Transplant Immunology

The Cellular and Molecular Basis, Consequences, and Clinical Management of Self-/Non-Self Discrimination

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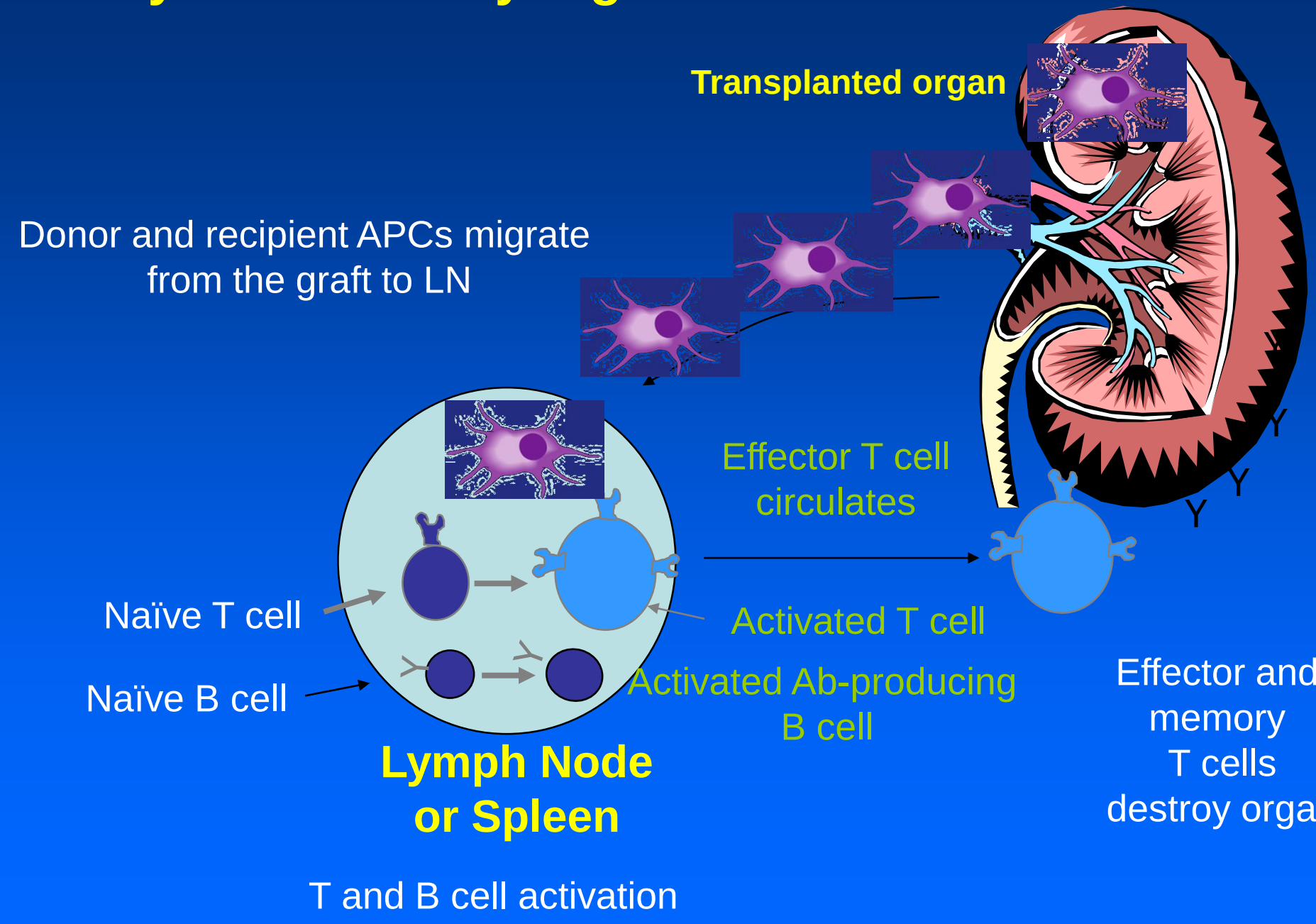
Allorecognition

Rejection

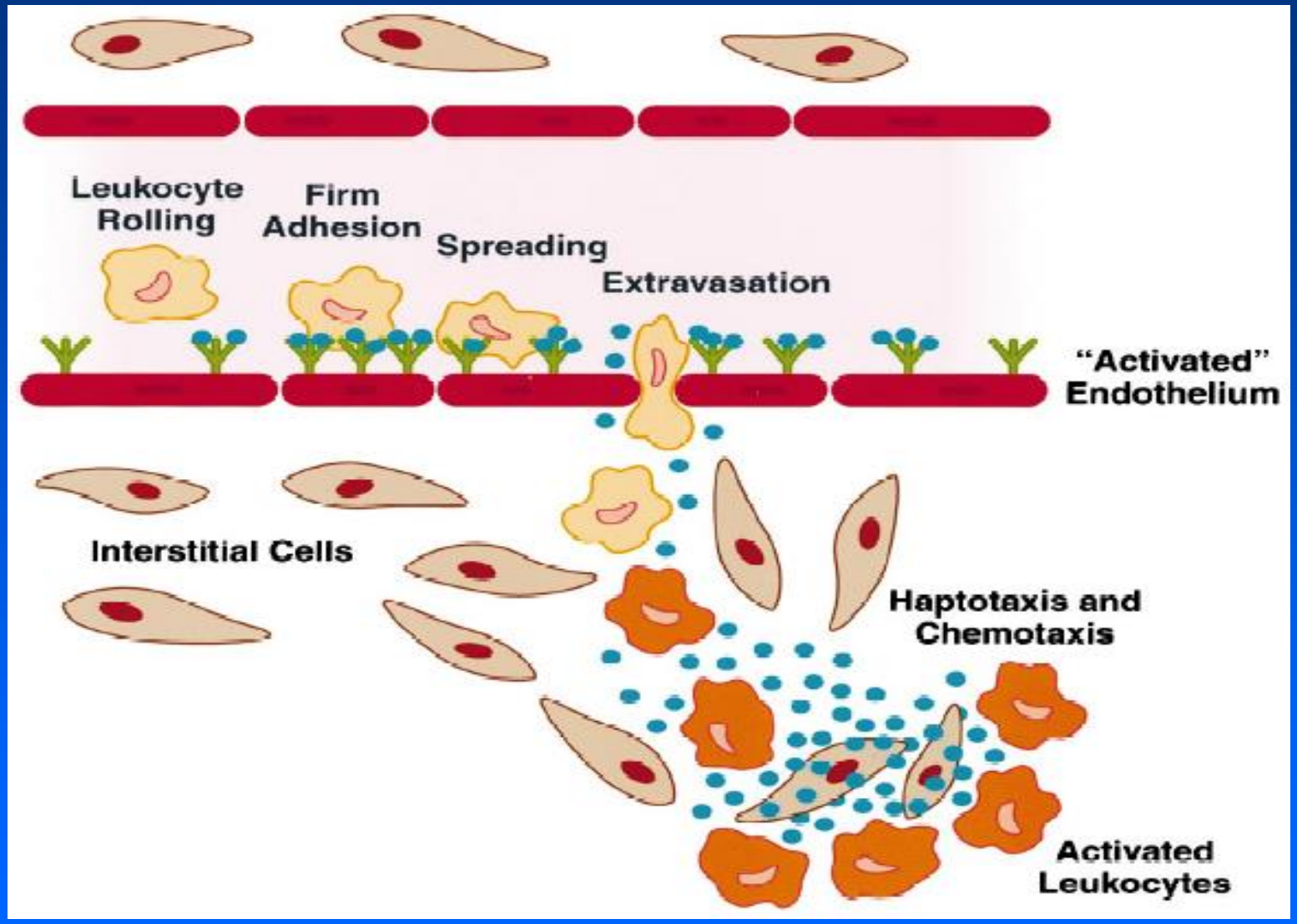
Allorecognition

- Immunity that develops against the antigens (proteins, carbohydrates, lipids) of another individual of the same species

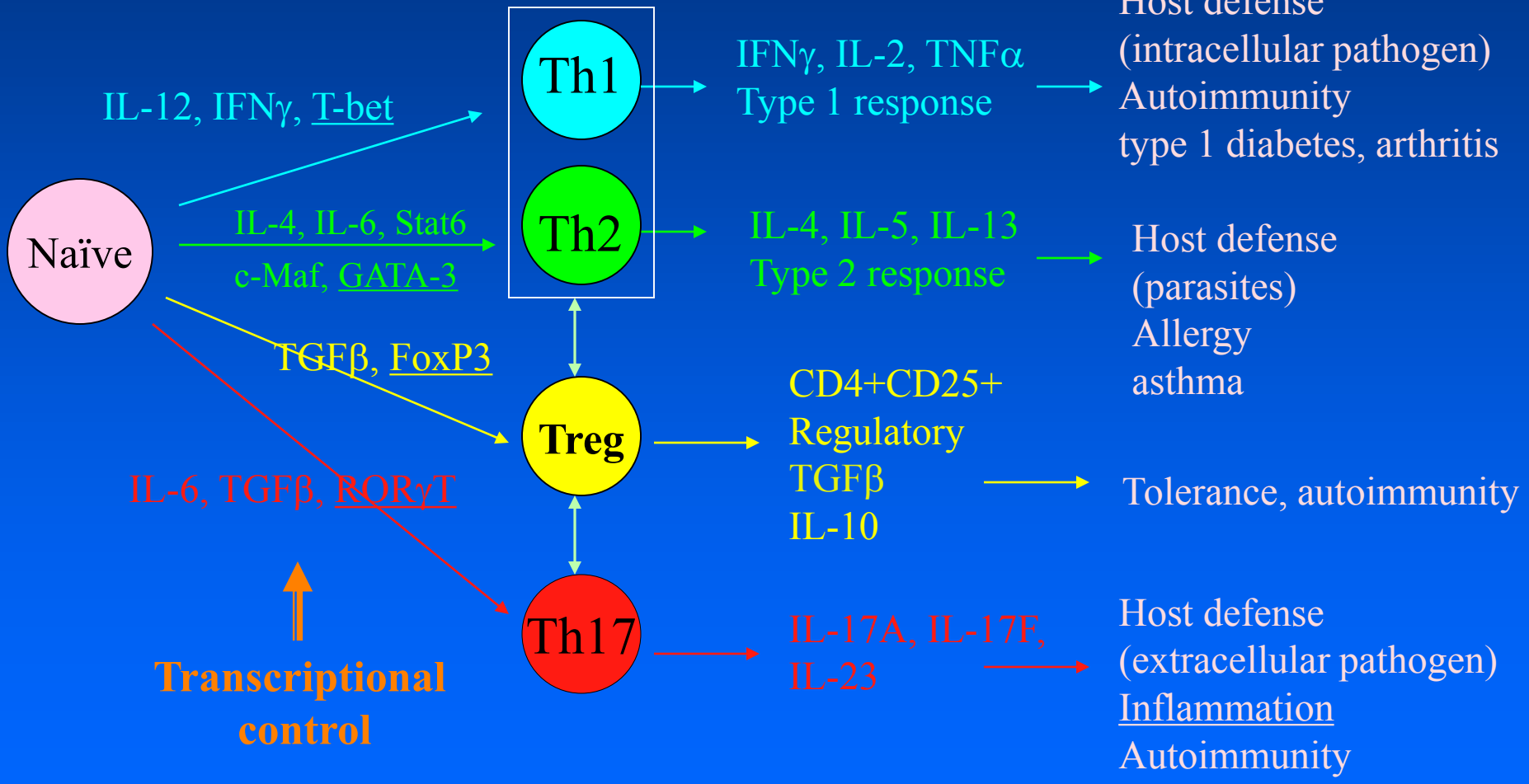
Early Inflammatory Signals



Entry into tissues, organs, lymph nodes

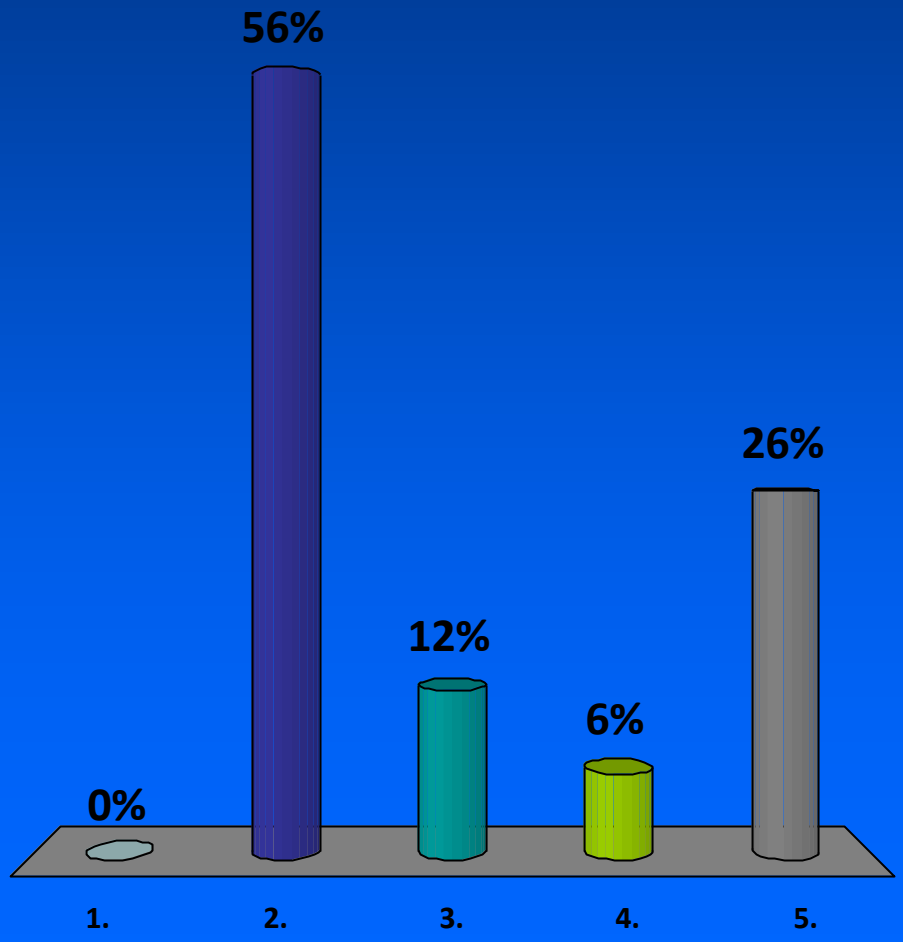


CD4 T Cell Development

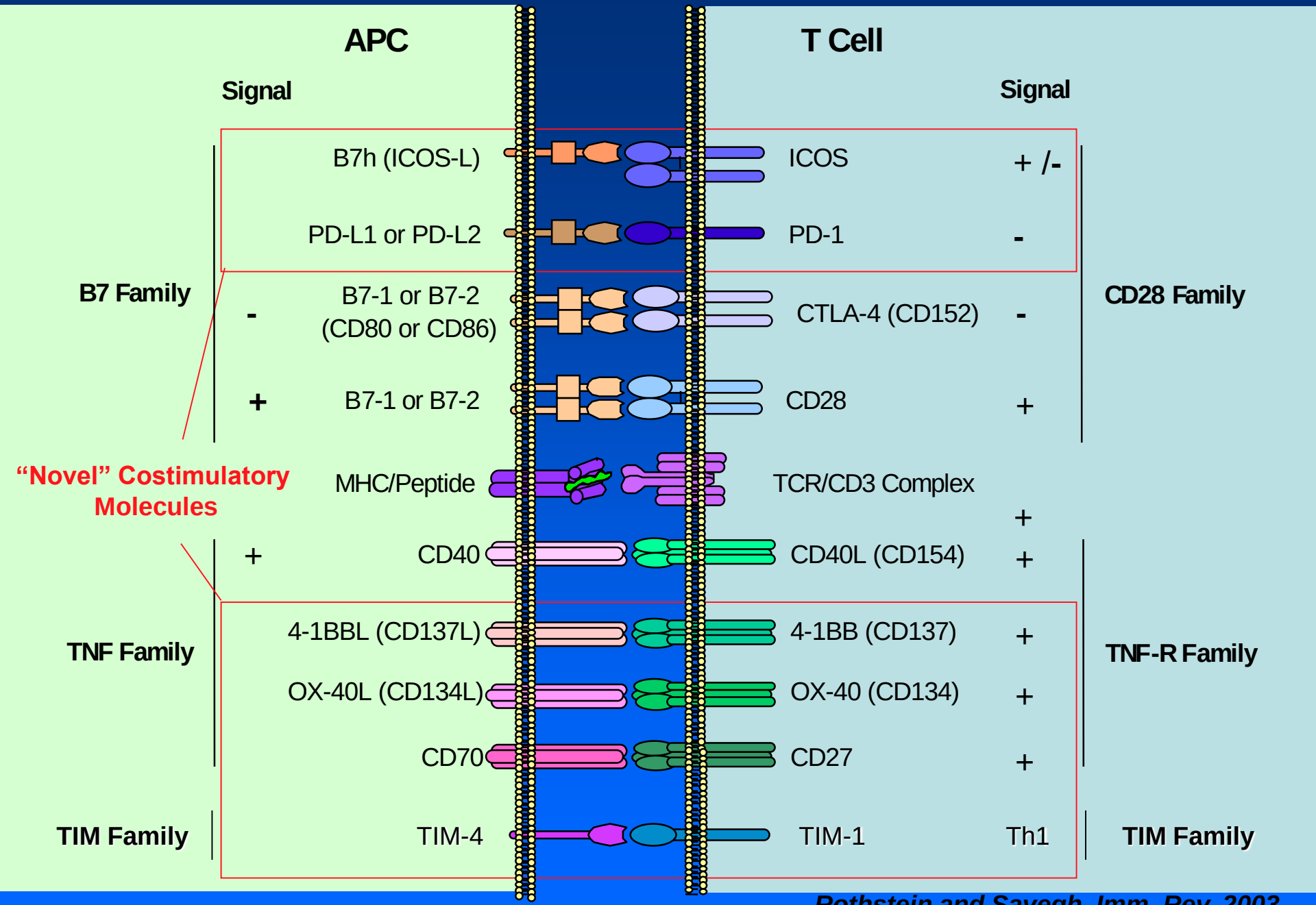


Costimulatory blockade is not tolerogenic because:

1. No drugs exist
2. Too many targets
3. No drugs approved
4. Humans don't express these molecules
5. It is tolerogenic



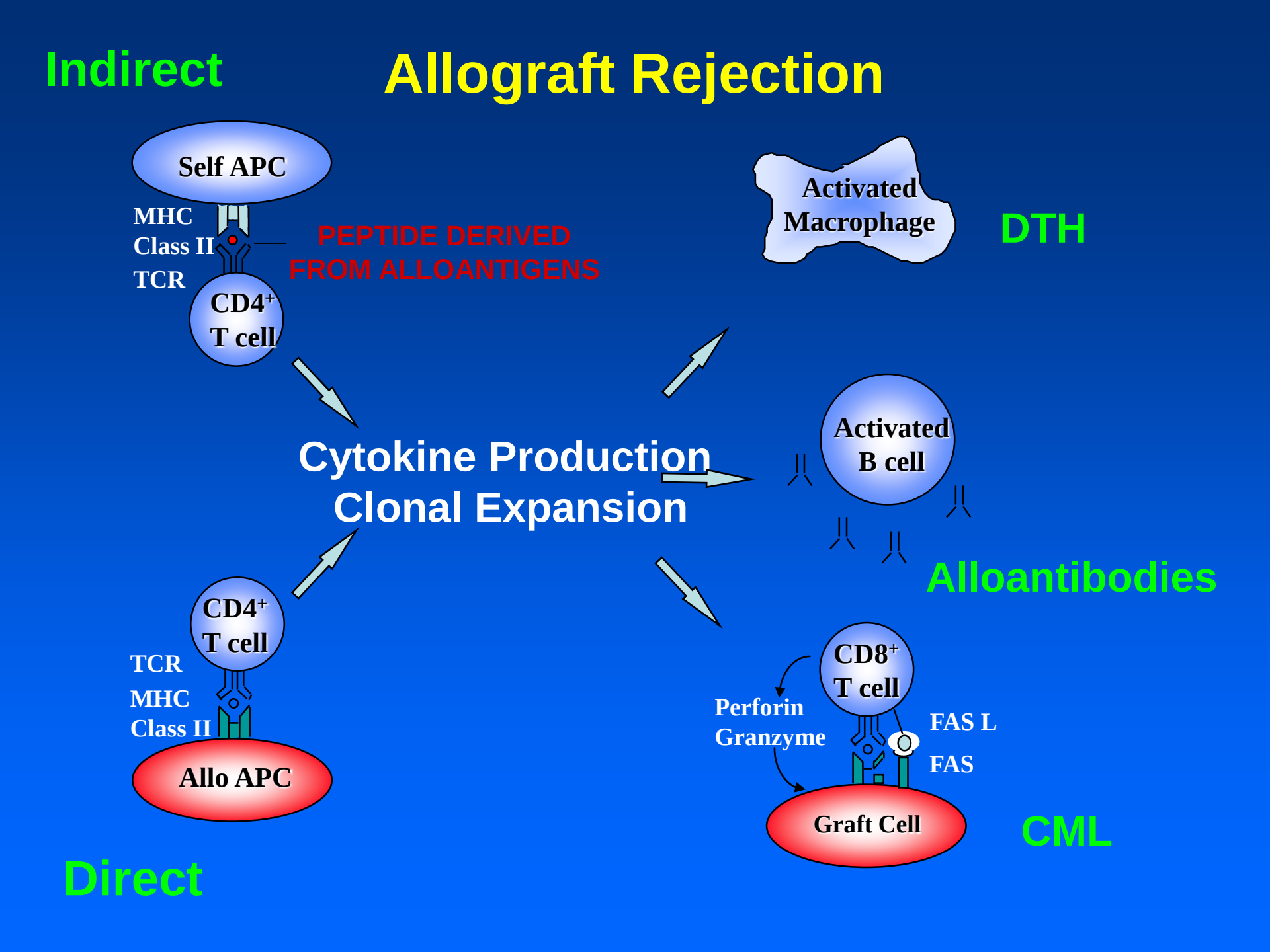
Costimulatory Molecules



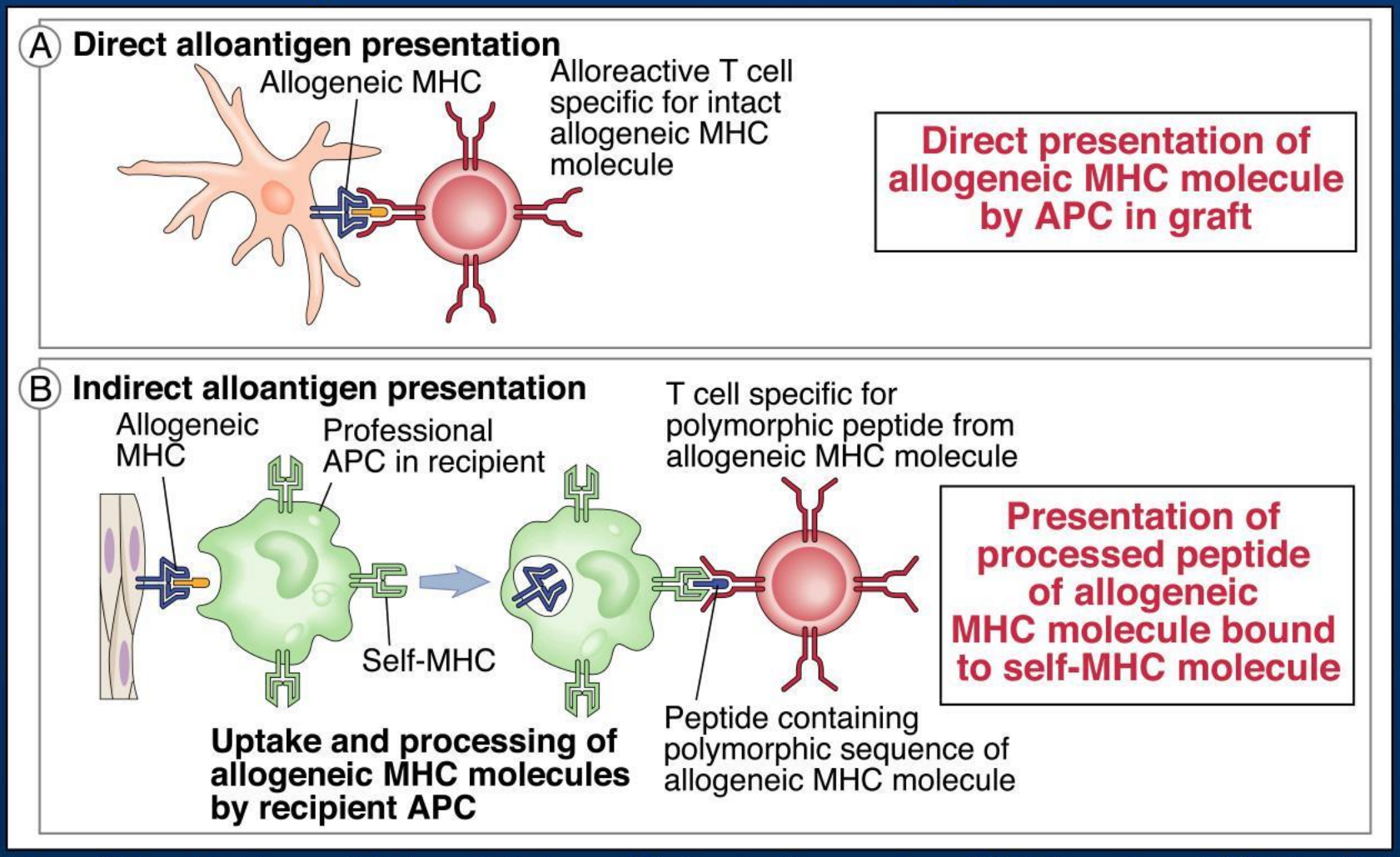
Rothstein and Sayegh, Imm. Rev. 2003

Direct and Indirect Alloantigen Presentation

Allograft Rejection

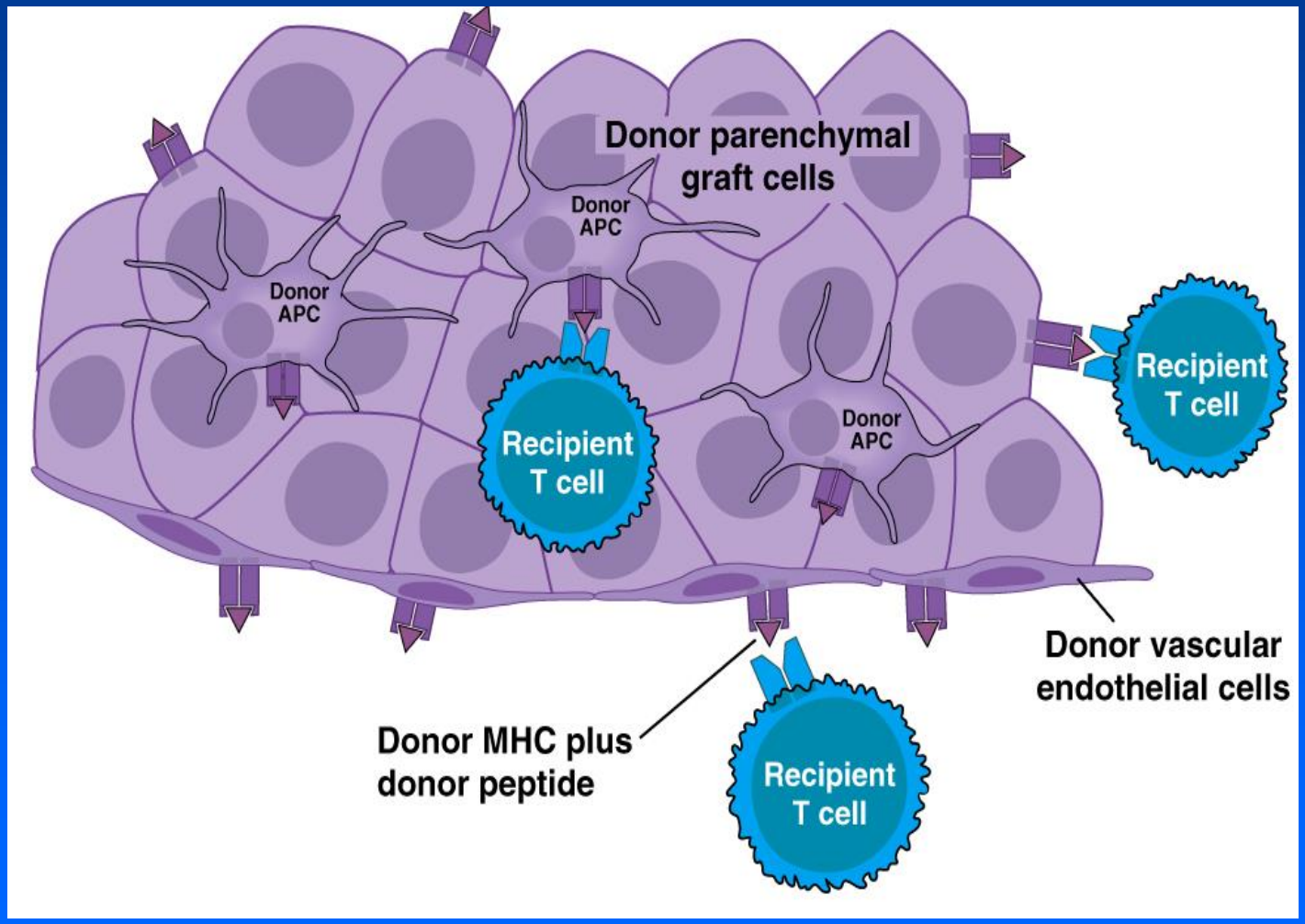


Direct and indirect presentation of alloantigens

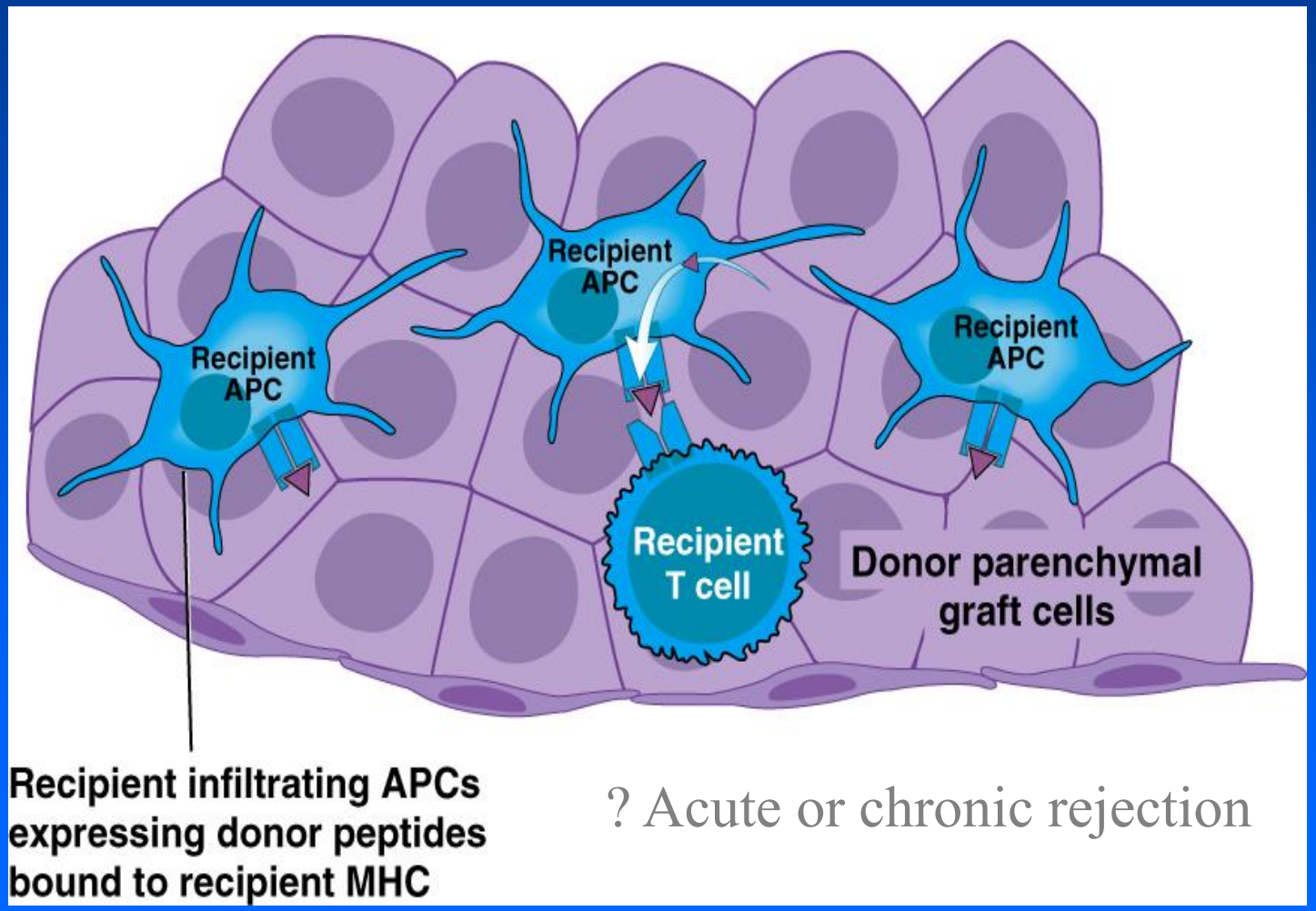


From Abbas, Lichtman, & Pober: Cellular and Molecular Immunology. W.B. Saunders, 1999, Fig. 16-3

T cells responding through the direct pathway may account for acute cellular rejection



T cells responding through the indirect pathway may contribute to acute and chronic rejection



? Acute or chronic rejection

Special Nature of T cell responses to MHC alleles

- T cells with high-affinity TCR for "new" antigens are rare (10^{-5}-10^{-7}), but persist in larger numbers after prior exposure: immune "memory"
  - secondary response more rapid
  - specific to the original challenge (third party response still "primary")
  - long-lasting response to "self MHC+X": indirect Ag presentation

- In contrast, a large fraction (~2-10%) of naive T cells are capable of responding DIRECTLY to mismatched MHC, because allo MHC "looks" like "self MHC+X"

Ischemia-Reperfusion Injury

Innate Immunity

Donor Brain Death and Inflammatory Response

- **Early-phase inflammatory process during organ retrieval**

  Kidney biopsy specimens were obtained during organ retrieval from BD (n=27) and living organ donor controls (n=34). Analyzed by IHC, RT-PCR.

  <u>Results</u>: After brain death, ↑ E-selectin, Hsp70, MCP-1, interstitial leukocyte invasion

  Unclear which factors trigger brain-death related graft injury

CIT and Inflammatory Response

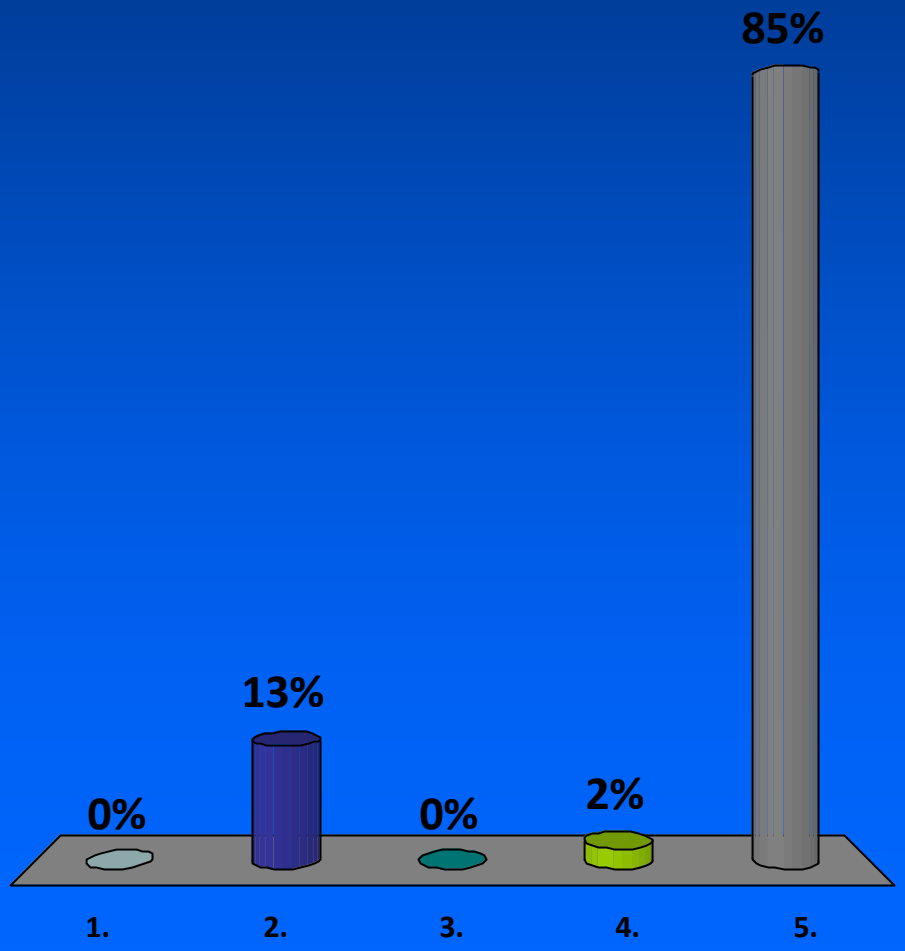
- **Increased chemokines during reperfusion of living donors (LD) and deceased donors (DD) renal allografts**

  Specimens obtained before and 30 min after reperfusion of the donor allograft from DD (n=19) and LD (n=20). Analyzed by RT-PCR.

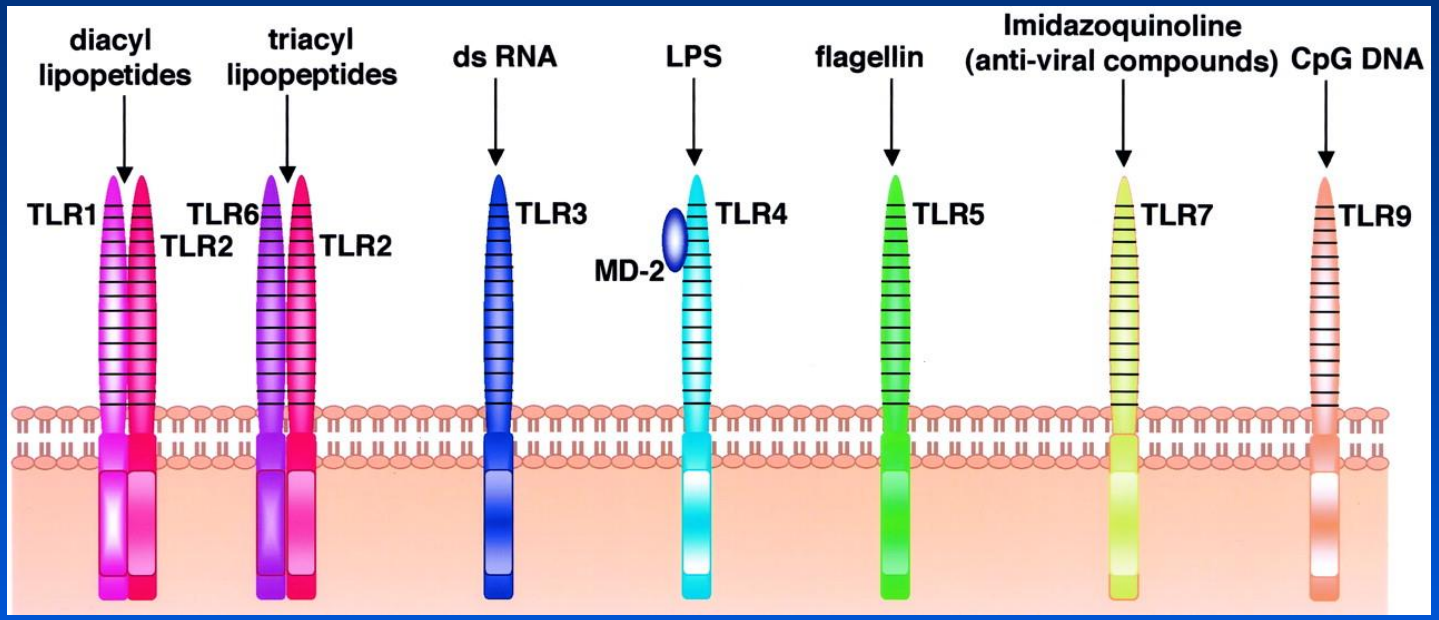
  <u>Results</u>: IL-8/CXCL8 (binds to neutrophil receptors) expression increased 50% from ischemia to reperfusion in LD but increased more than 13-fold during reperfusion of DD.

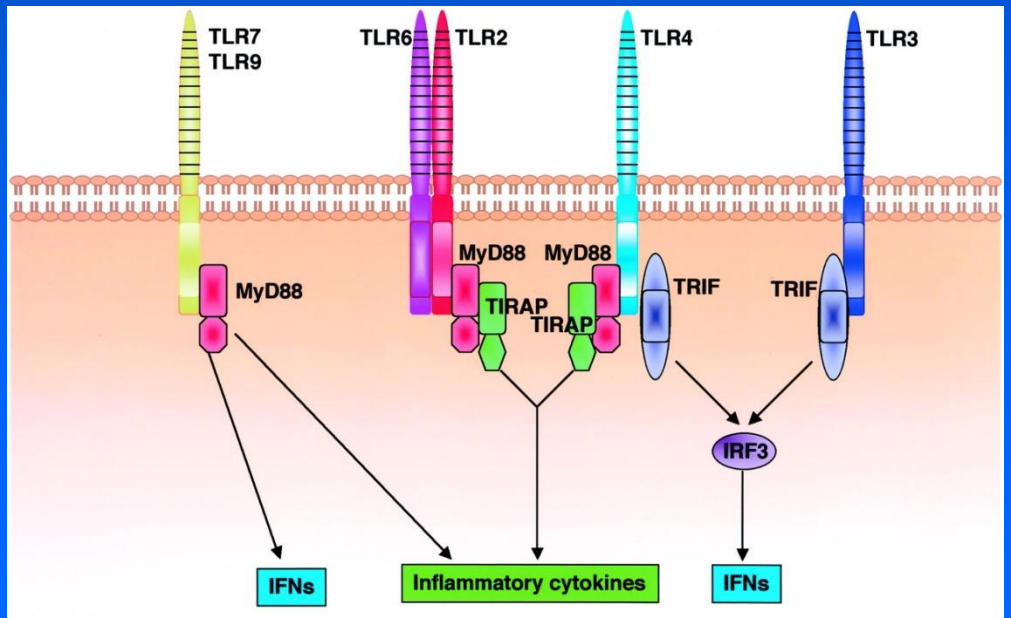
Toll like receptor blockade…

1. Is easy to do
2. Has a limited number of targets
3. Is only important in infection
4. Is not important in rejection
5. Would require blocking too many ligands & receptors



Toll like receptors (TLR)



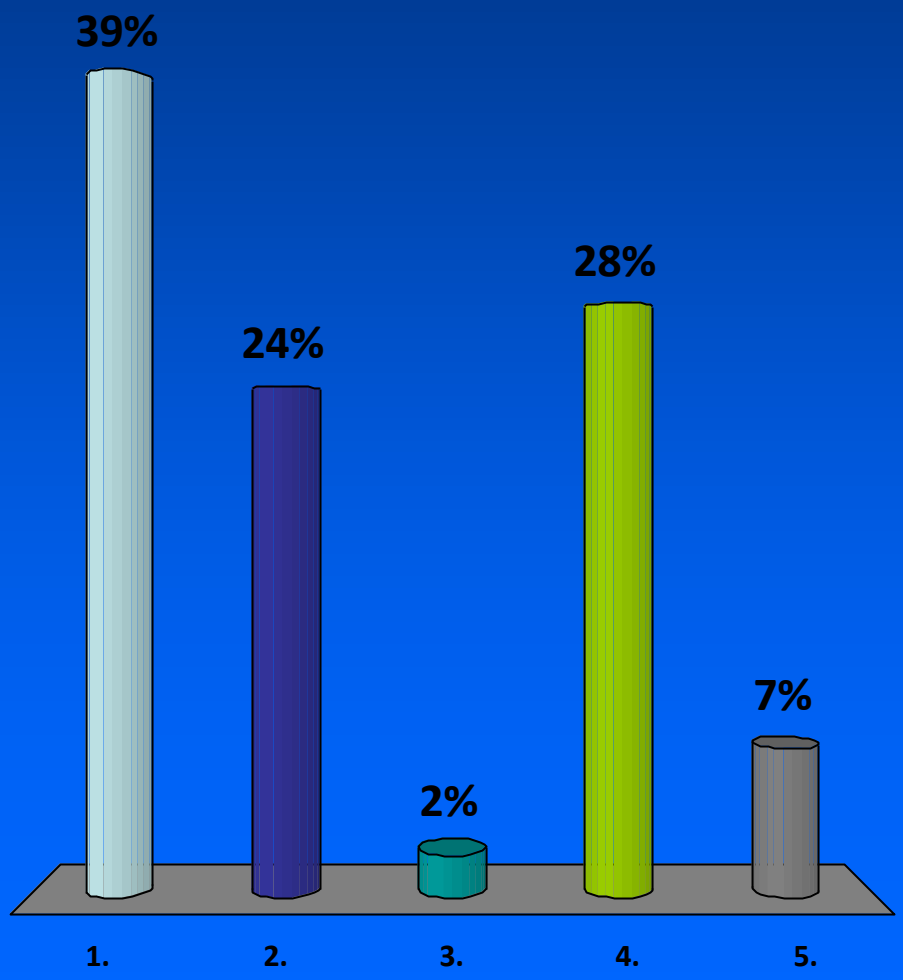


Endogenous Ligands of TLR

Ligand	TLR	Response
heat shock proteins: HSP60, HSP70, GSP96	TLR2 TLR4	DC maturation, increased cytokine production via NF-κB activation, stress responses
matrix components: fibronectin, fibrinogen, heparan, hyaluronan	TLR4	DC maturation, induction of inflammatory genes
products of necrotic cells	TLR2 TLR4	DC maturation, increased cytokine production via NF-κB activation, tissue repair gene induction
inducible defensins from urogenital epithelium, skin and respiratory tract: hBD1, hBD2, hBD3	TLR4	NF-κB activation, recruitment of DC and T cells

Chemokines and chemokine receptors:

1. Are blocked by many current drugs
2. Are activated by many current drugs
3. Are only important in infection
4. Show tremendous degeneracy
5. Are not important in alloimmunity



Chemokines

Antigen Dependent

- Acute Rejection
- Alloantibody

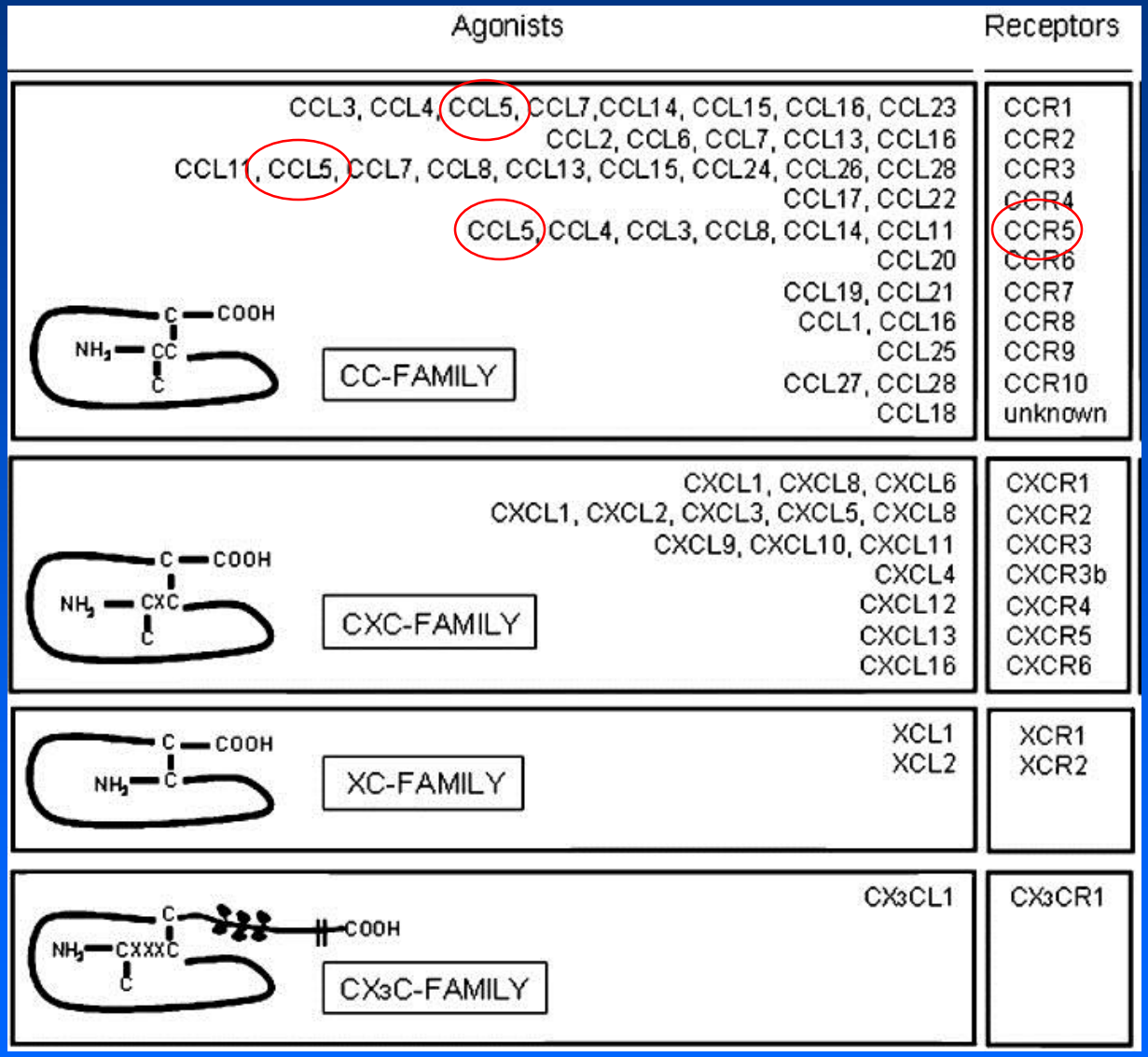
Antigen Independent

- Brain Death
- Ischemia/Reperfusion
- Low Nephron Mass
- Old Donor
- CNI
- Recurrent Disease
- Isolation/culture (islets)

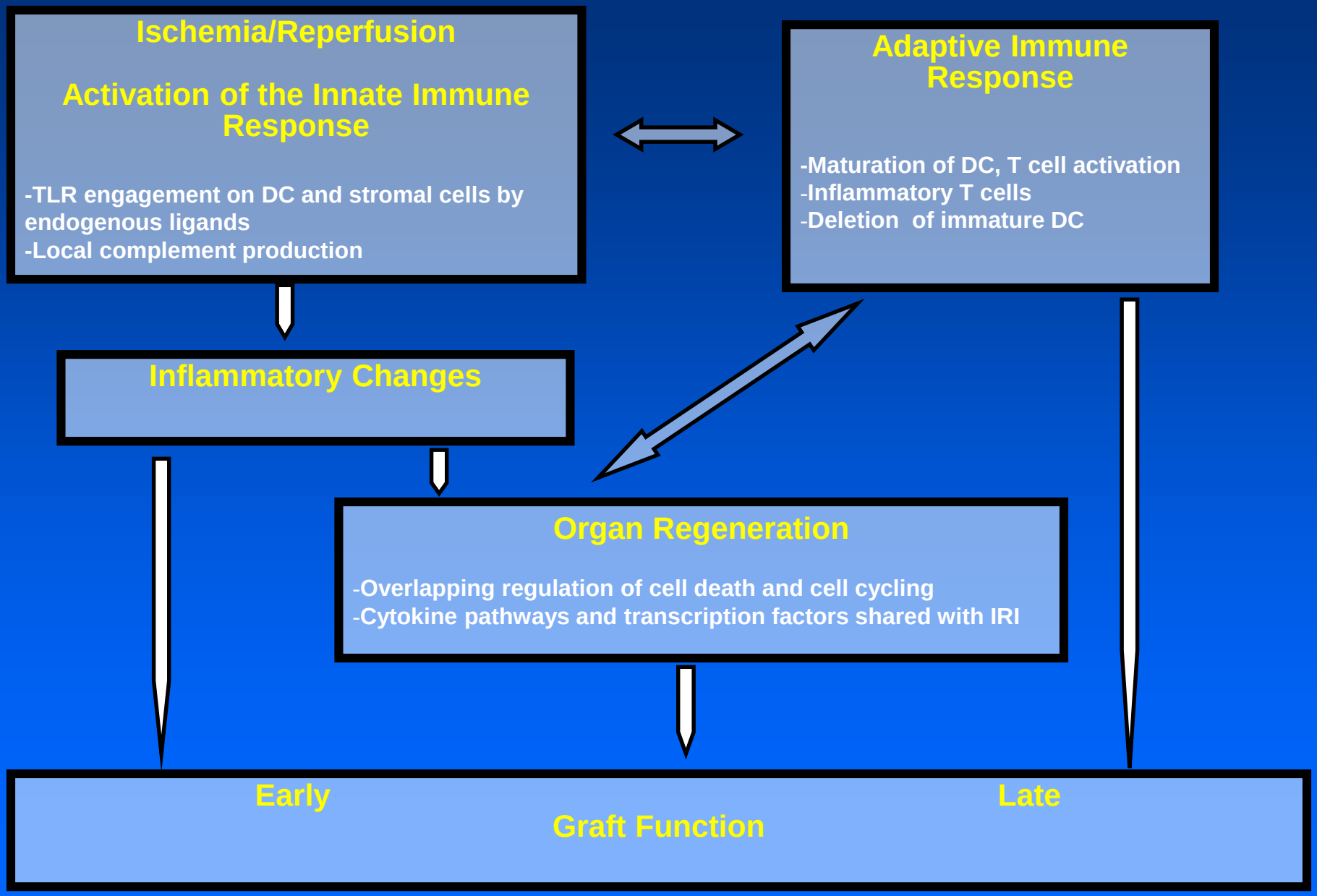
Tissue Injury

Chemokines

Degeneracy of Chemokine Ligands and Receptors



Interactions among Innate and Adaptive Immune Responses, Organ Regeneration, and Graft Function in IRI



Histocompatibility

Antigens: ABO, HLA, other

Measuring antigenic differences

Risk assessment

ABO compatibility and organ selection

-ABO identical or compatible

-UNOS regulations

-Organ type (liver vs. everything else)

-A2

Blood Group Compatibility for Solid Organ Transplantation

Donor Blood Group

Recipient Blood Group (IgM)	A	B	AB	O
A (anti-B)	Yes	X	X	Yes
B (anti-A)	X	Yes	X	Yes
AB (none)	Yes	Yes	Yes	Yes
O (anti-A and anti-B)	X	X	X	Yes

HLA Compatibility and Organ Selection

-HLA typing

-Determination of anti-HLA antibodies

  -Cross match (XM)

  -Panel reactive Abs (PRA)

  -Assay techniques (sensitivity, specifcity, function)

  -Historic, Current, Prospective Abs

-Risk stratification

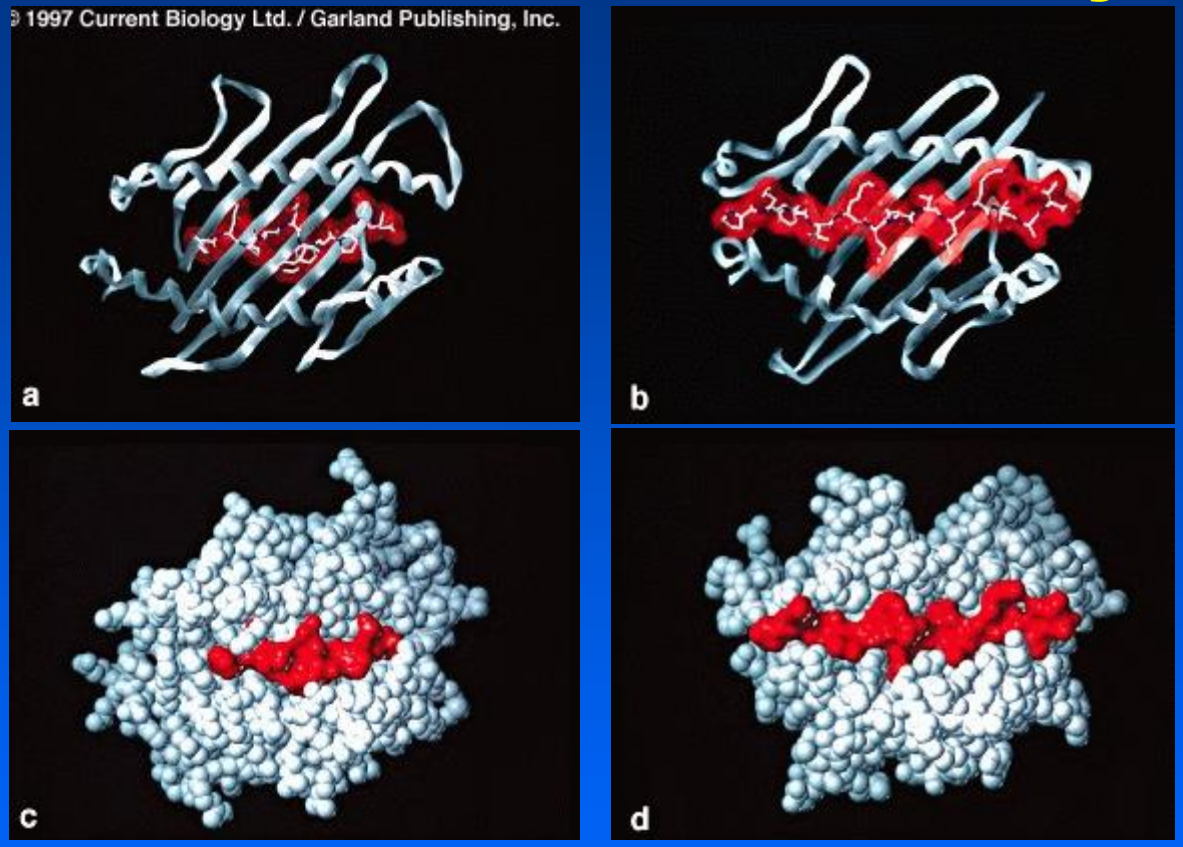
MHC Molecules

		Class I	Class II
Human	HLA	A B C	DR DP DQ
Rat	RTI	A	B D
Mouse	H-2	K D L	I-A I-E

Comparing MHC Class I and II

	Class I	Class II
ANTIGENS	HLA-A, B, C	HLA-DR,DQ,DP
TISSUE DISTRIBUTION	On virtually all cells	B cells, dendritic cells, macrophage
FUNCTIONS	Endogenous Ag presented to CD8 (cytotoxic)	Exogenous Ag presented to CD4 (helpers)

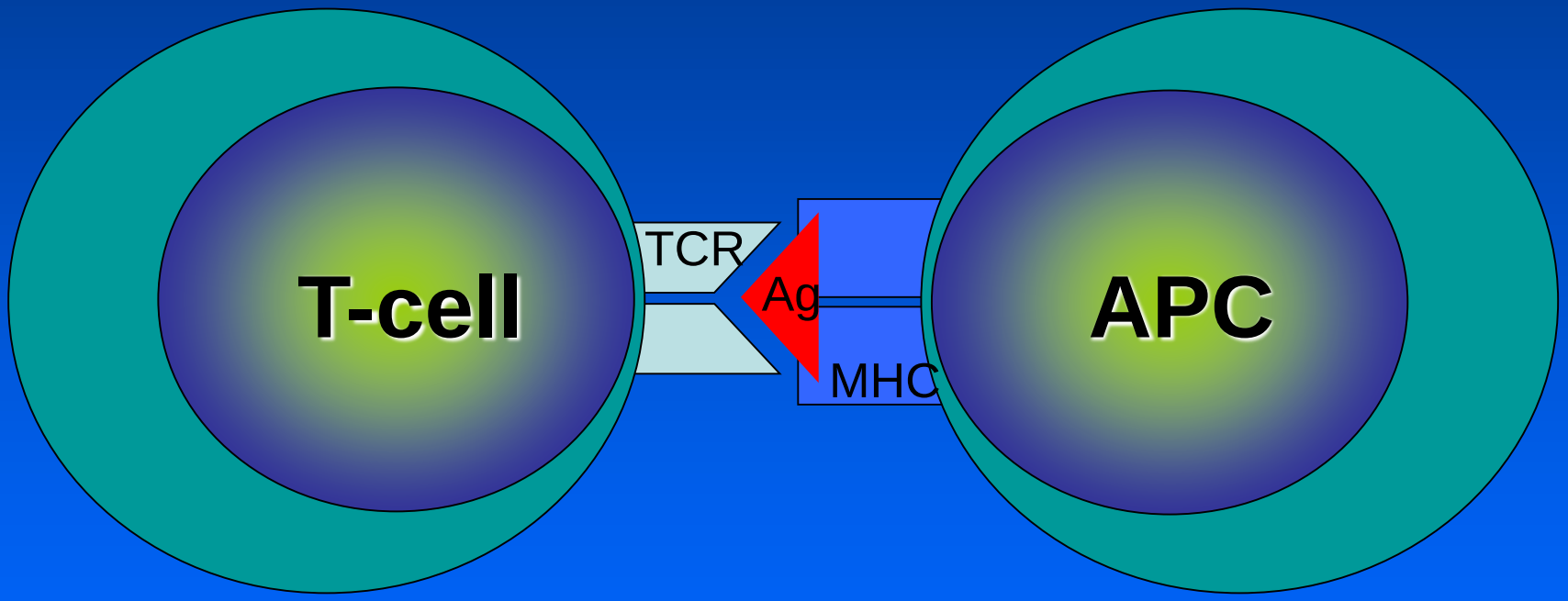
Peptides Fit into MHC I and II Molecules Differently



Class I Class II

Adapted from Janeway & Travers, *Immunobiology*

Function of HLA Gene Products



1. Determination of the repertoire of T cell antigen receptors (TCR) molecule
2. Presentation of peptides to T cells
3. The regulation of NK cell cytotoxic activity
4. Fetal allograft protection

Identification of HLA Antigens / Alleles

- Serological (old) - Tissue - lymphocytes

  CDC – Complement Dependent Cytotoxicity

- Molecular (new) - Tissue - any nucleated cell

  SSP - Sequence specific PCR

  SSOP - Sequence specific probes

  RSCA - Reference Strand Conformation

  SBT - Sequence based typing

Molecular Typing – Level of Resolution

- Low resolution
  - equivalent to serologic typing
  - include many members of broad family
  - used for typing recipient/donor for solid organ transplantation
- Intermediate resolution
  - important for determining ambiguities in solid organ transplantation
  - Important for determining relevance of alloantibody specificities
- High resolution
  - determine each allele at each loci
  - assess recipient/donor compatibility for bone marrow transplantation (BMT)
  - minimize Graft vs Host Disease (GVHD) in BMT

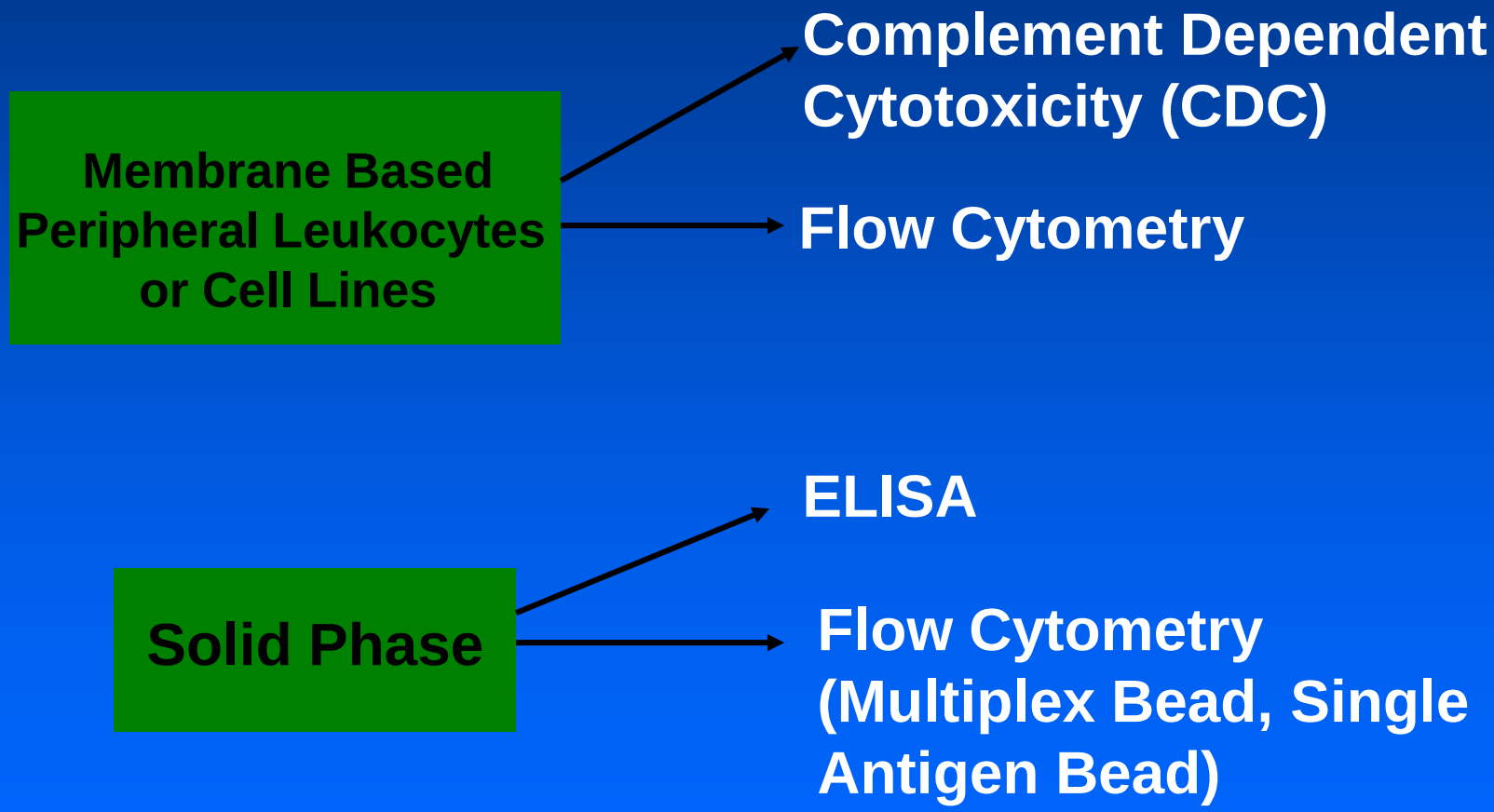
Goals in Antibody Detection

1. **Is HLA antibody present?**
   Sensitivity
2. **Is the antibody clinically relevant?**
   Specificity
   - **HLA vs Non-HLA**
   - **Which HLA – class, antigen, allele**
   - **Antibody Type – IgG subtypes, IgM**

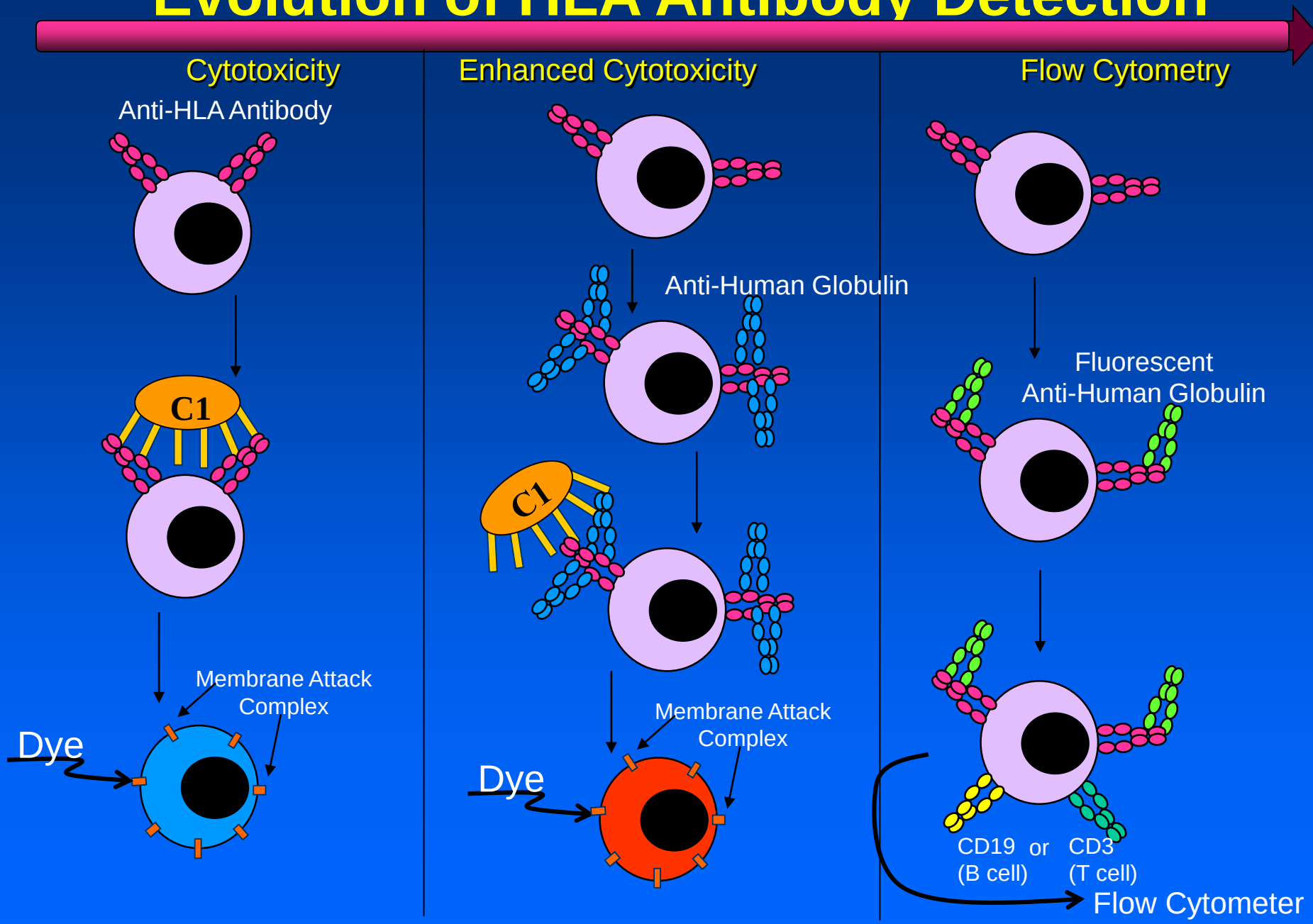
   Quantitative assessment
   - **Titer**

   Biological activity
   - **Complement fixation – CDC, C1q binding, activation**

Antibody Detection Methods



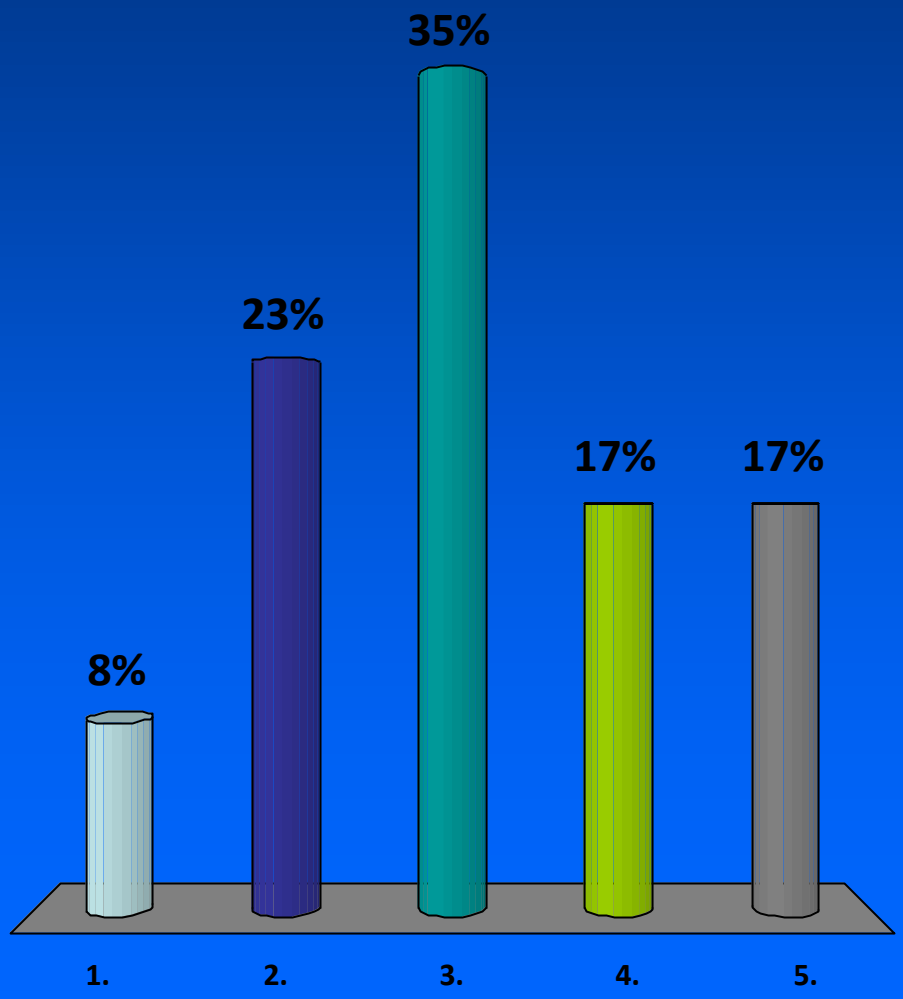
Evolution of HLA Antibody Detection



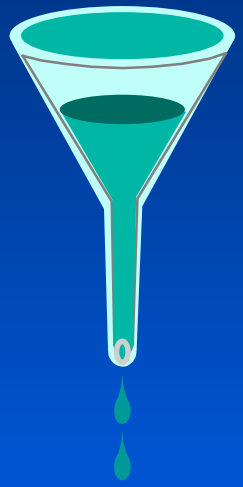
Bray et al Immunol Res. 29:41, 2004

Solid phase single antigen beads or single antigen testing of anti-HLA antibodies:

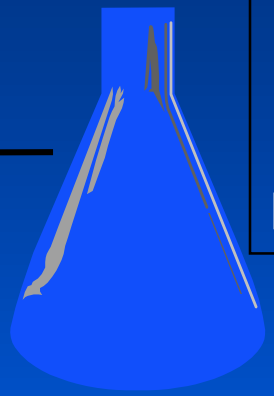
1. Is not quantitative
2. Is not functional
3. Is overly sensitive
4. Has a lot of variation
5. Has a lot of technical variability



Solid Phase, Antigen-Specific Assays



Extract and Purify
HLA Antigens

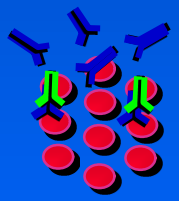


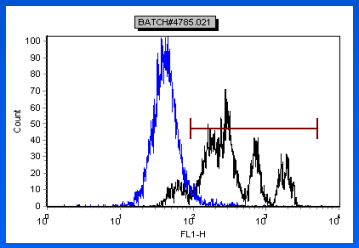
B cells + EBV =
B cell line
or
Peptide Synthesis

1. Class I HLA
2. Class II HLA
3. Individual HLA

Purified HLA

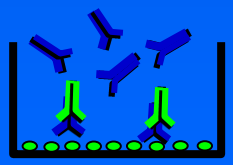
Microparticles





Flow Cytometry

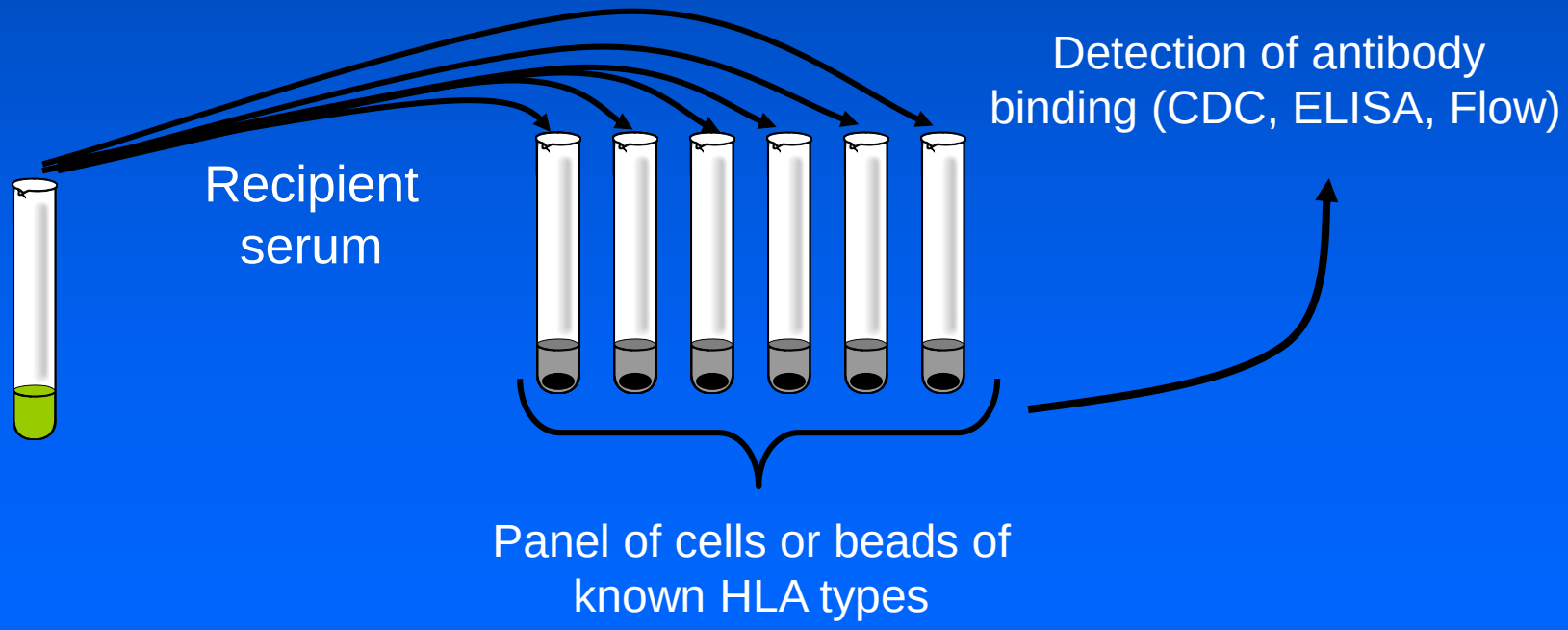
Plate



ELISA

Panel Reactive Antibody

A measure of the presence of multiple anti-HLA antibodies. The proportion of panel members with a positive antibody binding, or % PRA positive. Indication of sensitization, chance of positive cross match, chance of acute humoral rejection, chance of any rejection.

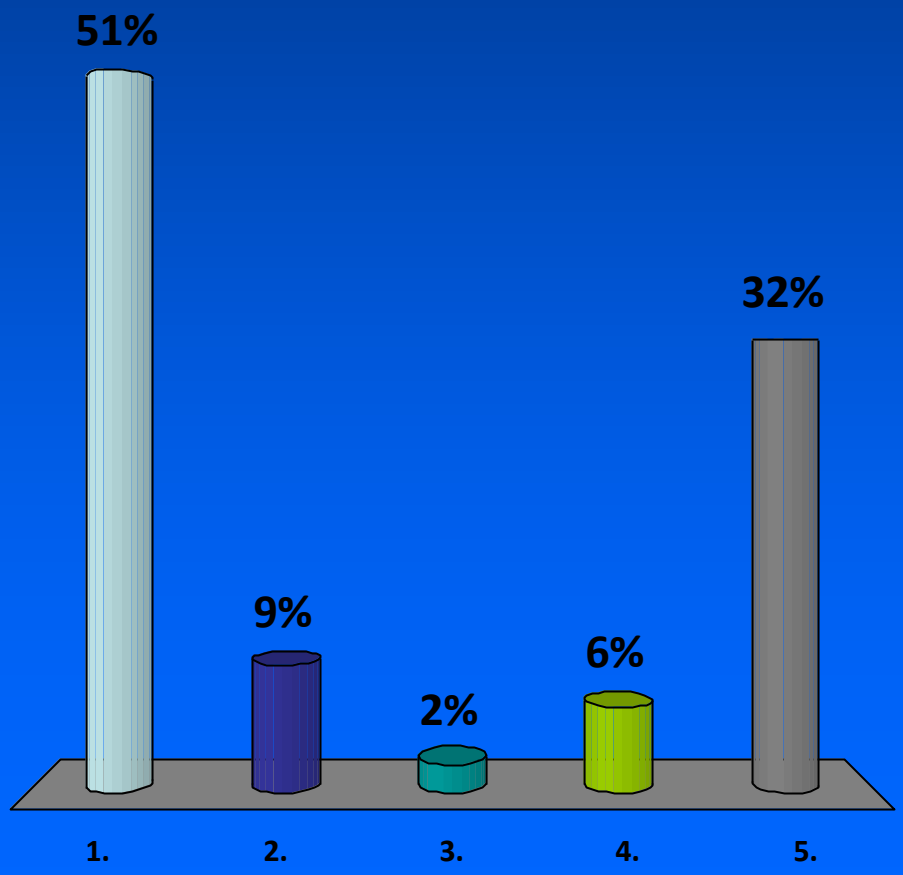


Consequence of HLA typing, antibody identification, and knowledge of population distribution of HLA types:

Virtual PRA
cPRA
Virtual Crossmatch

Single antigen testing is specific for:

1. HLA antibodies
2. Autoantibodies
3. Minor histocompatibility antigens
4. Endothelial cell specific antigens
5. All HLA specificities

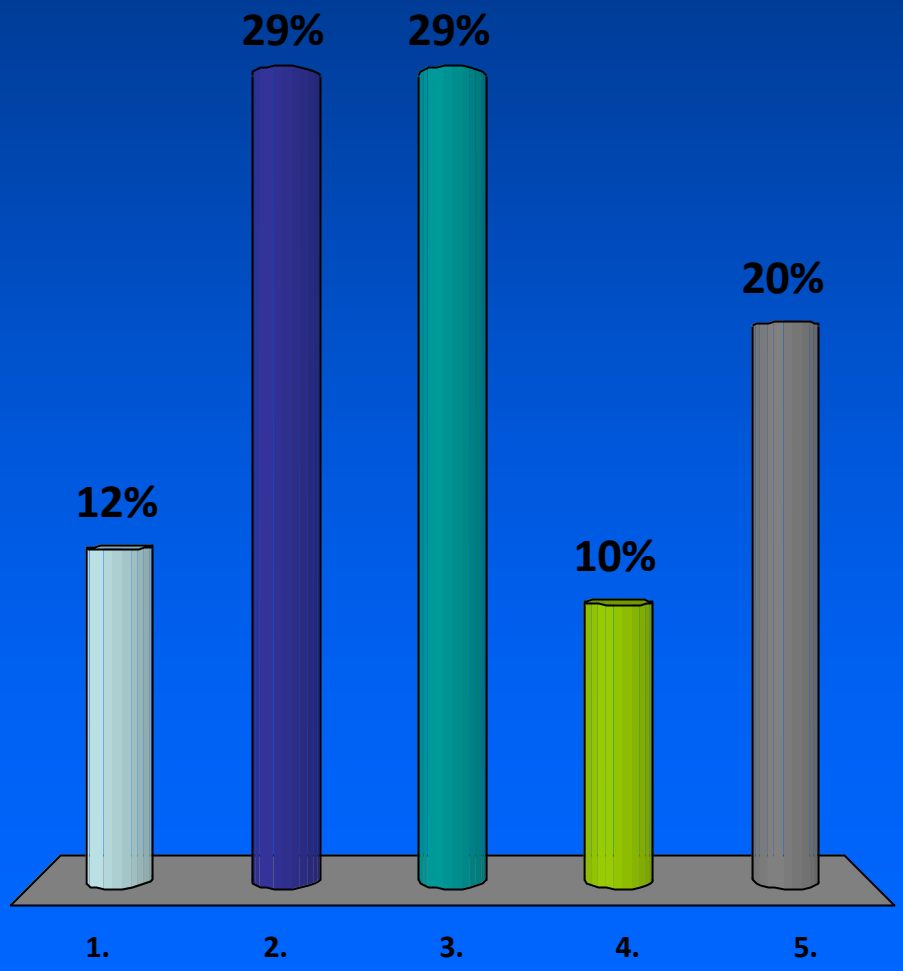


Target Antigens

- MHC molecules
  - HLA class I (A, B, C)
  - HLA class II (DR, DP, DQ)
- Non-classical MHC molecules
  - MHC class I polypeptide-related sequences A (MICA) and B (MICB)
- ABO blood group antigens
- Others:
  - Endothelial cell/monocyte antigens
  - Epithelial cells
  - Angiotensin receptors
  - Vimentin
  - Myosin

Current crossmatch techniques fail to detect:

1. Some HLA antigens
2. Autoantigens
3. Minor histocompatibility antigens
4. T cell alloreactivity
5. NK cell alloreactivity



Specificity

Antigen Non-specific

Complement-dependent cytotoxicity (CDC):

- Direct CDC (Standard)
- Modifications
  - Washes
  - Extended incubation
  - AHG-CDC
  - DTT/DTE
  - Heat

Flow Cytometry

- T cell
- B cell
- C’ fixation

Antigen Specific

ELISA

Flow PRA

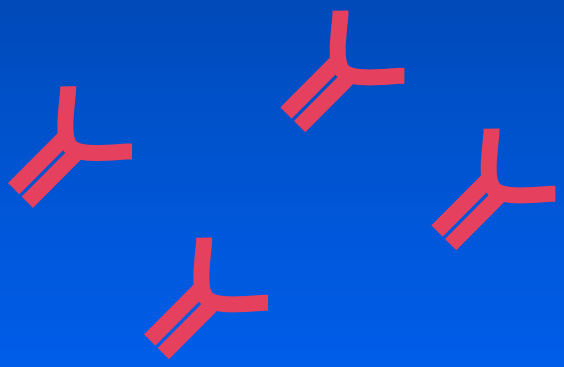
Flow Single Antigen Beads

C1q binding

Kinetics of Humoral Alloreactivity

Pre Tx

Preformed Abs



historic current

Kinetics of Humoral Alloreactivity

Pre Tx

Preformed Abs

Post Tx

De novo Abs

historic

current

de novo

time

Sensitivity of Anti-HLA Antibody Analysis by Different Methods

	Positive	Negative
CDC	102	162
AHG-CDC	116 (+13%)	148
ELISA	127 (+10%)	137
FLOW-PRA	139 (+10%)	125

Gebel and Bray. Transplantation 2000;69:1370

Limitations to Tests

- Not quantitative
- Not functional (? C1q binding)
- Overly sensitive
- Batch-to-batch variation
- Machine and technical variation are high
- False positives and false negatives
- Completely miss non-HLA antigens

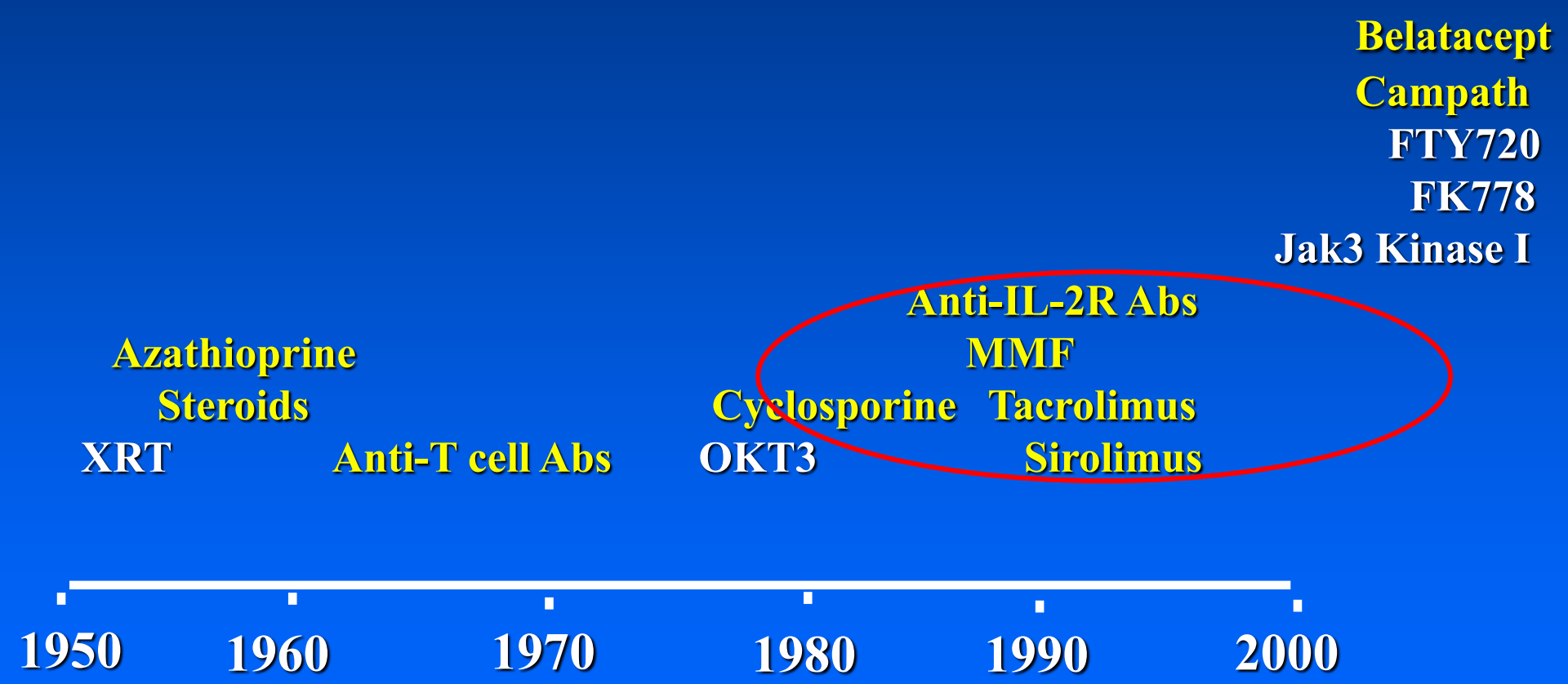
Areas of Uncertainty

- Sensitivity
- Specificity
- Function
- Pathogenicity

Bottom Line

- Negative CDC XM is good
- Positive CDC XM is very bad
- Positive flow XM with high titre DSA is probably very bad
- Positive flow XM with medium titre DSA may be bad, or not. Low titre DSA? C1q?
- Negative flow XM with DSA may be ok, or not. C1q?
- Some positive tests plus some negative tests??

Immunosuppression



Immunosuppression

Maintenance

- Steroids
- Tacrolimus
- Mycophenolate mofetil
- Rapamycin
- Azathioprine
- Cyclosporine
- Belatacept

Induction

- Basiliximab
- Daclizumab
- Thymoglobulin
- Campath
- Atgam
- OKT3
- Belatacept

Categories of Agents

- **Induction agents**
  - **Monoclonal or polyclonal antibodies**
  - **Administered intravenously immediately following surgery**
- **Primary immunosuppressants**
  - **CNIs form the cornerstone of immunosuppressive therapy**
- **Adjuvant agents**
  - **One or more medications prescribed in combination with the CNI**

Individualizing Immunosuppression Based on Immunologic Risk

PRE-TRANSPLANT
IMMUNOMODULATION

INDUCTION
ANTIBODY THERAPY

TRIPLE THERAPY
MAINTENANCE

MINIMIZATION
PROTOCOLS

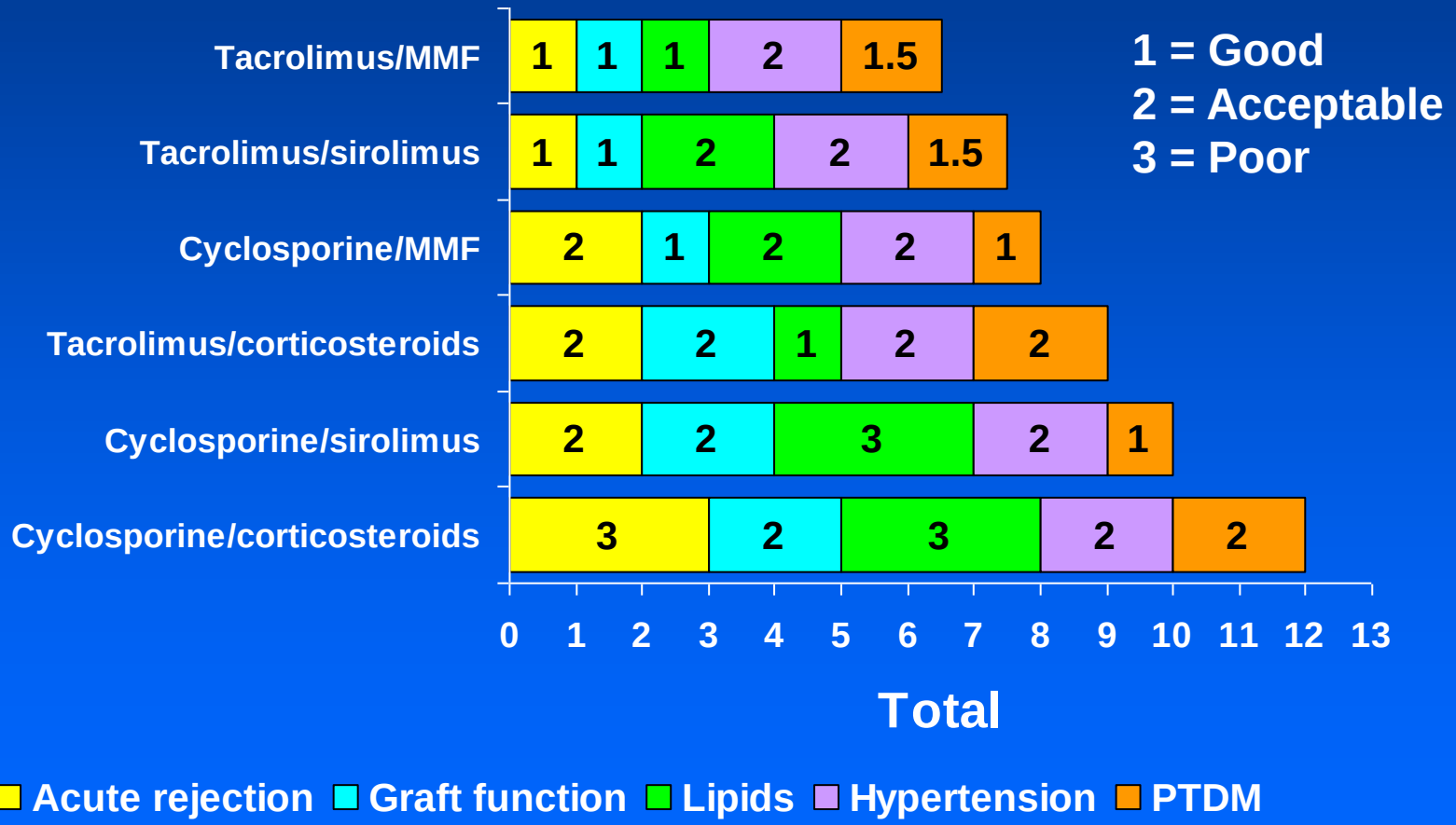
HIGH RISK

- HIGHLY SENSITIZED
- NON-PRIMARY TRANSPLANT
- AFRICAN AMERICAN/HISPANIC ETHNICITY
- CADAVERIC DONOR SOURCE
- POOR HLA MATCH

LOW RISK

- NONSENSITIZED
- ASIAN/CAUCASIAN ETHNICITY
- THE ELDERLY
- LIVING DONOR SOURCE
- GOOD HLA MATCH

Efficacy and Side Effect Profiles of Common Drug Regimens



Chan L, et al. *Am J Kidney Dis.* 2001;38(suppl 6):S2-S9.

Drug Monitoring

Goal: Maximize therapeutic index – immunosuppression vs. toxicity

Pharmacokinetic measurements: MPA, tacrolimus, rapamycin, cyclosporine – trough levels vs. AUC vs. 2-hr

[Prograf vs. generic tacrolimus; Rapamune vs. Zortress]

Pharmacodynamic measurements: Antibodies – flow cytometric cell counts – WBC, lymphocytes, platelets, CD3; alloantibody titres; graft response and outcome

Steroid Minimization, Withdrawal, and Avoidance

- Increased risk of acute rejection and CAN or IF/TA
  - Appropriate for patients with low risk of rejection
    - Living, 1-HLA+ donor
    - First transplant
    - Adult
    - Not of African-American ethnicity
    - No history of rejection
- Popular with patients because of steroid side effects

CNI Minimization, Withdrawal, and Avoidance

- CNI minimization, taper, withdrawal – prevent nephrotoxicity and/or prolong renal function
- CNI replaced with rapamycin
- Chronic Belatacept or other mAbs may be an alternative approach
- Good evidence that Pred/MMF/Rapa gives acceptable results, but Pred/MMF does not
- Recent evidence that CNI nephrotoxicity may be less common than we previously thought

Novel Combinations

- **Induction: Thymoglobulin + belatacept + steroids**
- **Maintenance: Beltacept + [MMF → rapamycin]**

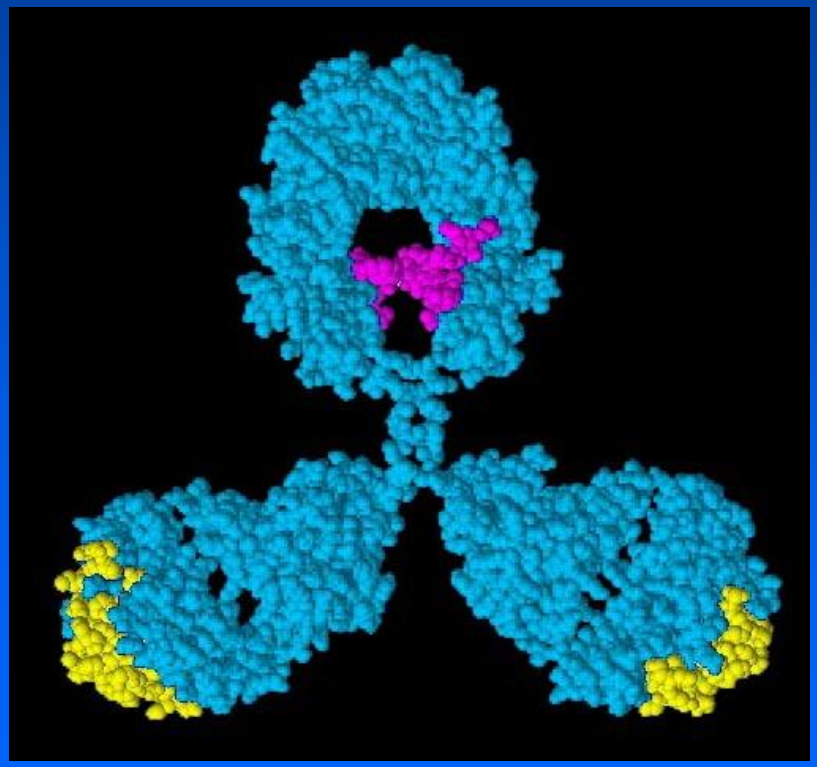
Causes of Allosensitization

- Traditional sensitizing events
  - Transfusion of blood products
  - Pregnancy
  - Prior transplantation
  - Severe infection
  - Autoimmunity
- Sensitizing events of particular importance in pediatric cardiac transplantation
  - Homograft exposure during repair of congenital heart disease

Investigational Immunosuppression

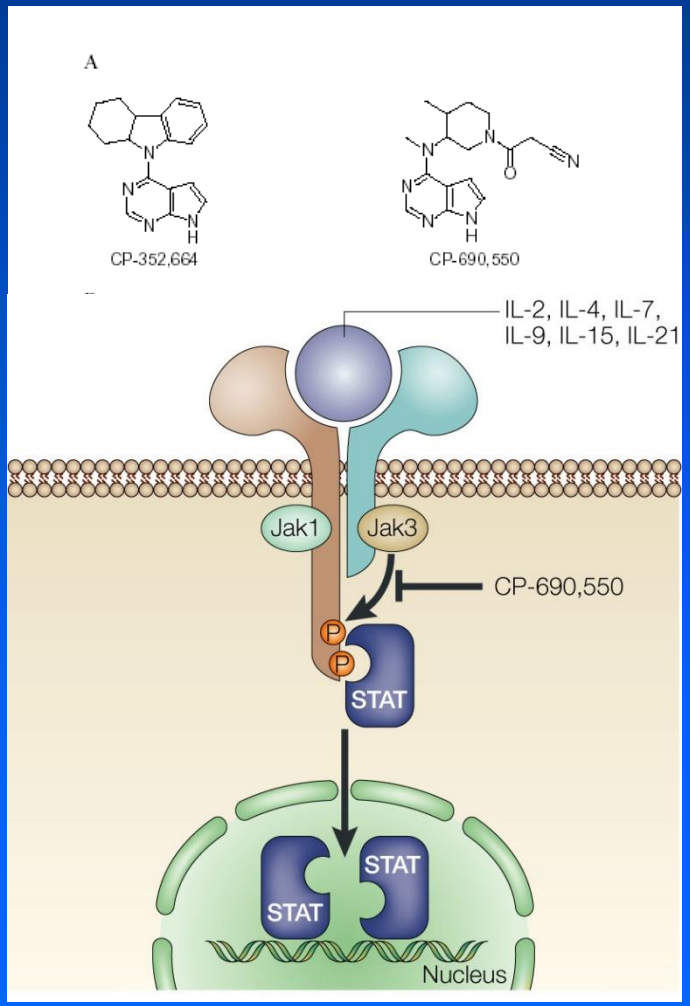
- CTLA4Ig, LEA29Y (Belatacept)
- Campath-1 (Anti-CD52)
- FTY720 (S1PR agonist/antagonist) (Fingolimod)
- FK778 (leflunamide prodrug)
- Jak3 Kinase Inhibitor (CP-690,550)
- Anti-CD3 immunotoxin; non-activating anti-CD3
- Alefacept (Amevive) – LFA3-Ig (anti-CD2)
- Anti-LFA-1 (Efalizumab, Raptiva)
- AEB071 (PKC inhibitor)
- Anti-CD40

Alemtuzumab (Campath-1H)



- Humanized CD52-specific IgG1
- Rapidly and specifically depletes T-cells, B-cells, and some monocytes.
- Indicated for lymphoid malignancies

Inhibition of Lymphocyte Proliferation: JAK 3 Kinase Inhibitors



- Regulates IL-2 receptor signaling via the gamma chain (γc)—which includes signaling by IL-2, 4, 7, 9, 15, and 21
- Defects in γc or in JAK3 kinase result in abnormal cytokine signaling.
- Is expressed on both lymphoid and myeloid lineages with high levels in NKT cells and thymocytes, and is inducible on activated B and T cells but not resting cells.
- Pfizer drug abandoned for transplant. Many more under development in oncology

Types of Allograft Rejection

- **Hyperacute** **Avoidable**
  Antibody- and Complement-mediated
- **Acute** **Treatable, mostly**
  T cell mediated (macrophages): TCMR
  Antibody mediated: AMR
- **Chronic** **Untreatable?, Not fully understood**
  T cell-driven anti-donor antibody
  Current vogue is this is AMR

Methods to Decrease or Downregulate Antibodies (anti-HLA or anti-A/B) or their function

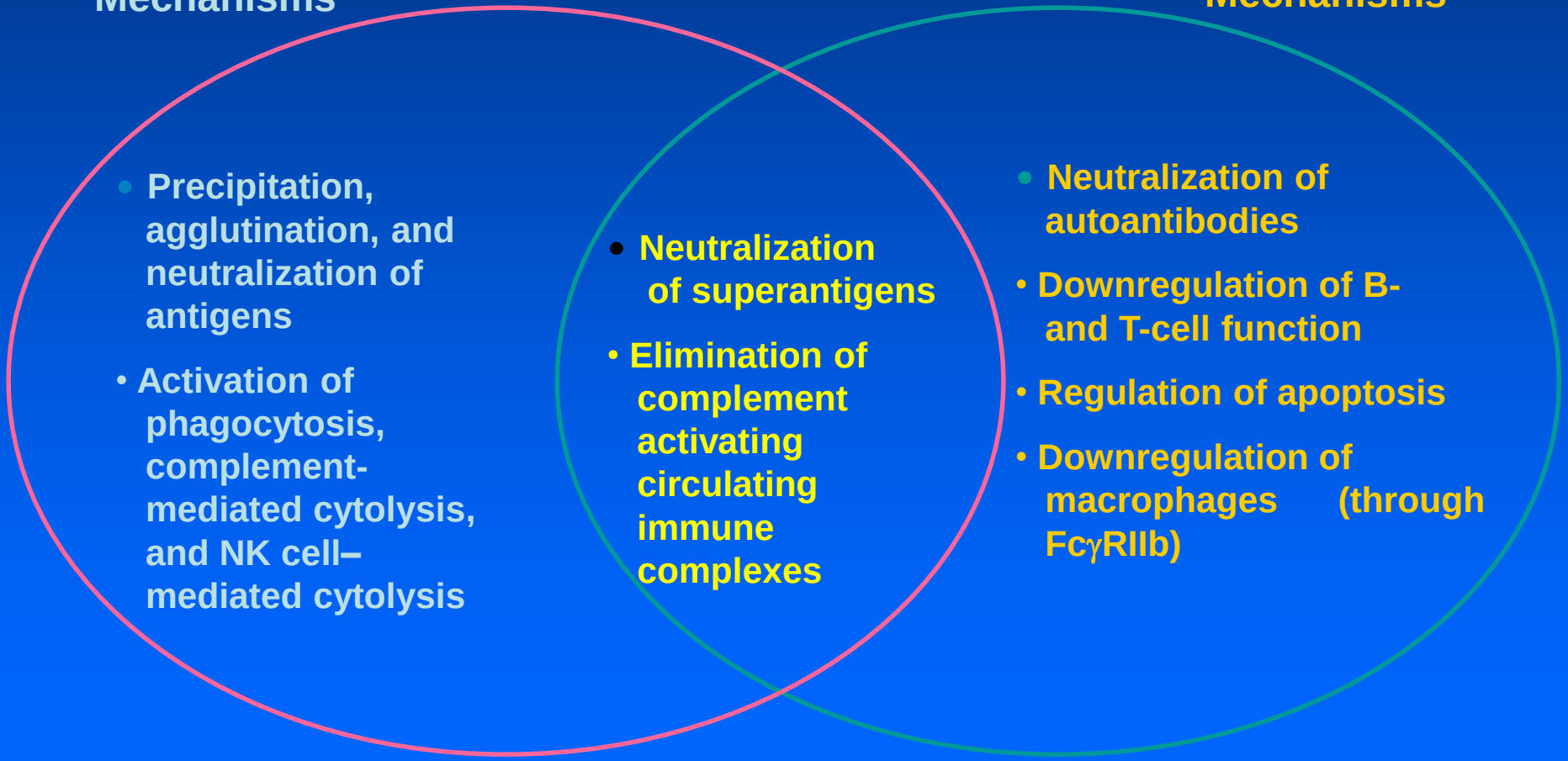
- Splenectomy
- Plasmapheresis
- Rituximab (anti-CD20 mAb)
- Intravenous Immunoglobulin (IVIG)
- Bortezomib (proteasome inhibitor)
- Eculizumab (anti-C5)

Properties of Intravenous Immunoglobulin (IVIG)

- IVIG has immunomodulatory properties and has been used in the treatment of a variety of autoimmune and systemic inflammatory conditions
- IVIG is prepared from pooled plasma from 3,000 to 10,000 healthy blood donors
- IVIG contains contains entire spectrum of antibodies found in normal human serum (HLA class I and II, T-cell receptor idiotypes, CD4, CD5, CD40, and cytokines)
- >90% IgG and traces of IgM, IgA, $F(ab)_2$ fragments
- Half-life is 3 weeks

Mechanisms of Action of IVIG

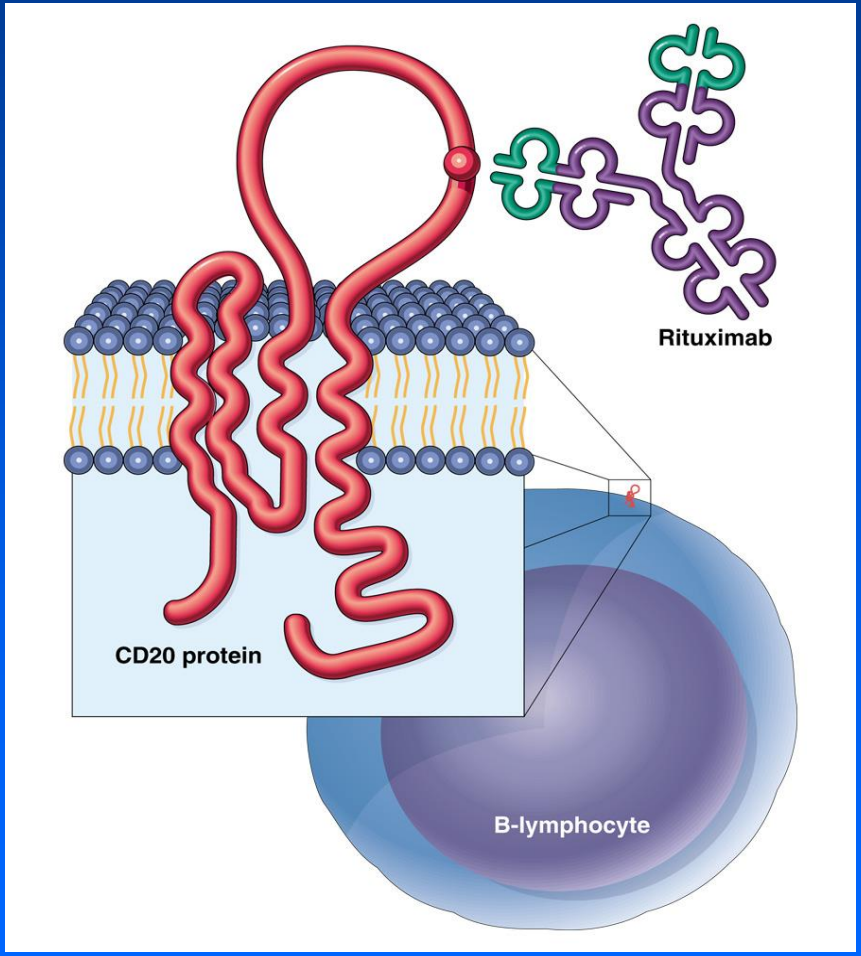
Mechanisms of action may overlap



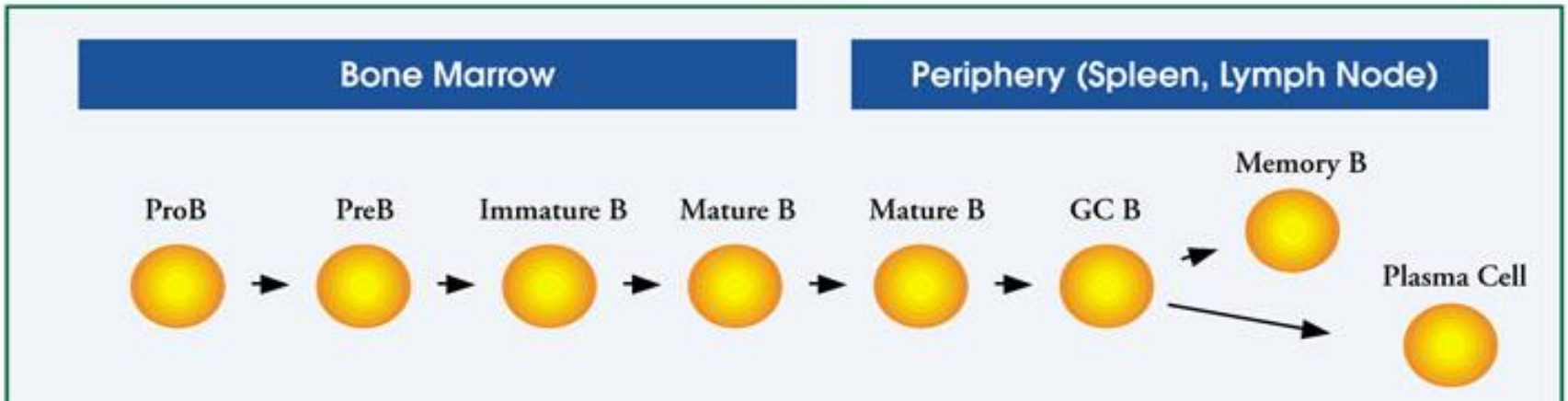
Kazatchkine MD, Kaveri S. N Engl J Med. 2001;345:747-755.

Rituximab: B Cell Depletion

- Genetically engineered chimeric murine/human monoclonal antibody
- Variable light- and heavy-chain regions from murine anti-CD20 antibody (IDEC-2B8)
- Human IgGκ constant regions
- First monoclonal antibody to be approved by the FDA for treatment of cancer



Antigen Expression During B Cell Development



CD19	+	+	+	+	+	+	+	-
CD10	+	+	+/-	-	-	+	-?	-
CD20	-	-	-/+	+	+	++	+	+/-
CD38	++	++	+	+	+	++	+?	++
mIg	-	-	-/+	+	+	+/-	+	-
IgH	G	R	R	R	R	R/M	R/M	R/M
IgL	G	G	R	R	R	R/M	R/M	R/M

Bortezomib (Velcade)

- Proteosome inhibitor
- Specific for mitotic cells (not just B cells)
- Chemotherapy
- Neurotoxicity common
- Uncontrolled evidence for B cell desensitization effect

New Additions to B Cell Armamentarium

- Epratuzumab (anti-CD22)
- Many new anti-B cell mAbs under development
- Atacicept (APRIL, BAFF)
- Belimumab (BAFF (BLyS))
- Oprozomib, carfilzomib (proteosome inhibitors)
- Many new preteosome inhibitors under development

Contact information

Your feedback is most welcome!

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