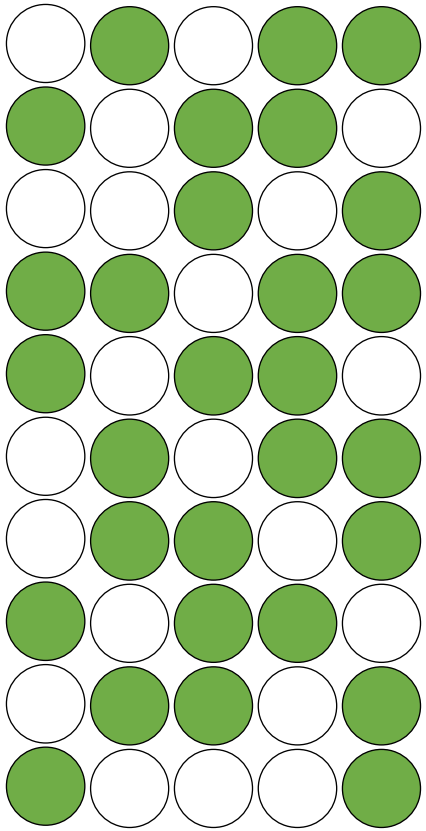
Add recipient serum
Add complement





CELL LYSIS
(loses fluorescence)

21/50 cells lysed = 42% PRA
42% of donors will NOT match



Calculated PRA (cPRA)

- Measure anti-HLA antibodies in the transplant candidate's serum
- cPRA is calculated based on the frequency of particular HLA antigens in the donor population
  - Testing directly against cells from blood donors is no longer done

- cPRA indicates how hard it will be to find a matched donor
  - cPRA of 42% means that 42/100 organs will not match

Clinical impact of high cPRA

- Patients with high cPRA will have to wait longer for an appropriate donor
  - Helps set expectations for time on waitlist or likelihood of finding an appropriate living donor match
  - A cPRA of 50% will double the average wait time compared to a non-sensitized patient
- High cPRA impacts clinical decisions for care
  - Initiation of mechanical support (ECMO, dialysis, etc)
  - In setting of other comorbidities, may make transplant not a realistic option

A few caveats to anti-HLA antibody measurements

- Substantial variability in positive cutoffs for MFI reporting
- Each center and organ has different risk tolerance for how much antibody is allowable
- Detection of antibody using a bead-based assay may not always reflect what actually happens in the bloodstream
  - Ab detection may not predict immunologic memory

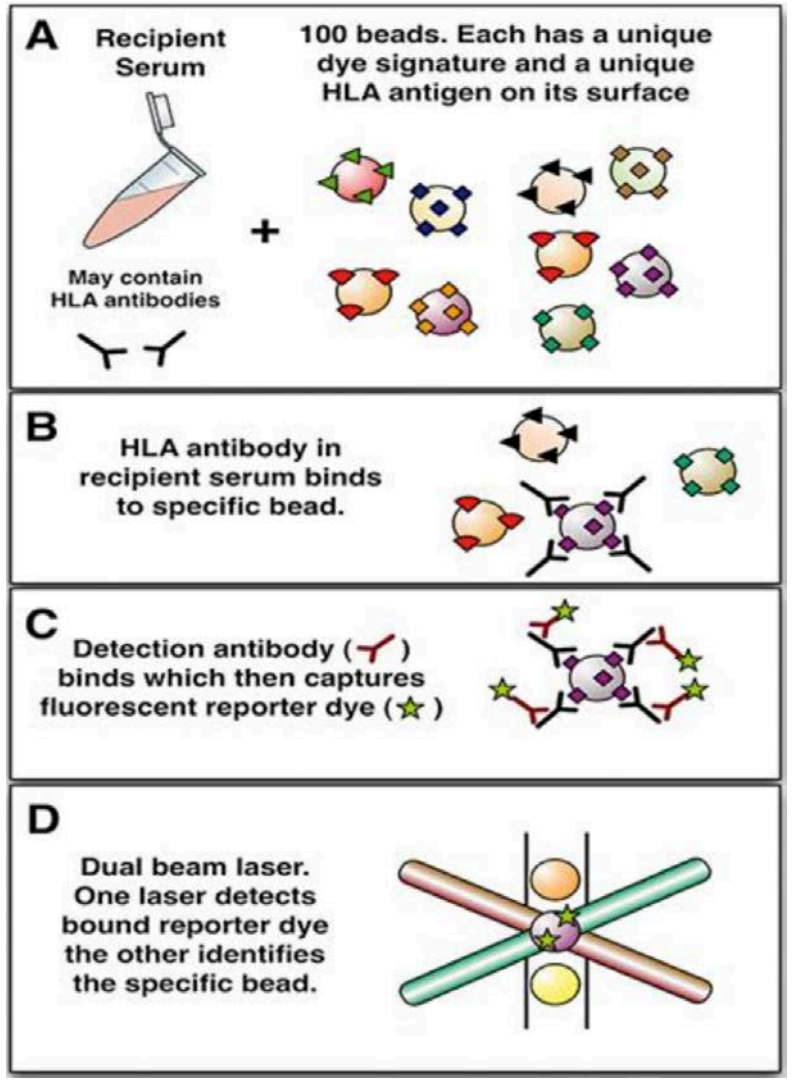
Clinical applications

- Before transplant or organ offer
  - cPRA to define recipient sensitization
  - HLA alleles are determined by sequencing

- Virtual crossmatch
  - Does your potential recipient have any known antibodies to that specific donor's HLA?
- Flow crossmatch
  - Does the recipient have any antibodies that bind to donor lymphocytes?
- Cytotoxic (CDC) crossmatch
  - When the antibodies bind, can they kill donor cells?

Virtual crossmatch (Luminex)

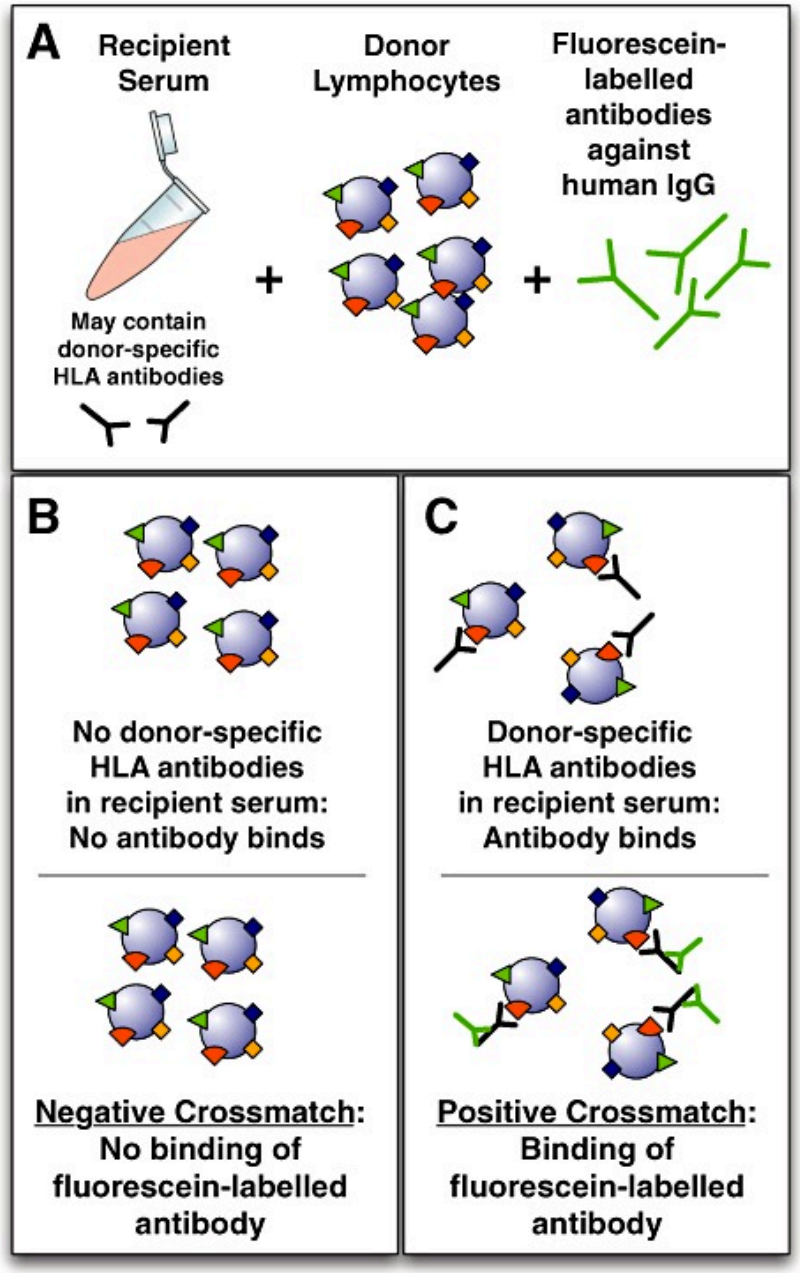
- Are HLA antibodies in the potential recipient's blood?
- Each bead has an HLA antigen, marked with different fluorophores
  - This test does *not* include donor cells
- Negative: no anti-donor antibodies are detected
- Positive: antibodies are present
  - Need more details (HLA, MFI, titer) for clinical decision making



Kumar, *J Kidney*, 2017

Flow crossmatch

- Recipient serum + donor cells
- Does the recipient have pre-formed antibodies against their particular donor?
  - Differs from cPRA because this is not calculated against the overall donor pool
- Does *not* test for complement activation or cell death

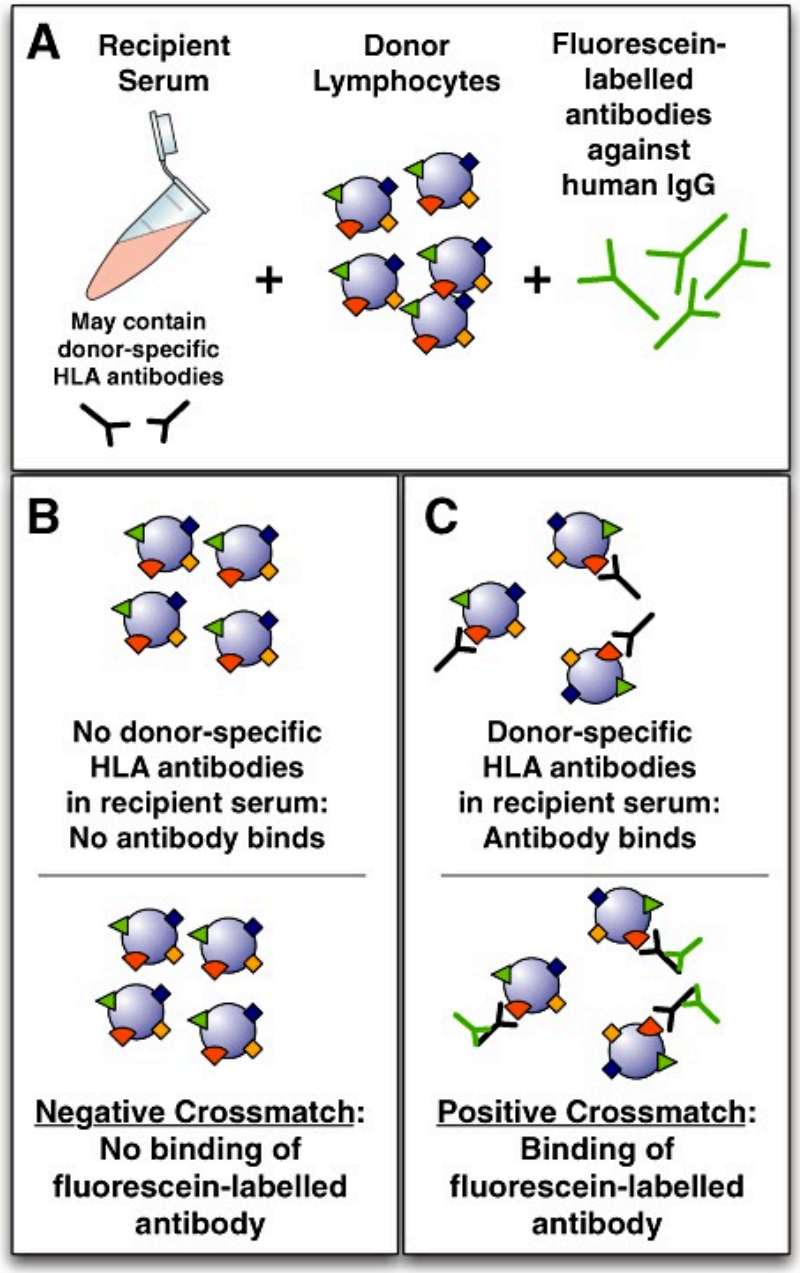


Mulley, Nephrology, 2011

Flow crossmatch

- Negative flow XM
  - Highly unlikely to have a positive cytotoxic (CDC) crossmatch

- Positive flow XM
  - Can predict a positive CDC-XM
  - Causes that have a negative CDC-XM
    - Non-complement fixing antibody
    - Non-HLA antibody
    - Low level antibody

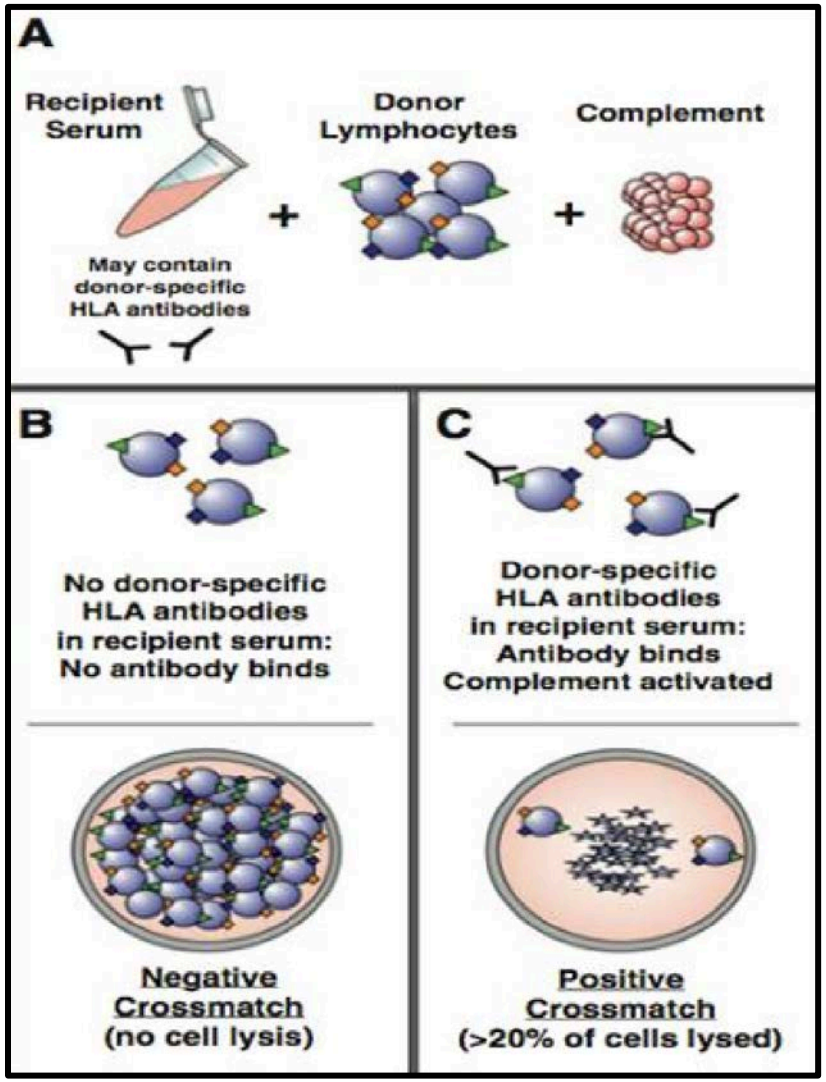


Mulley, Nephrology, 2011

Complement-dependent cytotoxicity crossmatch (CDC XM)

- Antibodies bind donor cells
- Activate complement
- Cause cell lysis

- T and B cells can be tested separately
- >20% cell death = positive



Kumar, *J Kidney*, 2017

Objectives

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- Desensitization options and considerations

Desensitization

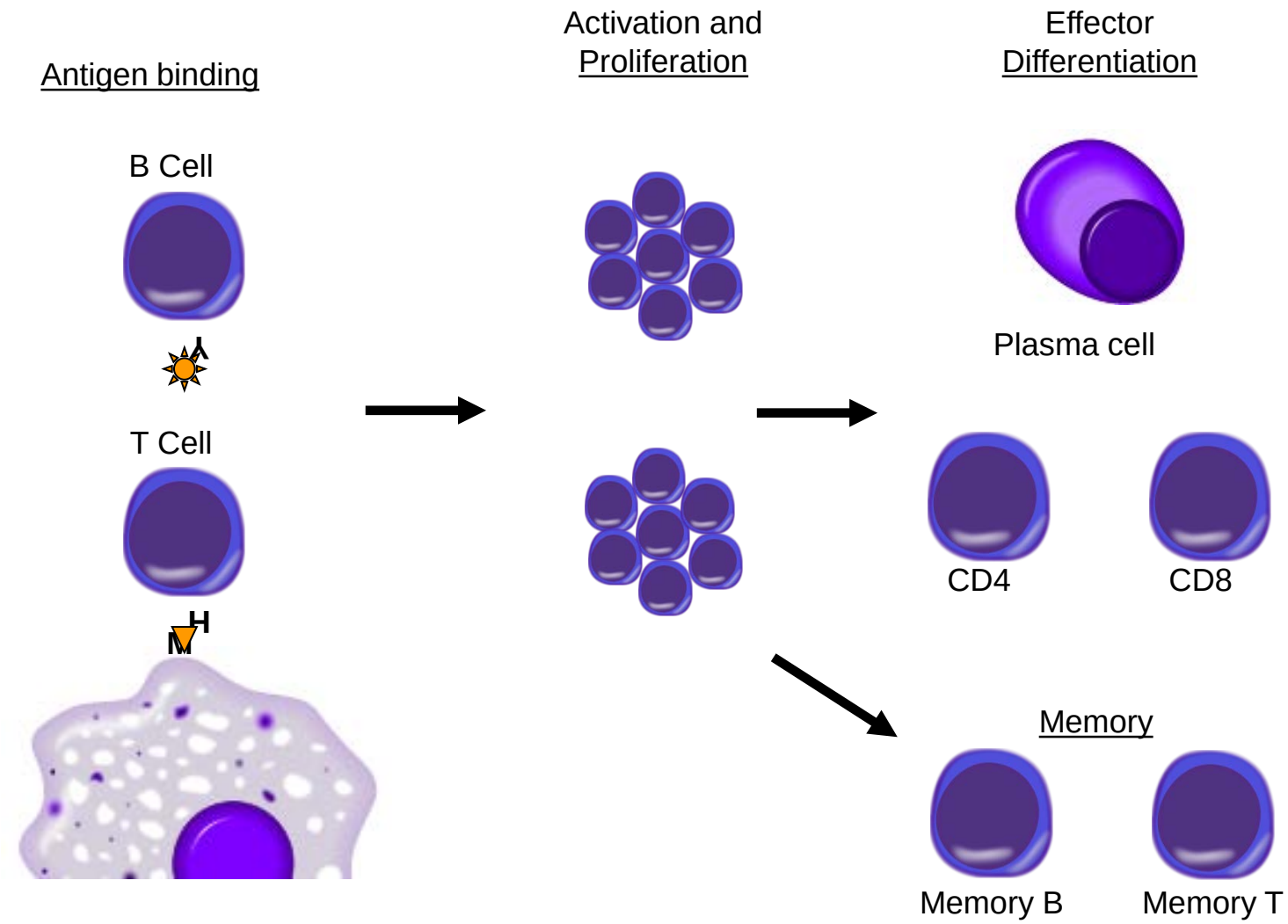
- Removal and/or reduction of anti-HLA antibodies
  - Can be performed on day of transplant or prior to organ allocation

- Goals of desensitization
  - Improve organ access for sensitized patients
    - Even a small improvement in cPRA can be life-changing
  - Remove antibody prior to a planned transplant

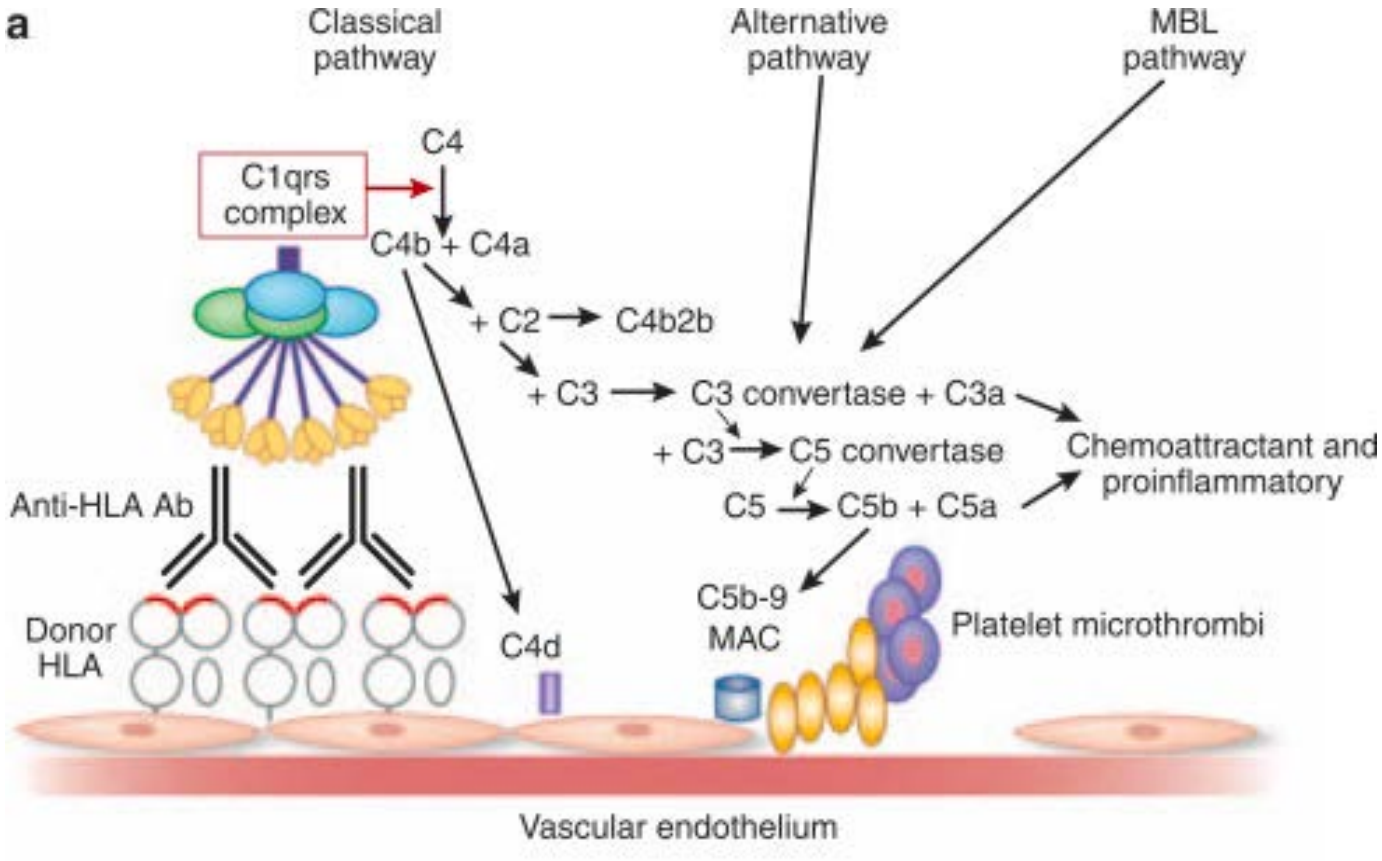
Data to guide treatment decisions

- There are no FDA approved agents for desensitization
- Every center and organ does this a little bit differently
- There is no real consensus of the optimal protocols
- Many treatments are expensive
- Many treatments have only transient effects

What triggers antibody production?



How do anti-HLA antibodies cause injury?



1. Antibody binds HLA
2. Complement system is activated
3. Membrane attack complex is formed
4. Endothelial cell death
5. Releases more donor antigens → amplifies

Heeger, Kidney Intl, 2010

Desensitization strategies

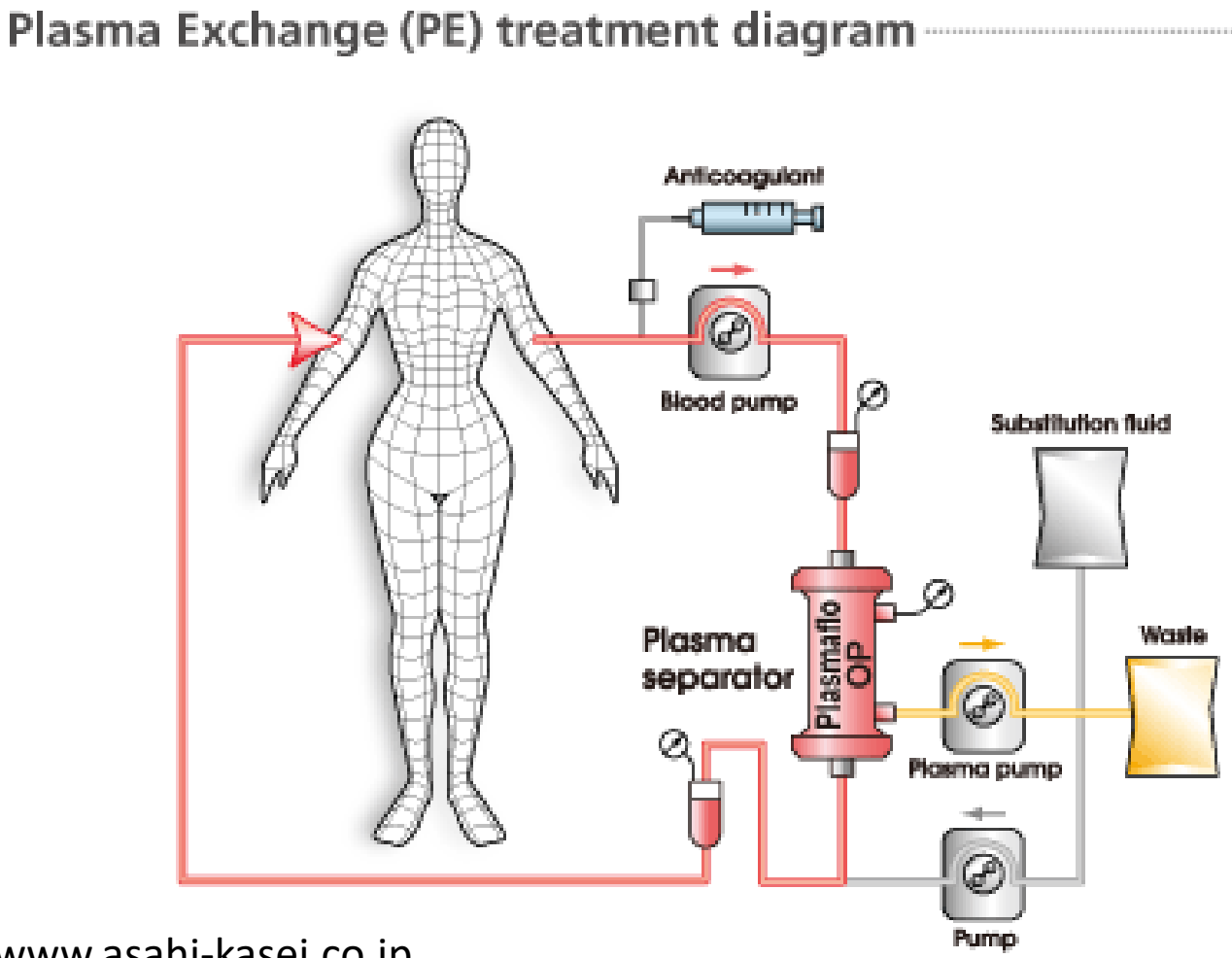
- Remove existing pre-formed antibodies
- Stop production of additional antibody
- Suppress signals driving antibody production
- Stop complement activation

Removing pre-formed antibodies

- Plasmapheresis
  - Removes (all) antibodies from the circulation

- IVIg (immunoglobulin)
  - Binds and facilitates removal of existing antibodies

Plasmapheresis



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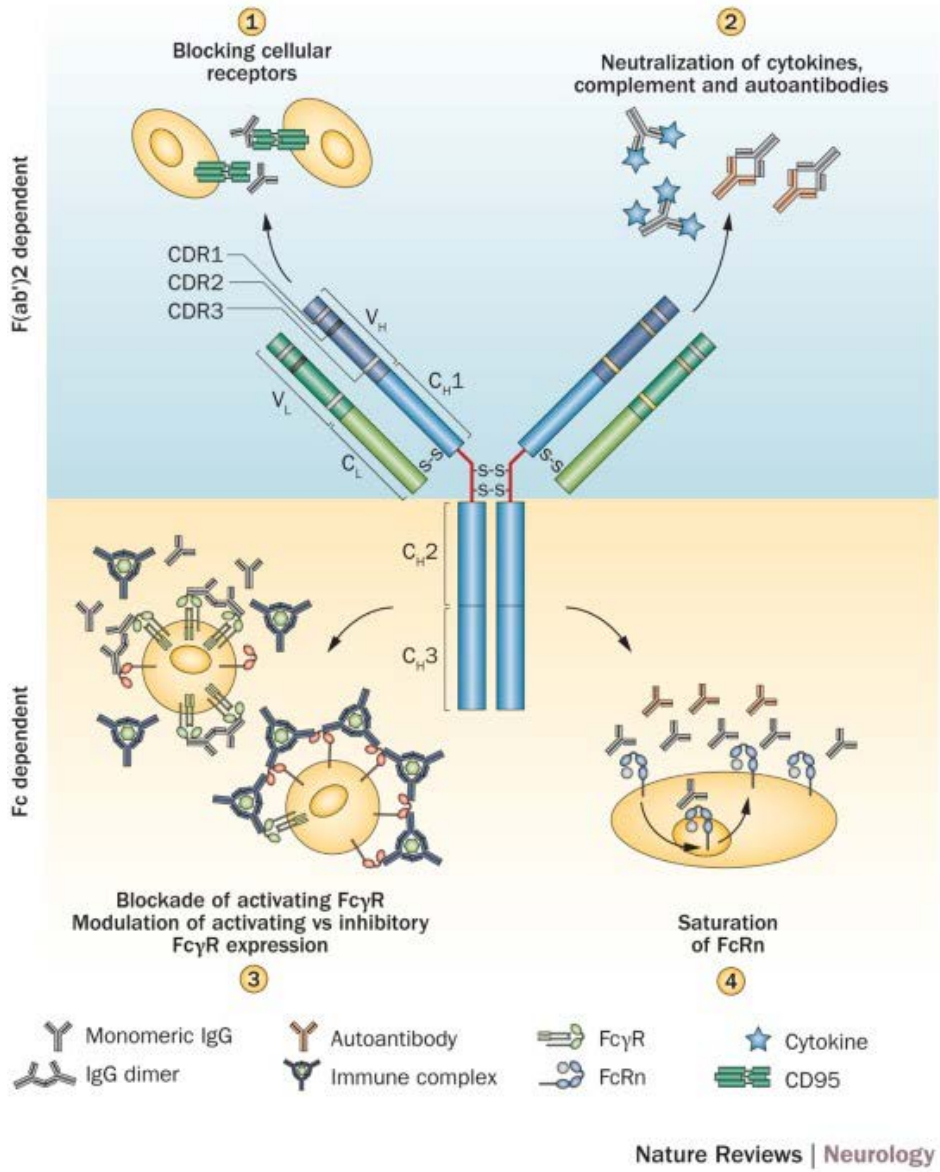
- All antibodies (pathogenic and protective) are affected
- Replace volume with FFP or albumin
- Can adjust the number of exchanges

IVIG

- Binds to circulating antibodies → neutralizes, facilitates immune complex removal
- Saturates FcRn → prevents recycling of Ab and facilitates degradation in lysosomes
- Blocks complement and other cellular receptors



Lunemann, Nat Rev Neurol, 2015

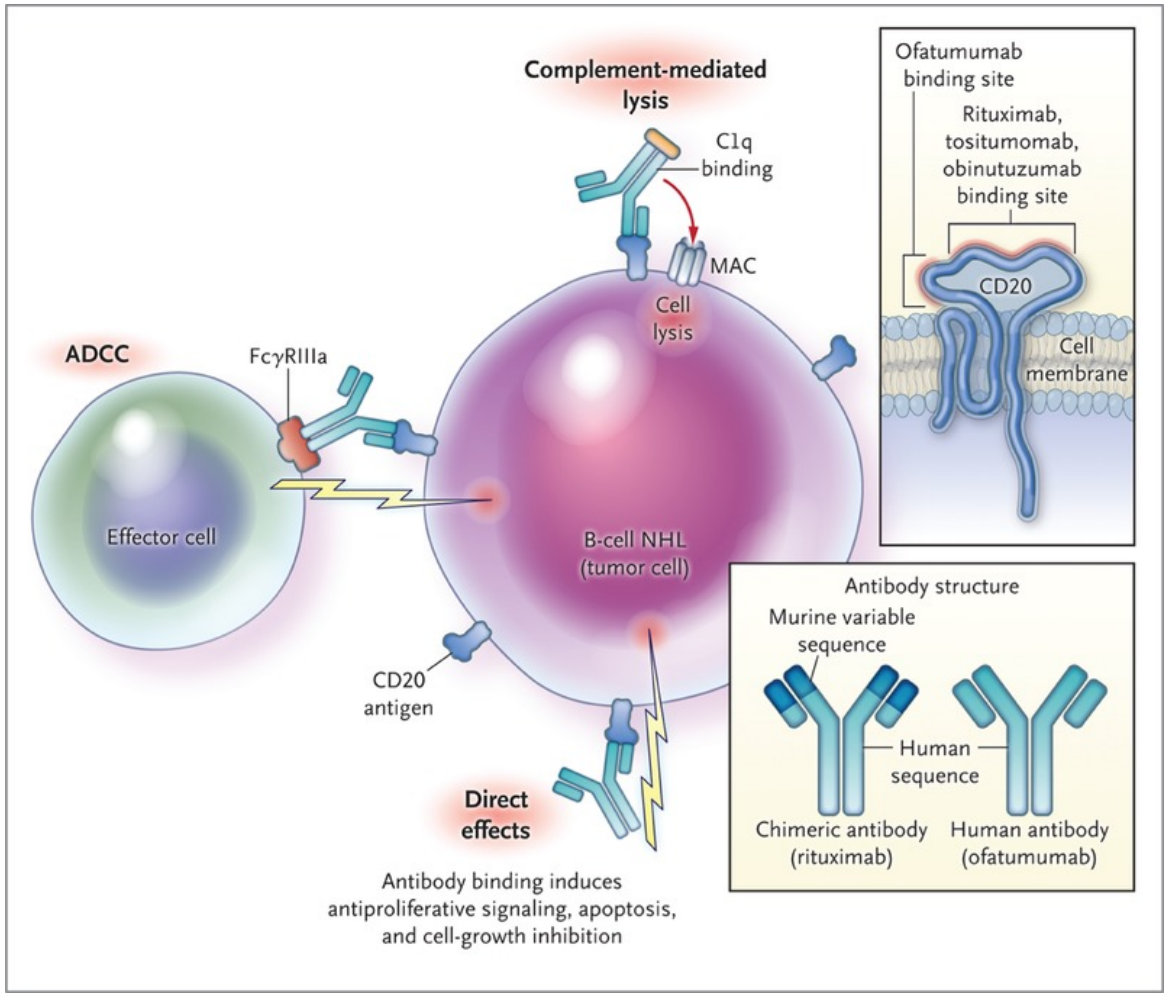
Stop production of additional antibodies

- Anti-CD20 – rituximab
  - Targeted removal of CD20+ B cells
- Proteosome inhibitors – bortezomib, carfilzomib
  - Apoptosis of plasma cells
- Anti-CD38 – daratumumab
  - Targeted removal of CD38+ plasma cells and NK cells

Rituximab

- Anti-CD20 antibody (B cells)
- Induces antibody-dependent cytotoxicity
- Activates complement-dependent cell lysis
- Antibody-dependent phagocytosis
- Causes apoptosis due to signal interruption

- Successfully reduces Ab levels and cPRA
  - Many grafts have Ab resurgence within 1 month (Vo, Transplantation, 2014)
  - Used in heart and kidney for desensitization

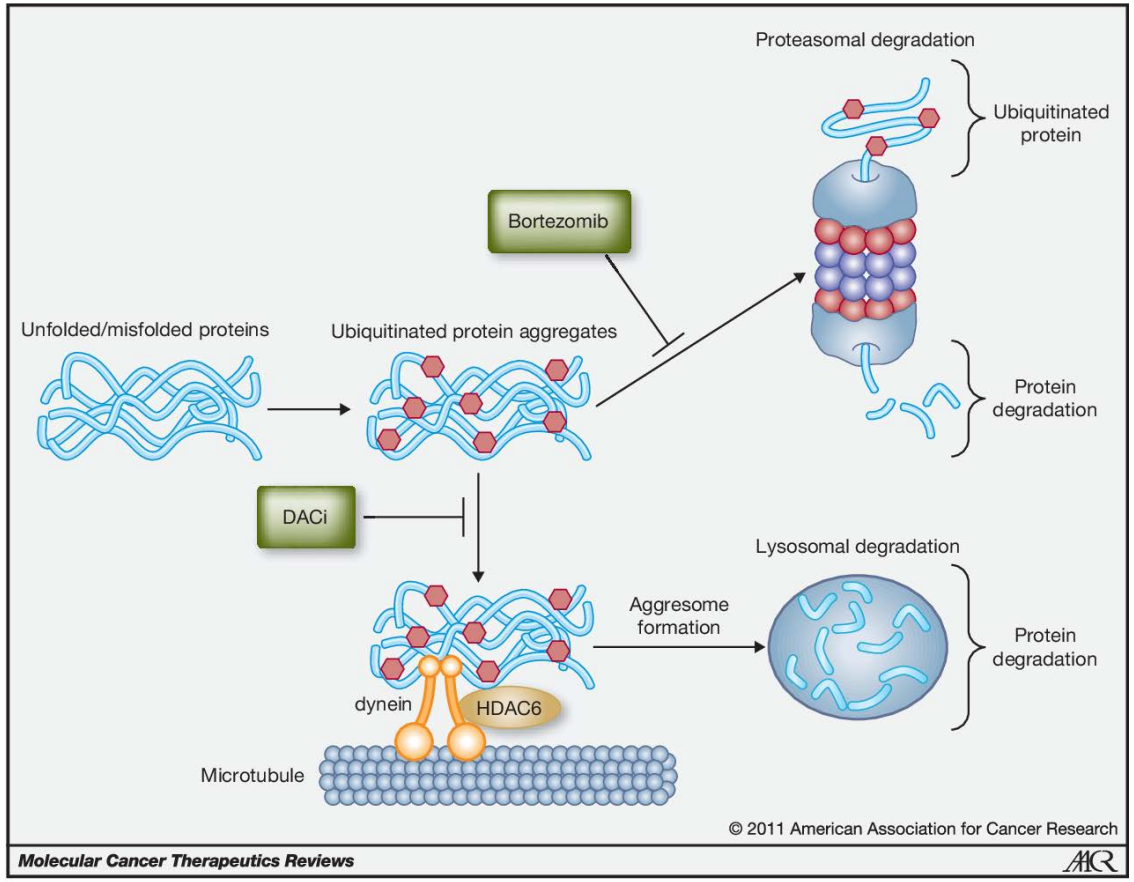


Maloney, NEJM, 2012

Proteosome inhibitors

- Bortezomib (reversible), carfilzomib (irreversible)
- Misfolded proteins accumulate → apoptosis
- Targets plasma cells because of their propensity to make enormous amounts of protein

- Numerous side effects
- Have been used in heart, kidney, and lung
  - Therapeutic effect lasts up to 6m and then rebounds



Hideshima, Mol Cancer Ther Rev, 2011

Daratumumab

- Anti-CD38 monoclonal antibody (plasma cells, NK cells)
- Mechanism of effect is similar to rituximab
  - Antibody-dependent cytotoxicity
  - Complement-dependent cell lysis
  - Antibody-dependent phagocytosis
  - Apoptosis
- Reduced Ab levels and improved graft survival, but had worse rebound in non-human primates (Kwun, Am J Soc Neph, 2019)
  - CD38 is also on regulatory B cells and some suppressor cells

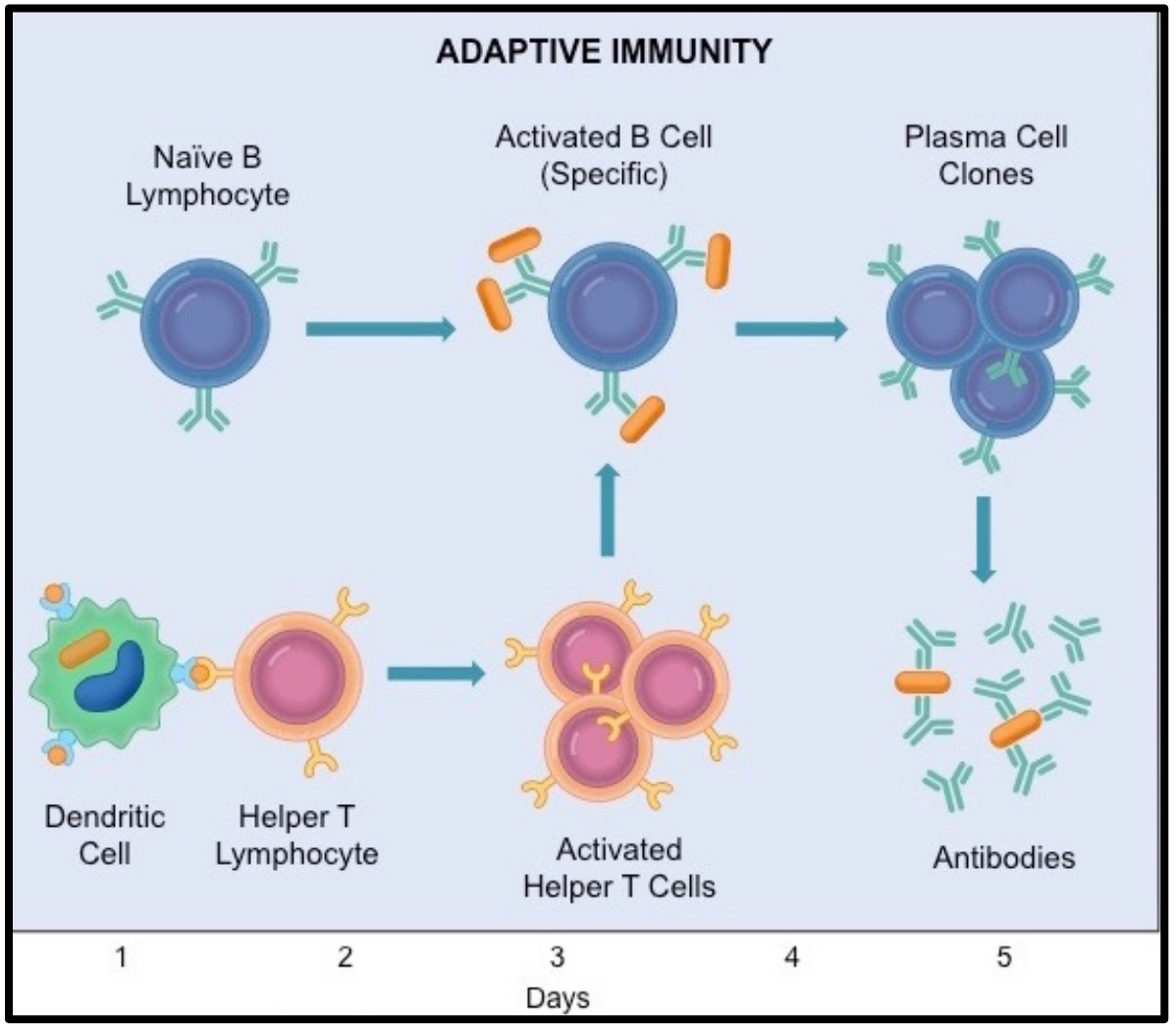
Suppress signals driving antibody production

- Lymphocyte depletion
  - Anti-thymocyte globulin
  - Alemtuzumab
- IgG cleavage proteins
  - Inflimidase
- IL-6 pathway therapies
  - Tocilizumab
  - Clazakizumab

Lymphocyte depletion or inhibition

- Anti-thymocyte globulin
  - Polyclonal antibody preparation
- Alemtuzumab
  - Anti-CD52

- Co-stimulation blockade
  - Belatacept



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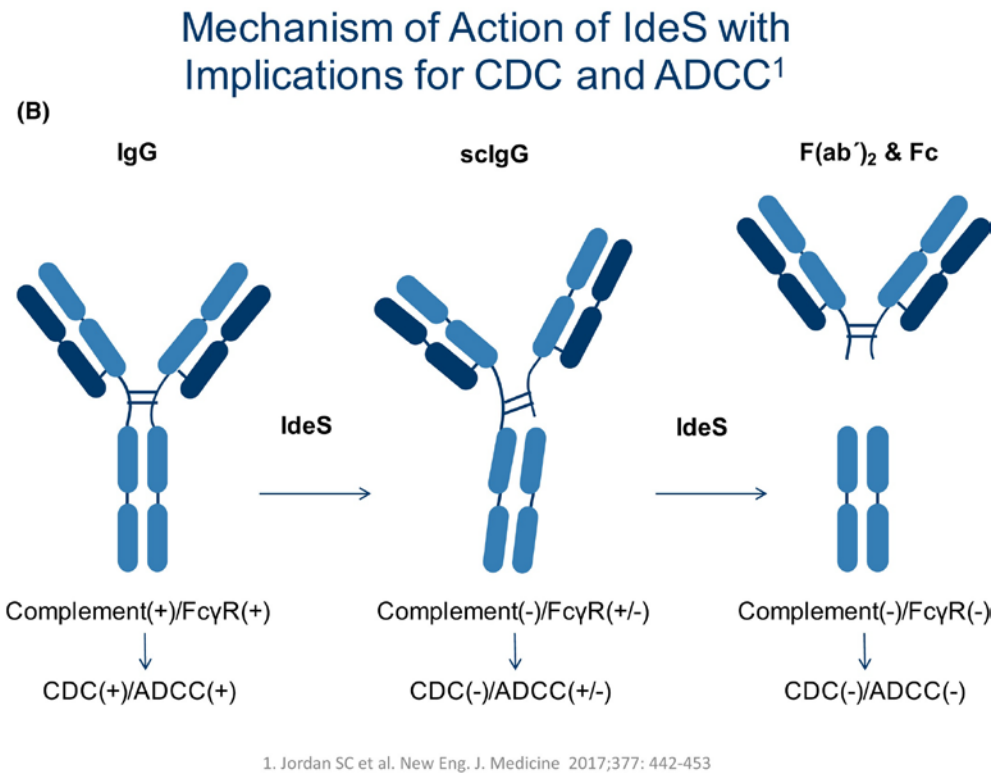
Inflimidase

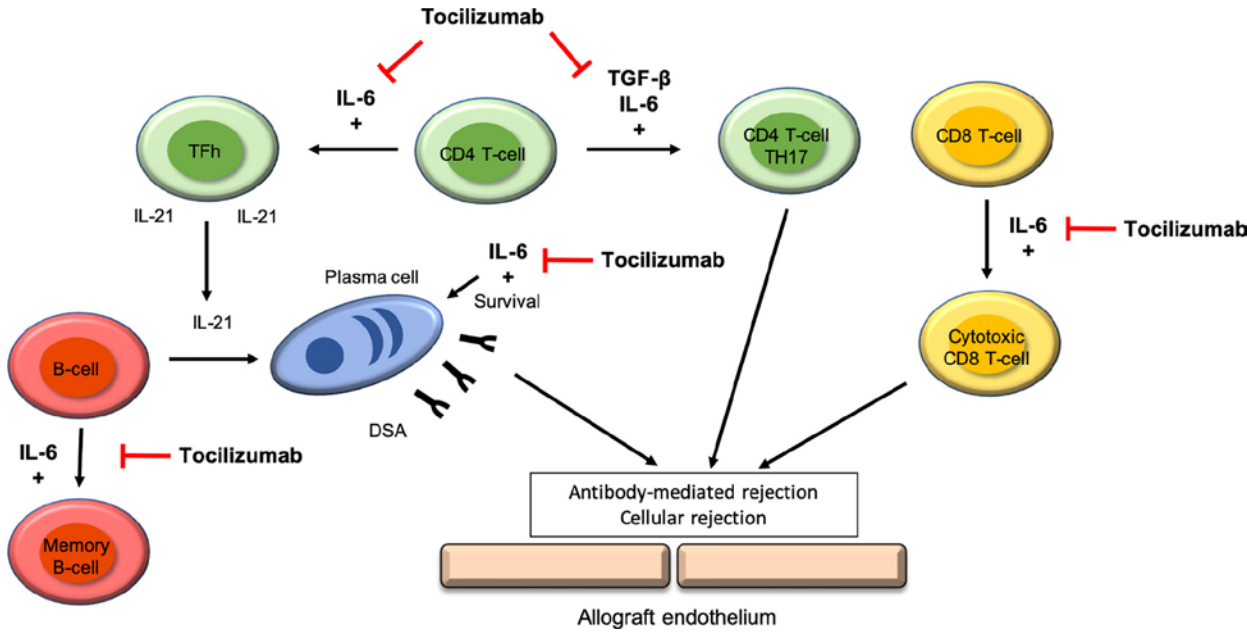
- Streptococcal protein
- Cleaves circulating IgG into F(ab) and Fc
  - Inhibits Ab-dependent and complement-dependent cytotoxicity
- Circulating antibody is depleted within 6 hours
  - Also cleaves B cell receptors → inhibits Ag binding, may reduce plasma cell differentiation
- Rebound IgG levels within 1-2 weeks
  - Used successfully in kidney, usually in combination with other agents



Huang, AJT, 2021

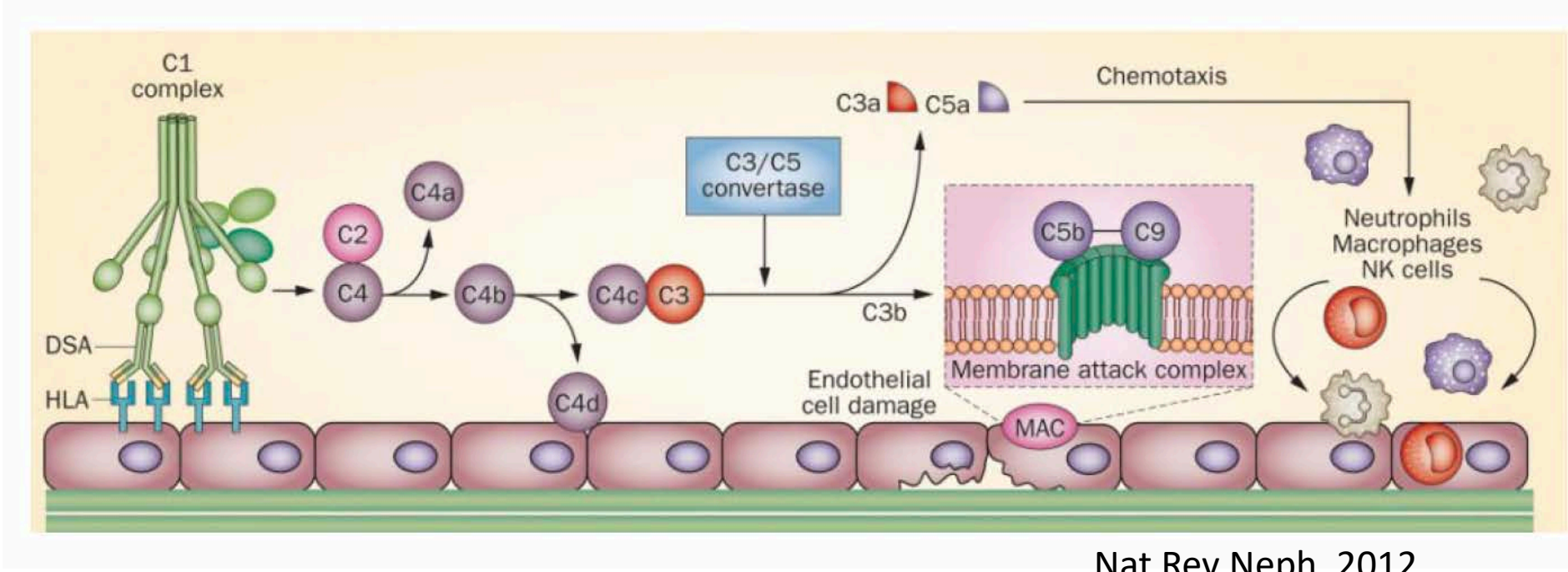
IL-6 pathway inhibition

- Tocilizumab (IL-6R antagonist)
- Clazakizumab (direct IL-6 inhibitor)
- IL-6 functions
  - Stimulates T helper, Th17, and CD8
  - Inhibits regulatory T cells
  - Promotes plasma cell survival
- Growing data in kidney transplant

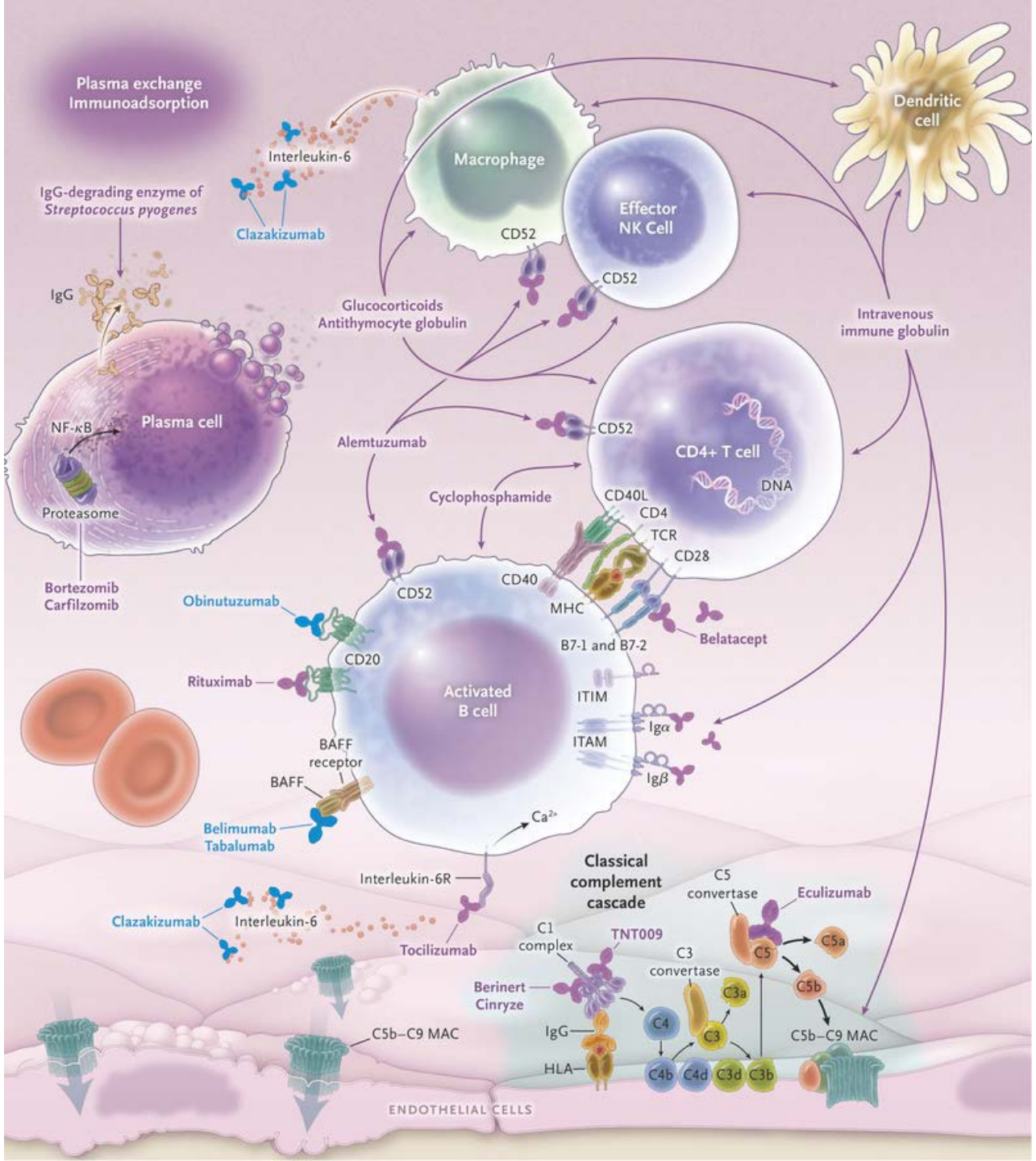


Stop complement activation

- Eculizumab
  - Anti-C5 antibody
  - No effect on antibody levels or binding
  - Prevents formation of MAC complex



Nat Rev Neph, 2012



Desensitization options

- Plasmapheresis
- IVIG
- Rituximab
- Bortezomib / Carfilzomib
- Daratumumab
- Thymoglobulin / Alemtuzumab
- Inflimidase
- Tocilizumab / Clazakizumab
- Eculizumab

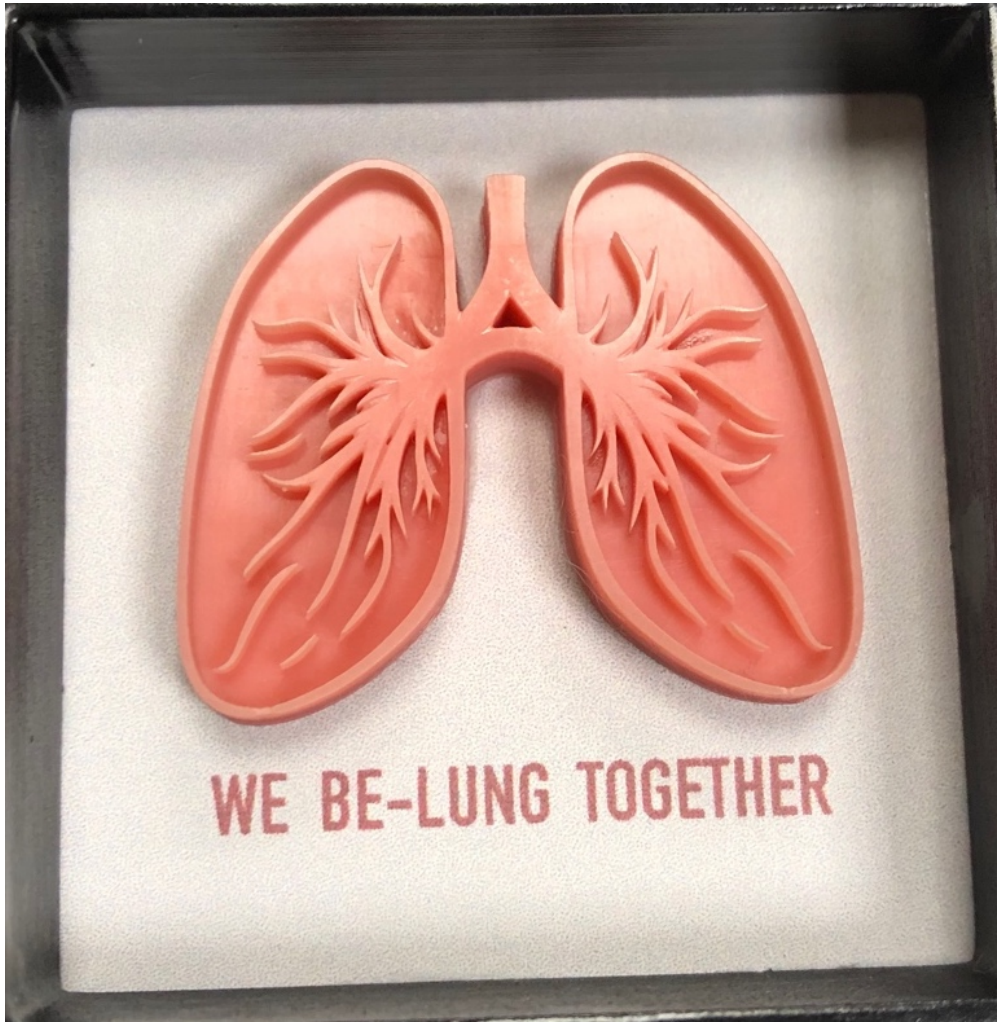
Desensitization – what do centers actually do?

- Which patients undergo desensitization?
  - cPRA trigger varies widely by organ
- Which combinations of medications are used on the day of transplant?
  - Plasmapheresis + IVIG is most common
  - Many centers add lymphocyte depletion or B/plasma targeting agents
- Which combinations are used when reducing cPRA is the goal?
  - Rituximab +/- IVIG

Key steps for achieveing successful solid organ transplant

- Before transplant day
  - HLA typing: identify potential recipient and define “self” by HLA
  - Antibody screening: assess for pre-existing anti-donor HLA antibodies
- Transplant day
  - Crossmatch between actual donor and the recipient
- After transplant day
  - Immunosuppress: avoid exposure of donor antigens to the recipient’s immune system

Questions?



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