Transplant Immunology

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

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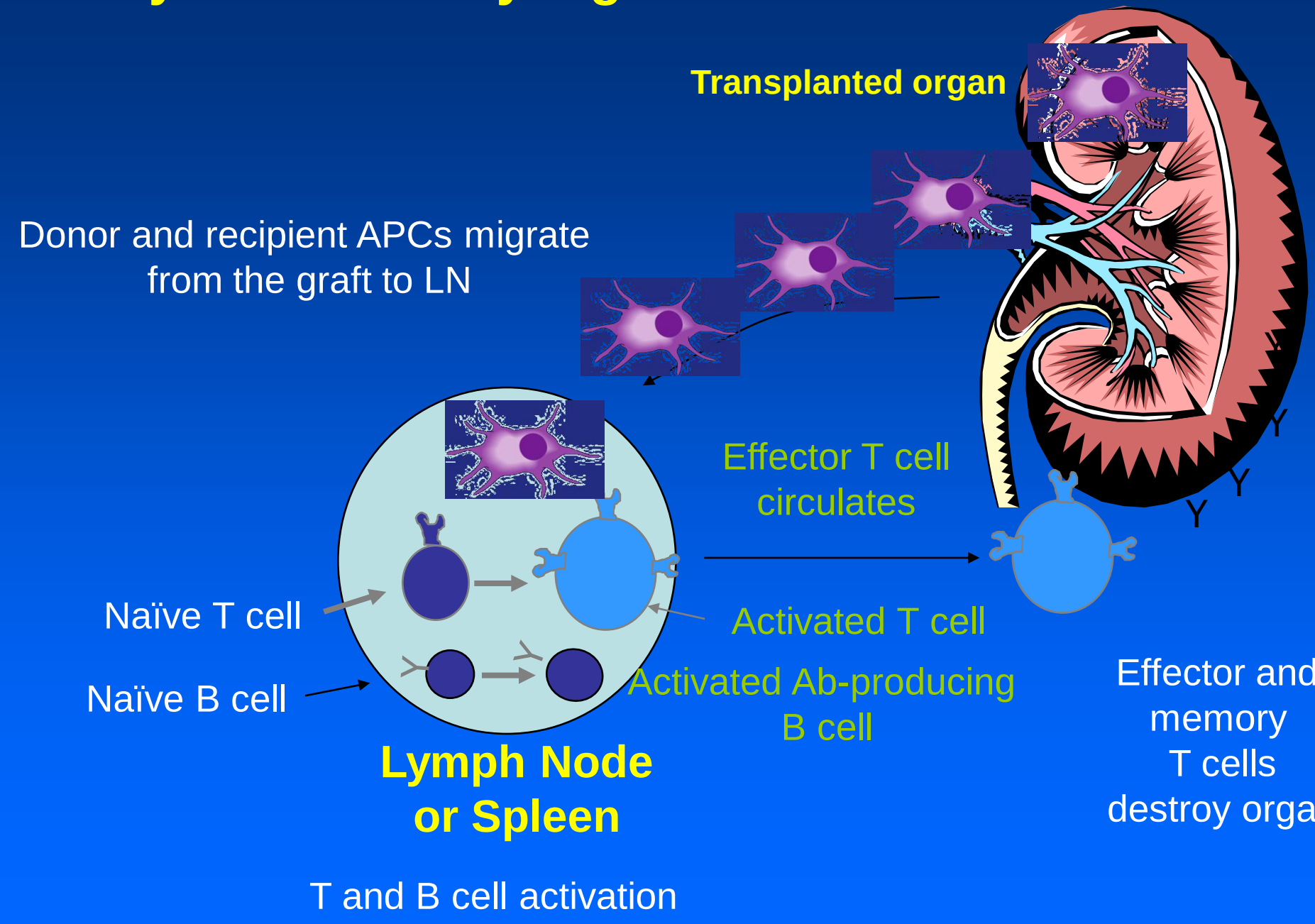
Allorecognition

Rejection

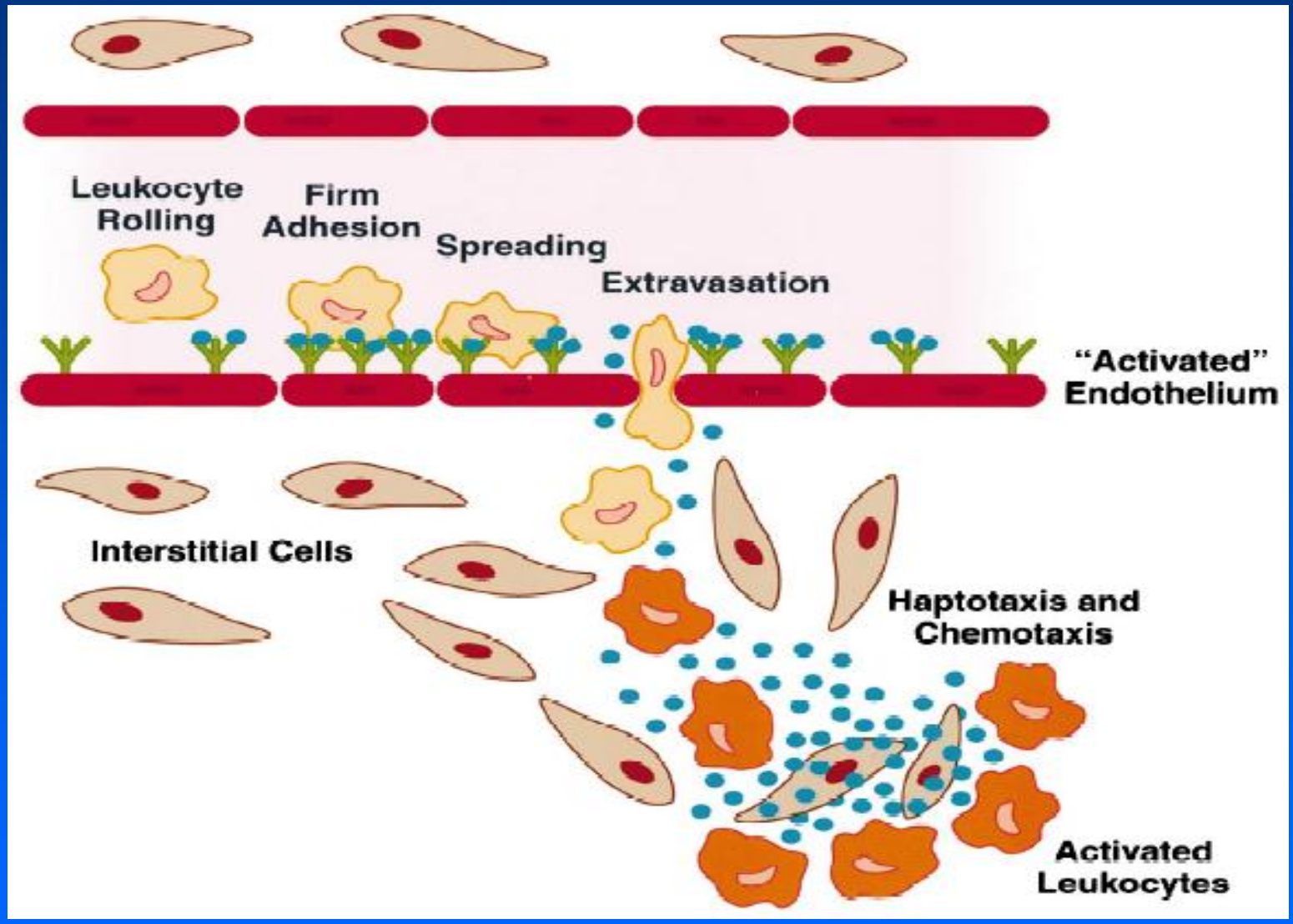
Allorecognition

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

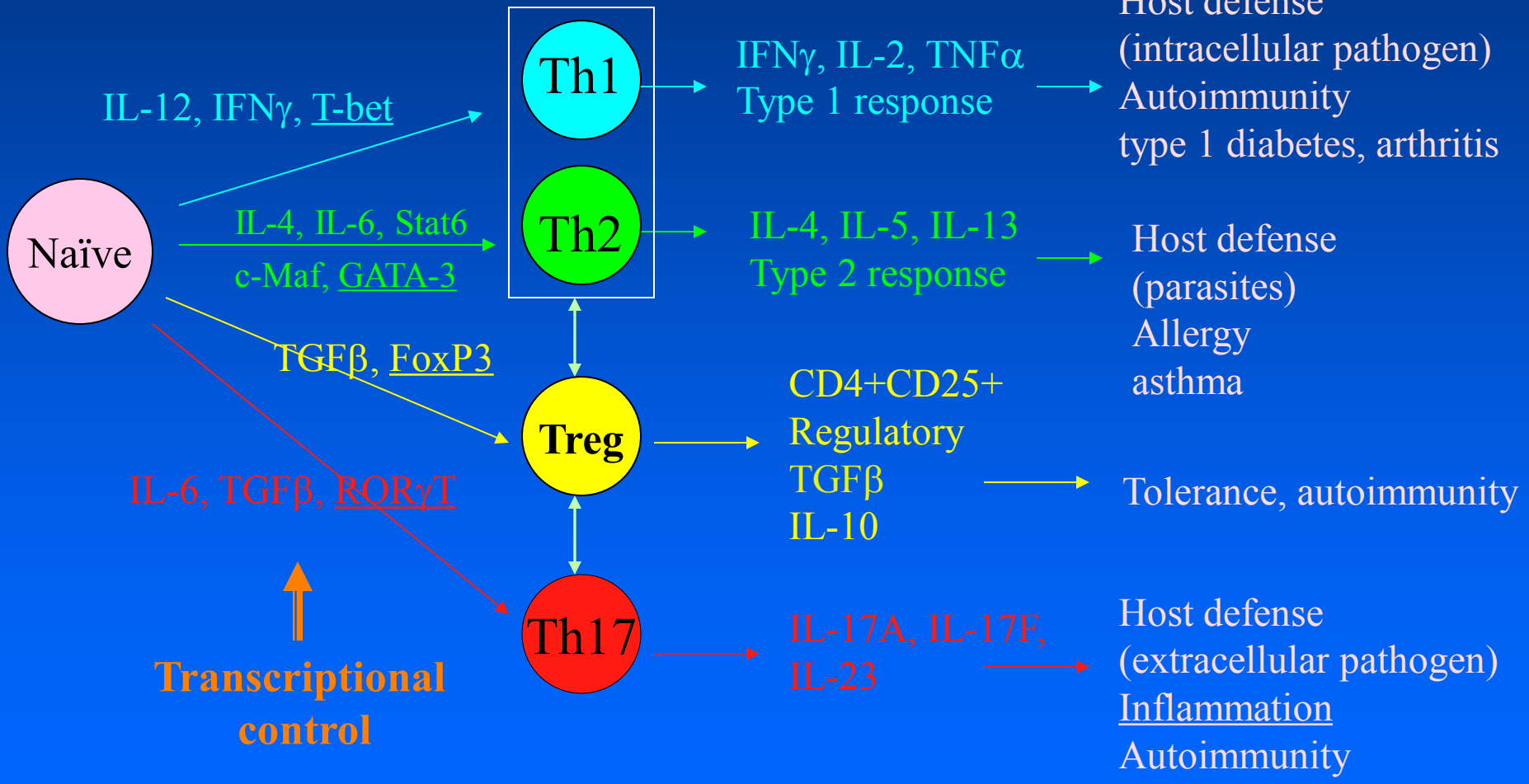
Early Inflammatory Signals



Entry into tissues, organs, lymph nodes

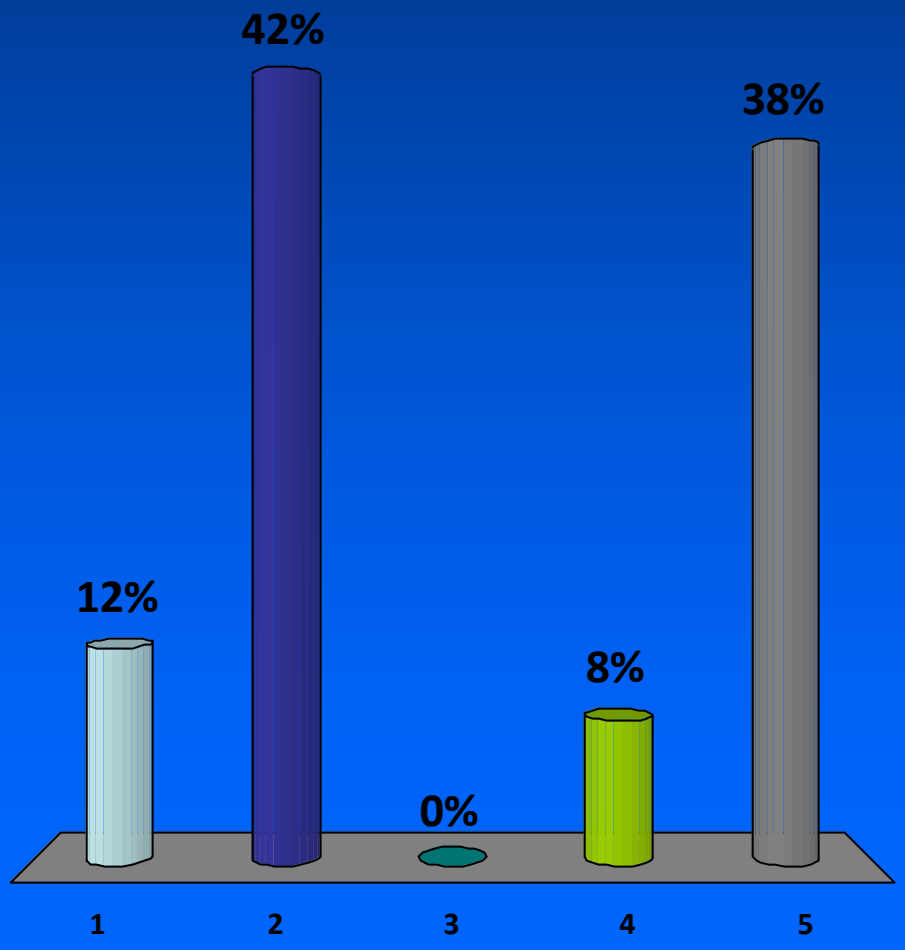


CD4 T Cell Development

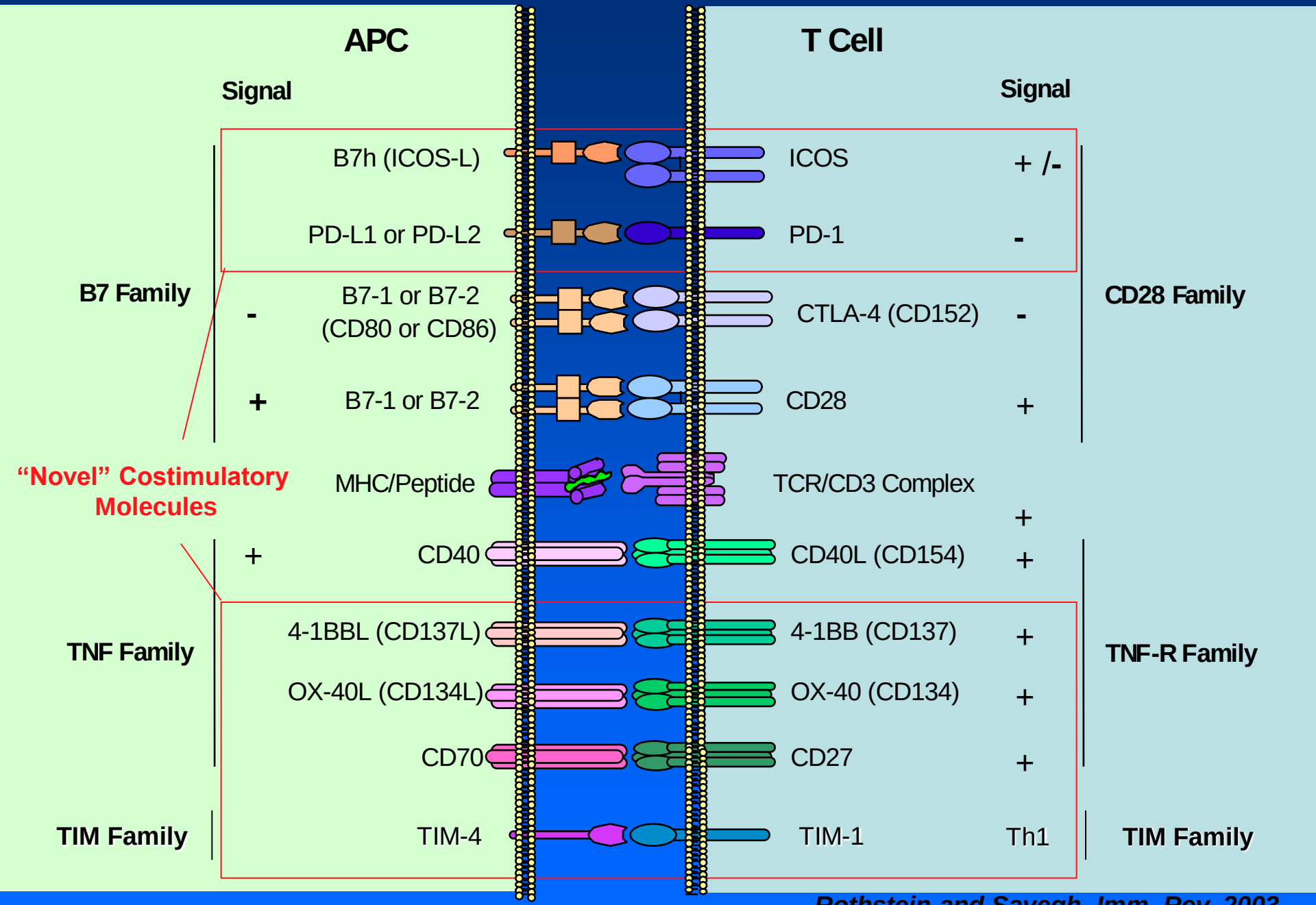


Costimulatory blockade is not tolerogenic because:

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



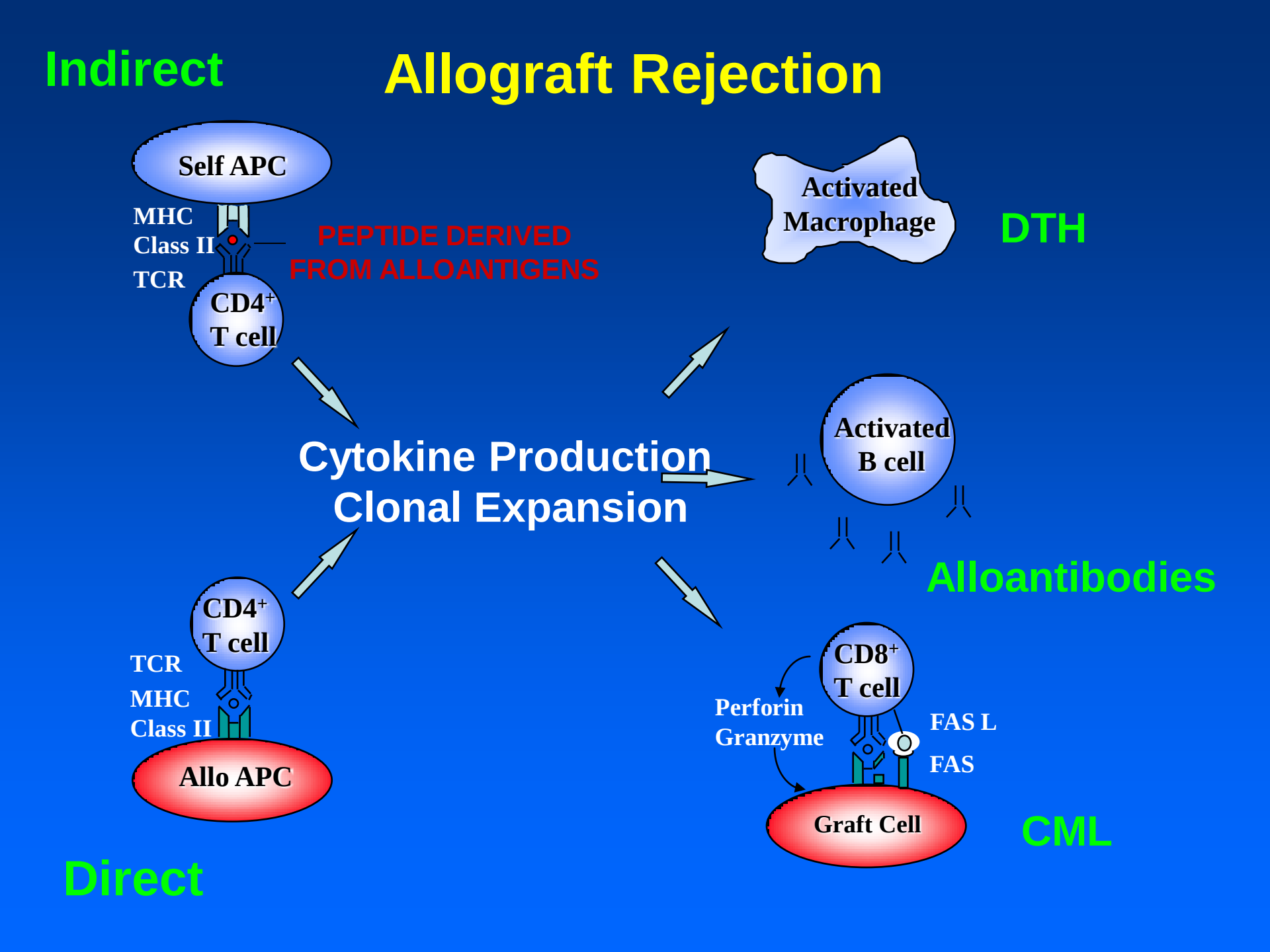
Costimulatory Molecules



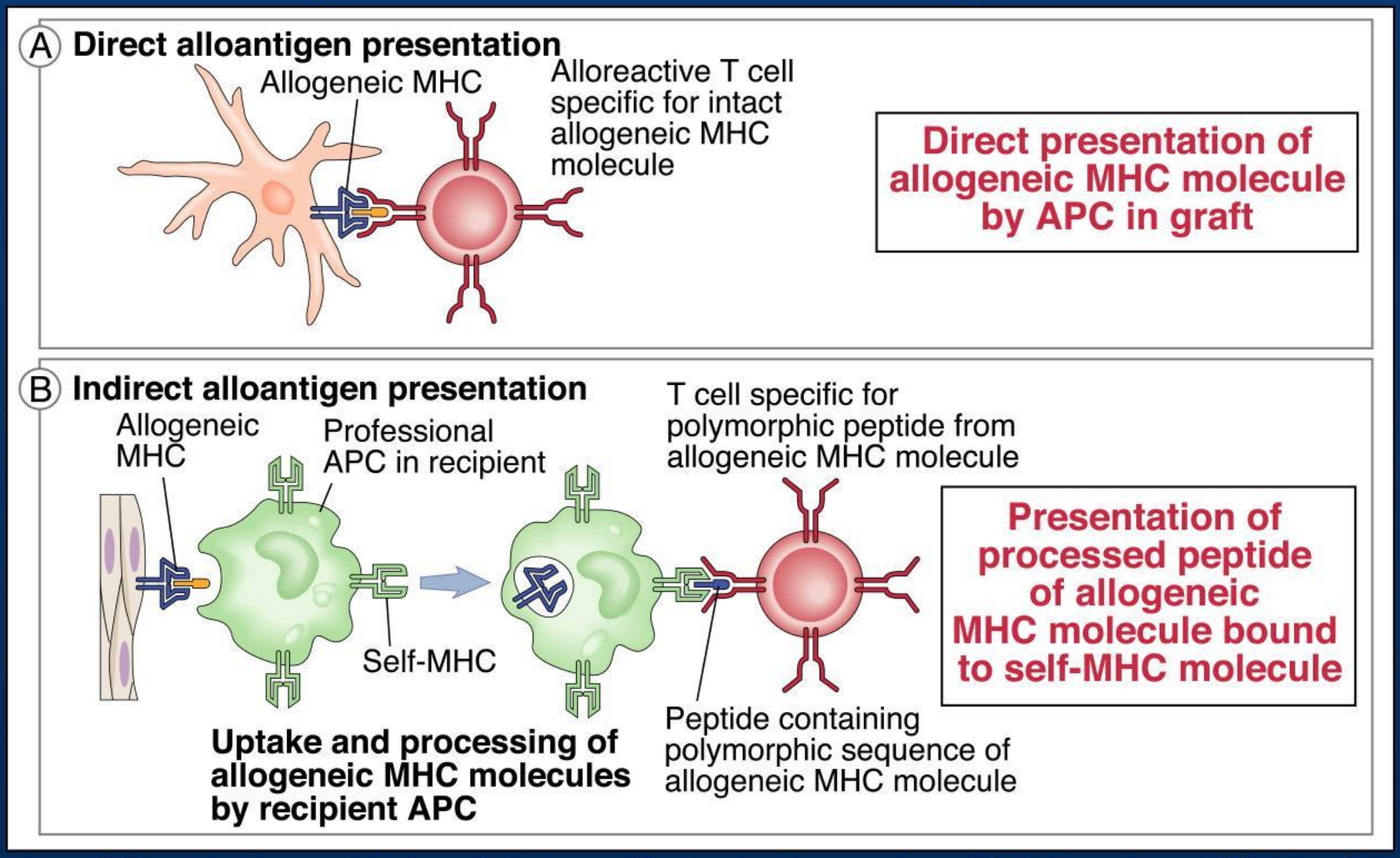
Rothstein and Sayegh, Imm. Rev. 2003

Direct and Indirect Alloantigen Presentation

Allograft Rejection

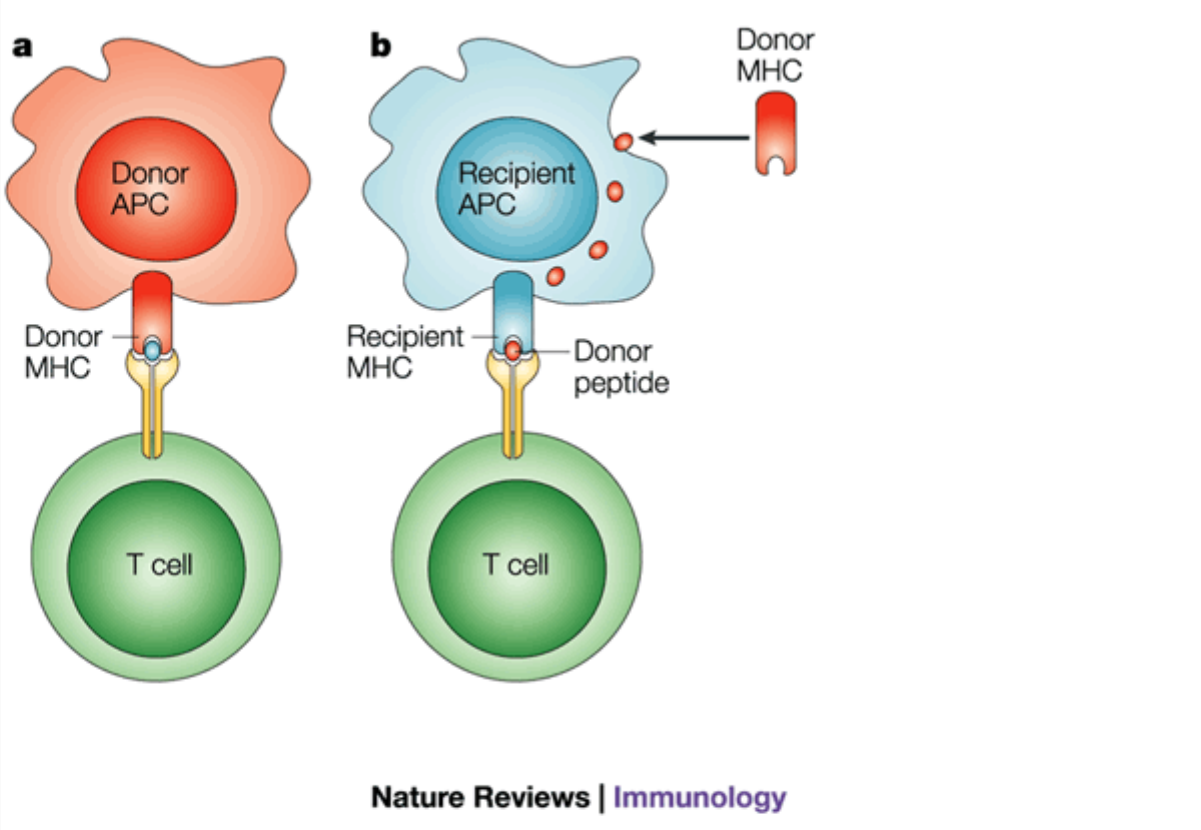


Direct and indirect presentation of alloantigens



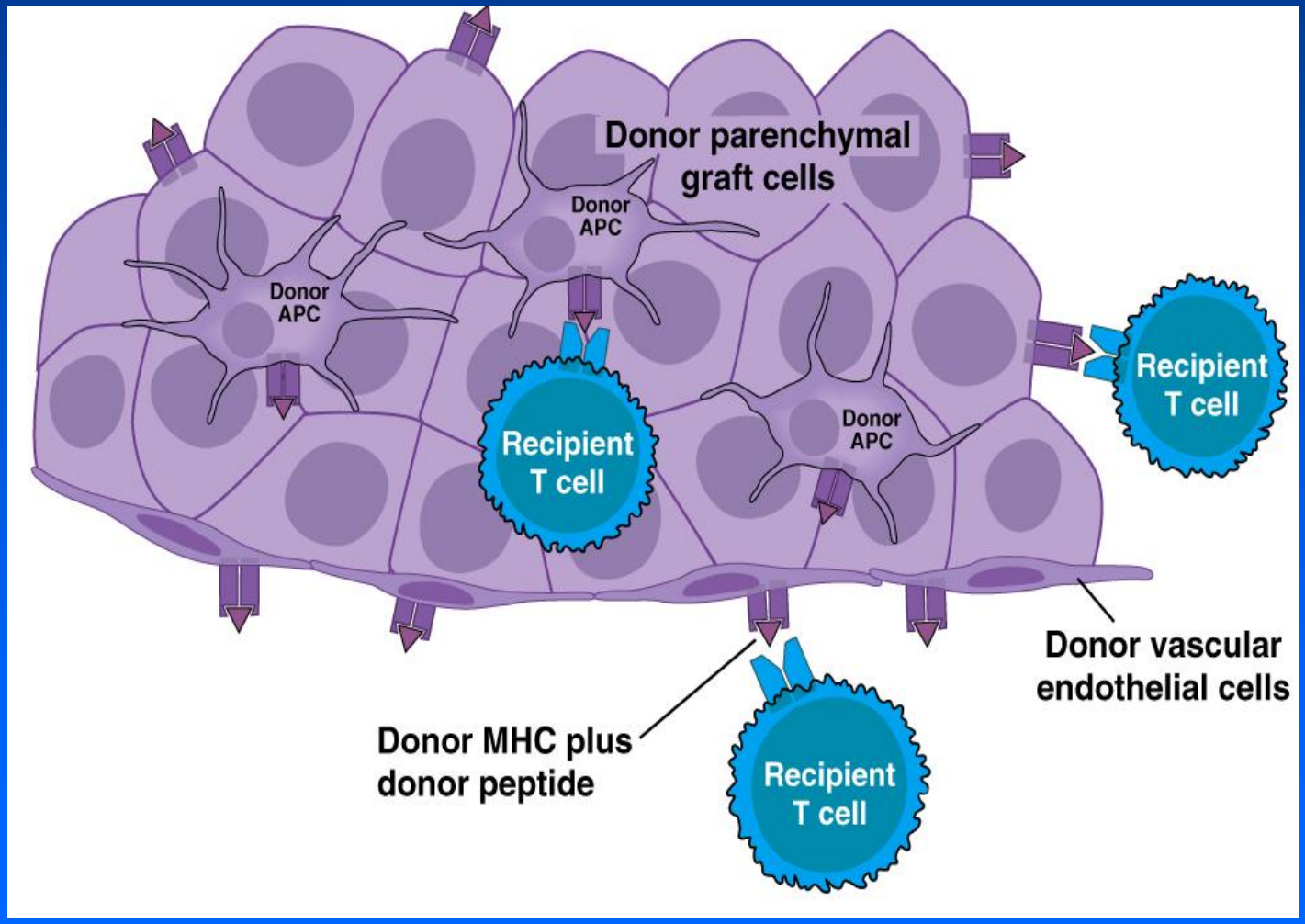
From Abbas, Lichtman, & Pober: Cellular and Molecular Immunology. W.B. Saunders, 1999, Fig. 16-3

Direct and Indirect Presentation

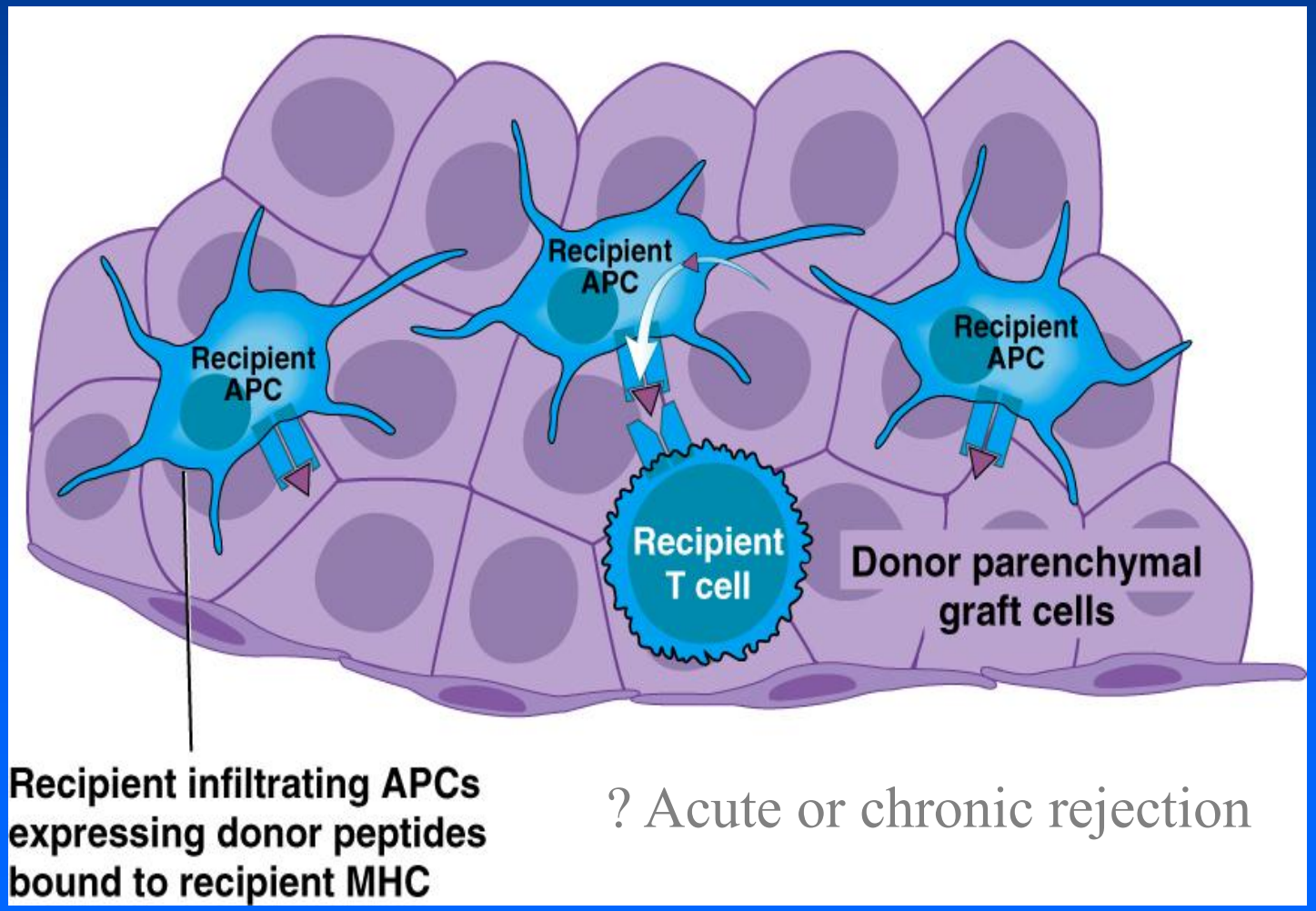


T cells recognize alloantigens by two distinct pathways, known as the direct and indirect pathways[103]. **a** | During direct allorecognition, which is unique to transplantation, T cells recognize intact allogeneic MHC molecules (together with bound endogenous peptide) on the surface of donor antigen-presenting cells (APCs) in the graft. **b** | During indirect allorecognition, which is analogous to the T-cell response to protein antigens, alloantigens are recognized as linear peptides in the context of recipient MHC class II molecules after they have been processed and presented by recipient APCs. Direct recognition by T cells of donor alloantigens on donor dendritic cells leads to full T-cell activation and graft rejection[104]. By contrast, direct allorecognition by T cells of intact MHC molecules expressed on the surface of parenchymal graft cells that lack co-stimulatory activity might render the T cells refractory to further stimulation — in other words, induce a state of T-cell anergy[105].

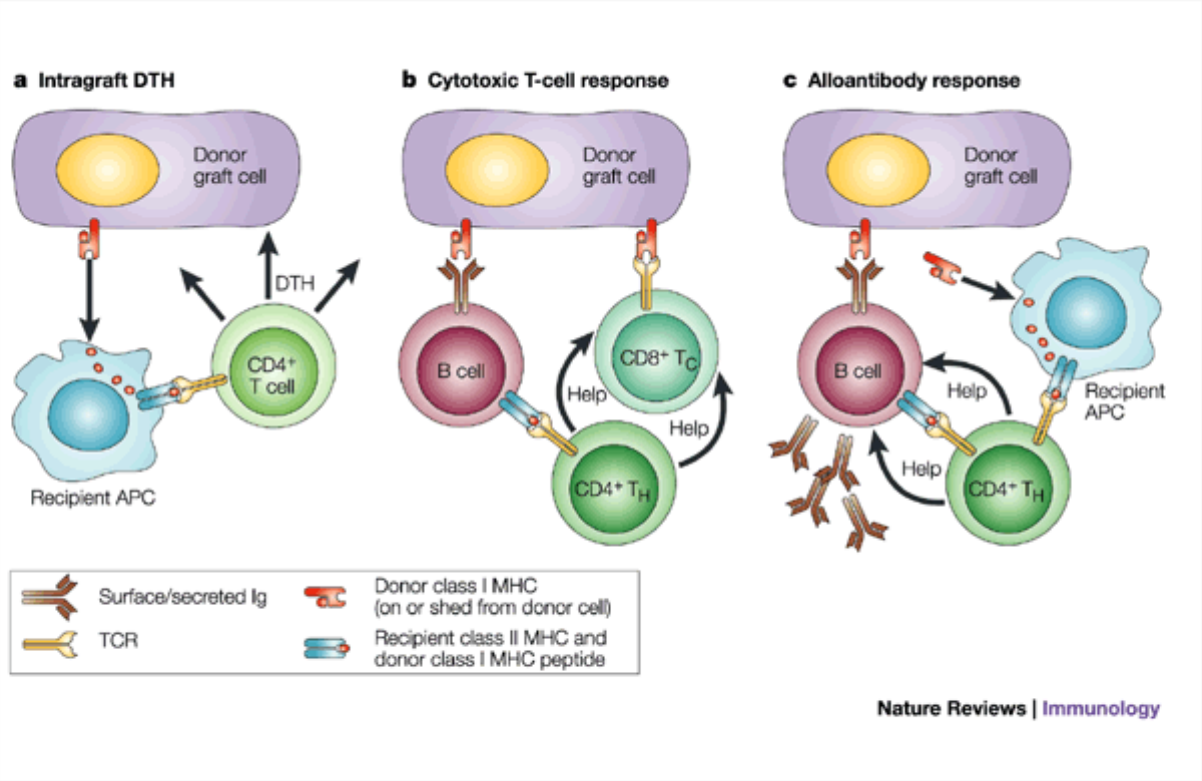
T cells responding through the direct pathway may account for acute cellular rejection



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



? Acute or chronic rejection



$CD4^+$ T cells with indirect specificity for donor HLA molecules would not generally recognize cell-surface target molecules expressed by donor cells in a graft (unless donor and recipient share the same HLA class II molecules). Potential cellular pathways by which such cells might recruit each of the three main effector mechanisms of allograft rejection are shown[106, 107, 108]. **a** | Delayed-type hypersensitivity (DTH). Recipient-HLA-class-II-restricted $CD4^+$ effector T cells might recognize shed alloantigens that have been processed and presented by recipient antigen-presenting cells (APCs; dendritic cells or macrophages) in the interstitium of the graft. Activated $CD4^+$ T cells might then mediate graft damage by the release of pro-inflammatory cytokines and/or the recruitment and activation of macrophages and other non-specific effector cells. **b** | Cytotoxic T cells. Self-restricted alloreactive $CD4^+$ T cells, although unable to recognize intact target antigen on target cells, might provide help for the generation of cytotoxic $CD8^+$ effector cells with specificity for intact HLA class I target molecules on donor cells in the graft. Effective cooperation between cytotoxic (T_C) and helper (T_H) T cells that have different antigen specificities is a well-described phenomenon *in vitro*, and if, as seems probable, this occurs *in vivo*, it would provide a mechanism for interaction between and amplification of the direct and indirect pathways of allorecognition. **c** | Alloantibody. $CD4^+$ T cells primed by indirect allorecognition could provide contact-dependent help for B cells to produce alloantibody by a classical cognate T-cell–B-cell interaction. Alloantibody might mediate graft damage by various mechanisms, including complement-dependent target-cell injury. hES, human embryonic stem cell; Ig, immunoglobulin.

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"

- In either case, the high-affinity T cell requires the right environment to proliferate: co-stimulation
  - danger (LPS: TLRs) + cytokines + ischemia/reperfusion
  - Co-stimulatory molecules: CD28/B7 (CD80, CD86); CD154/CD40

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

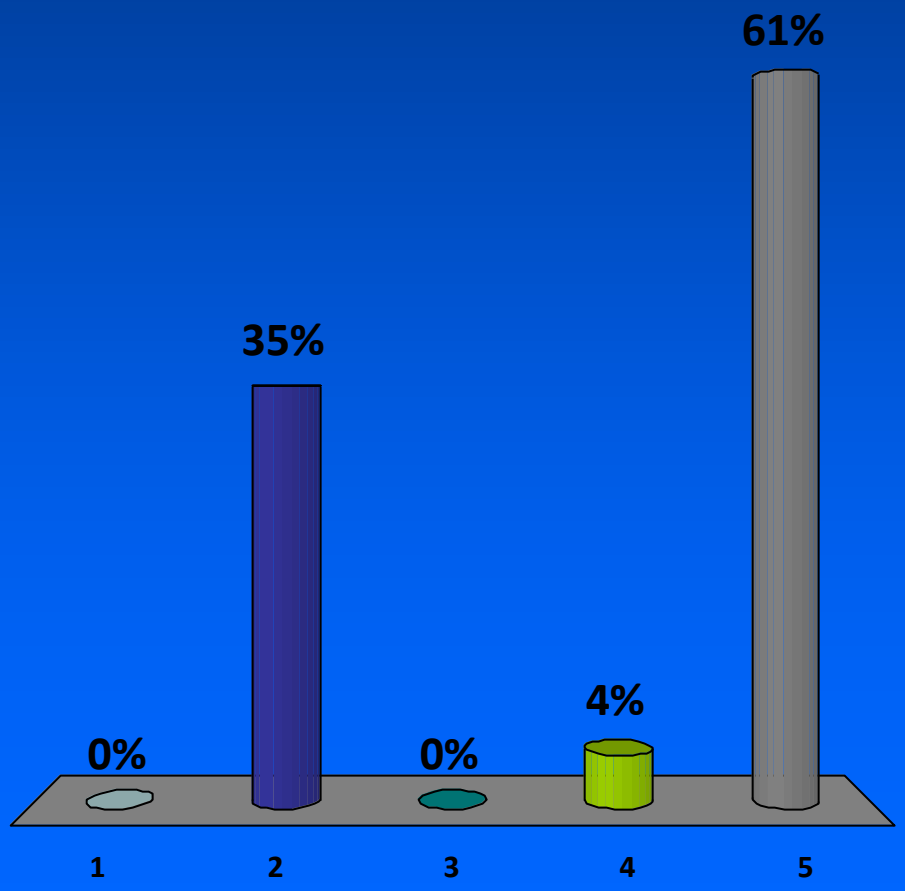
- **Increased chemokines (attracting neutrophils and macrophages) during reperfusion of living donors (LD) and deceased donors (DD) renal allografts**

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

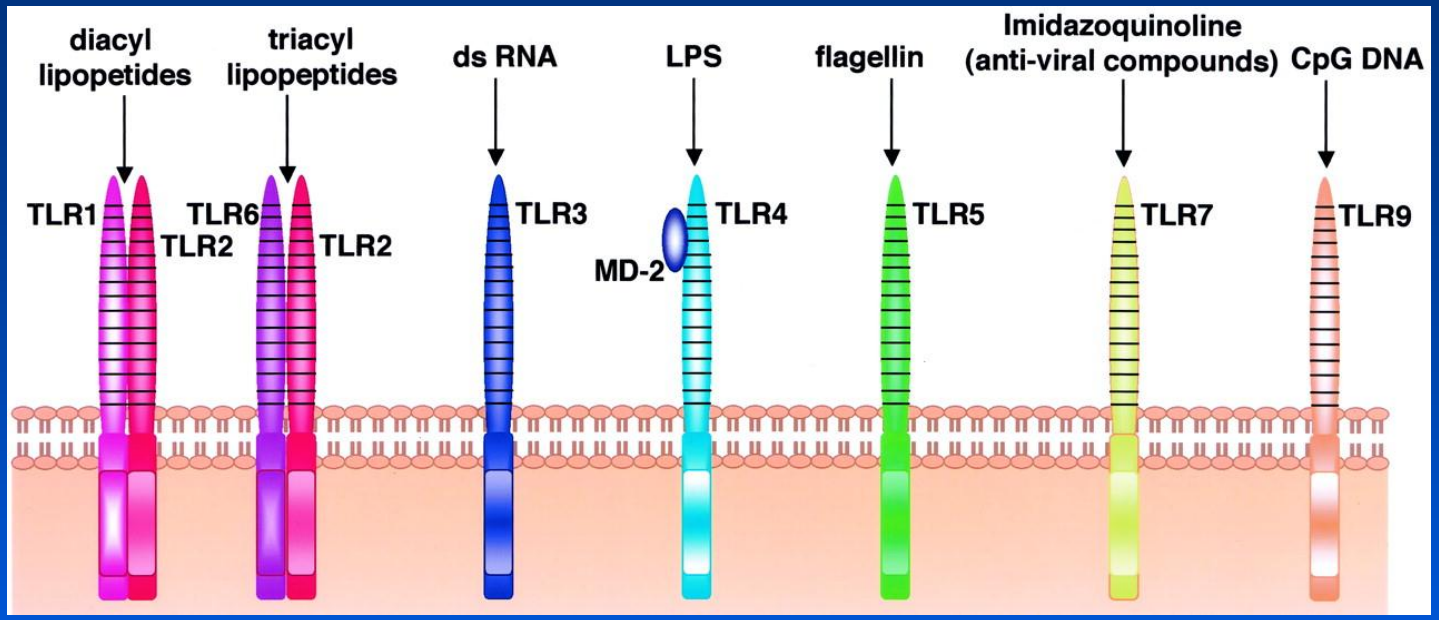
  RESULTS: IL-8/CXCL8 (binds to neutrophil receptors) expression increased 50% from ischemia to reperfusion in LD but increased more than 13-fold during reperfusion of DD.

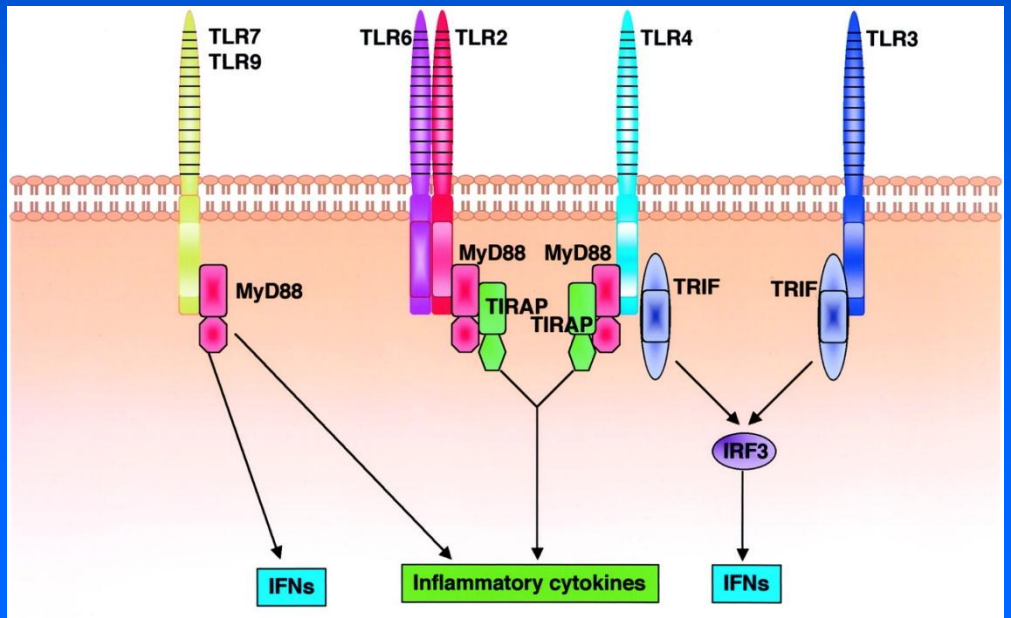
Toll like receptor blockade…

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)



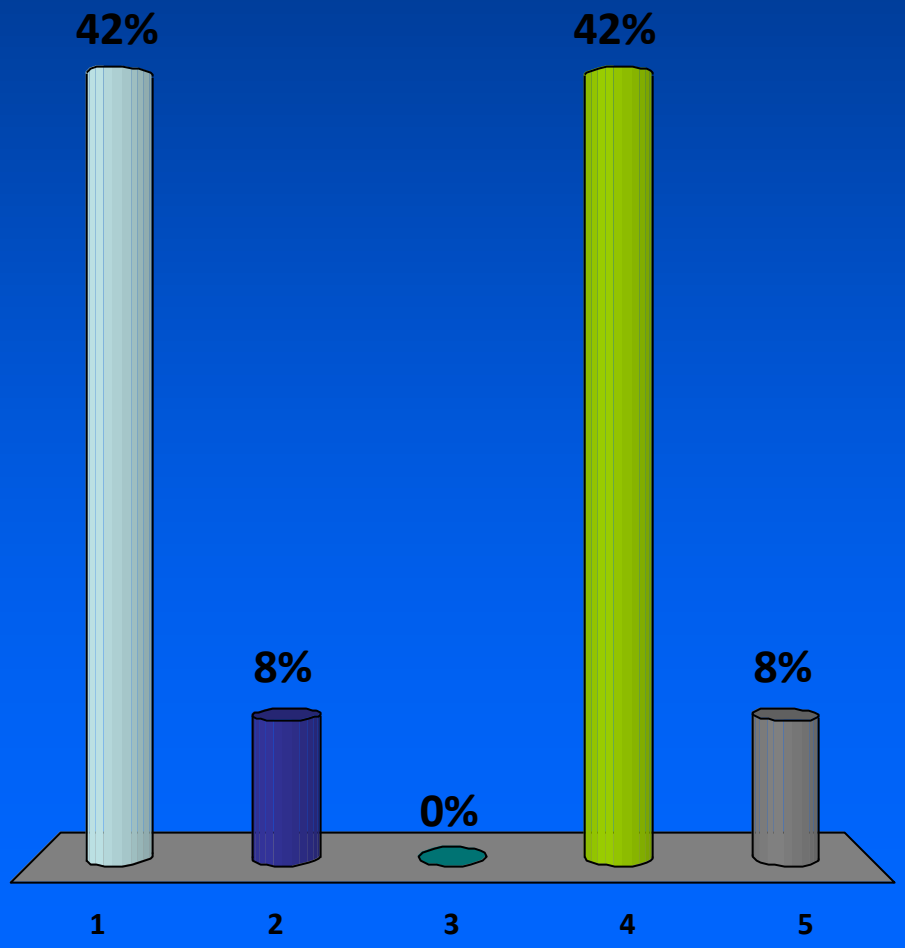


Endogenous Ligands of TLR

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

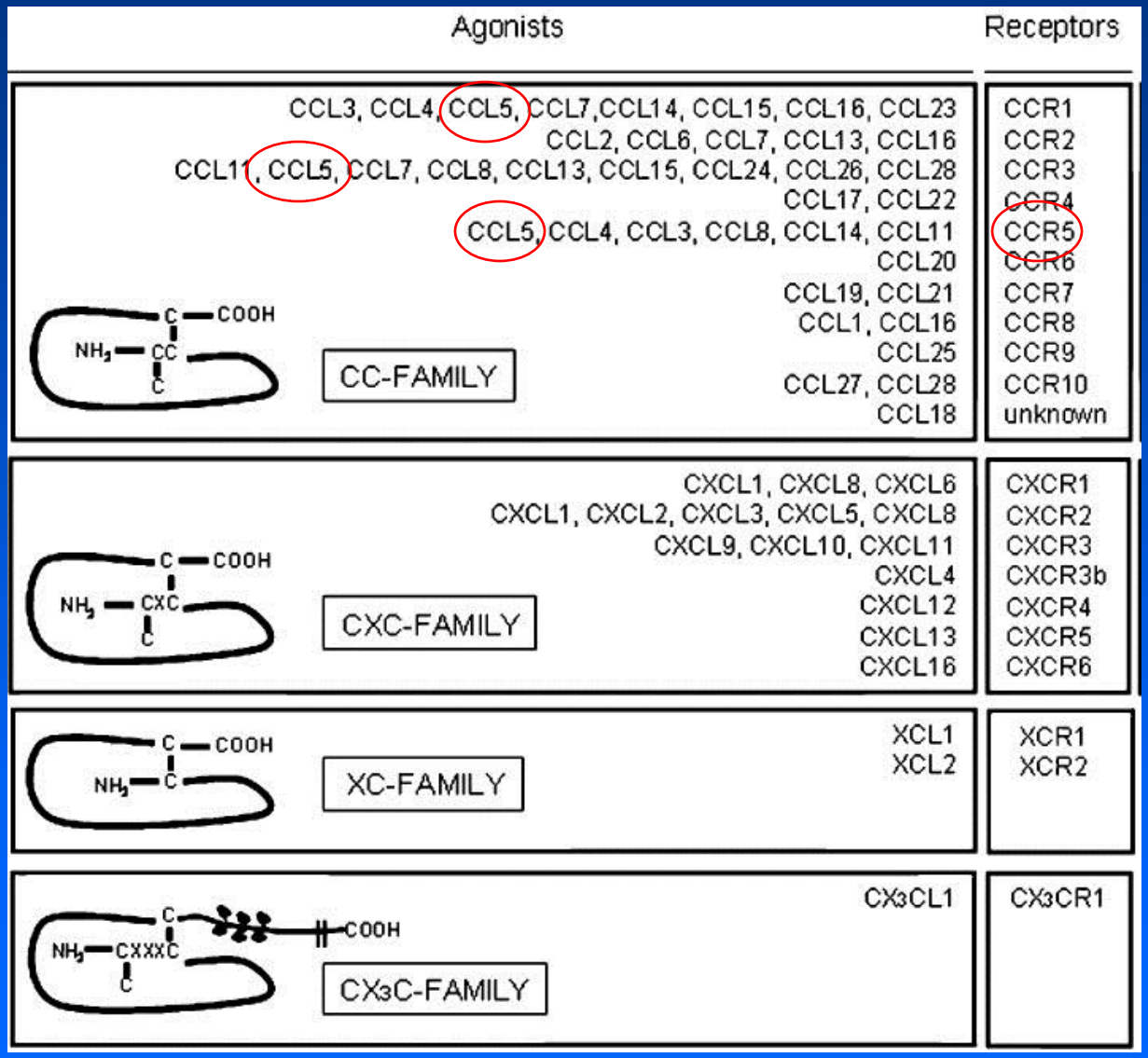
Antigen Independent

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

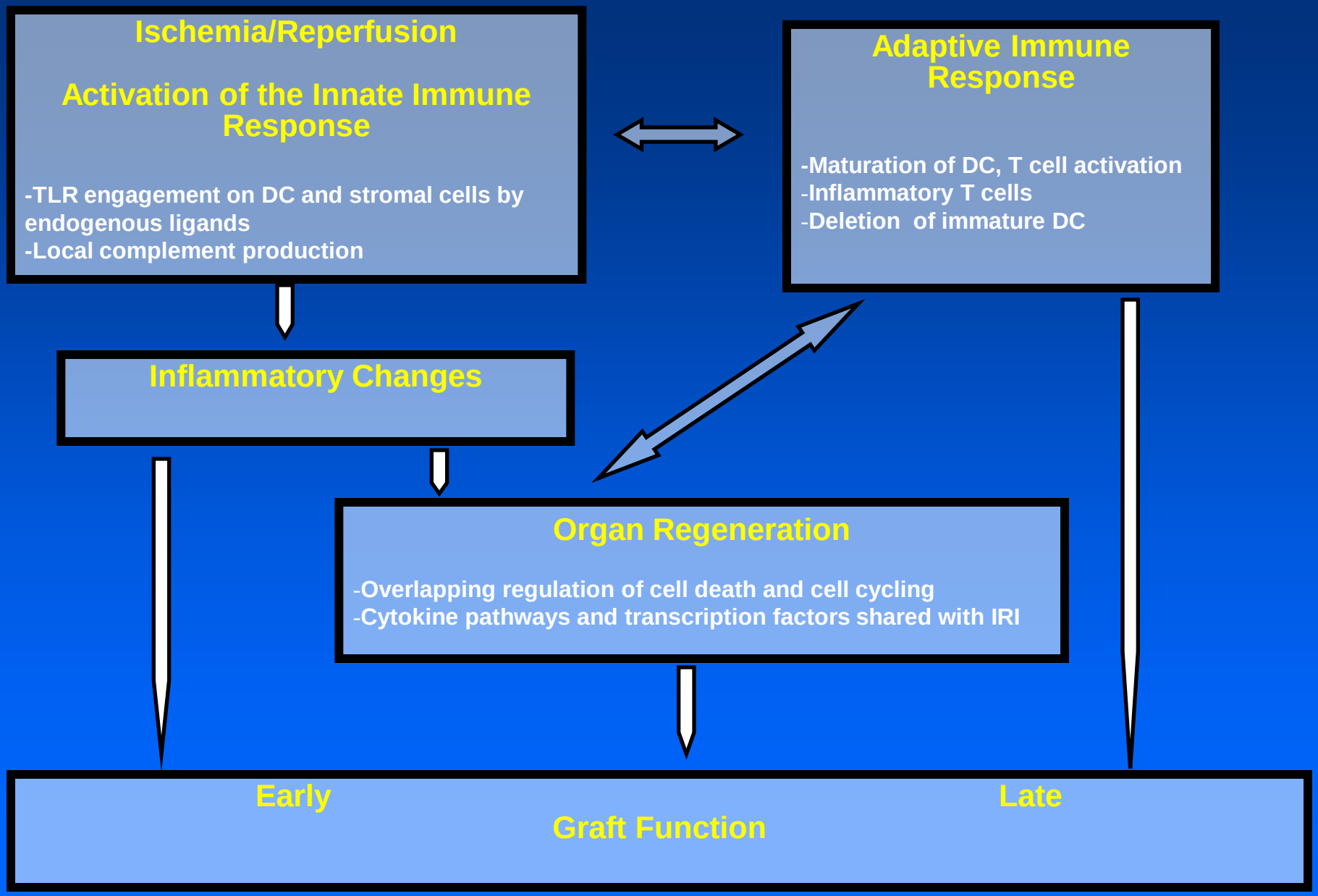
Tissue Injury

Chemokines

Degeneracy of Chemokine Ligands and Receptors

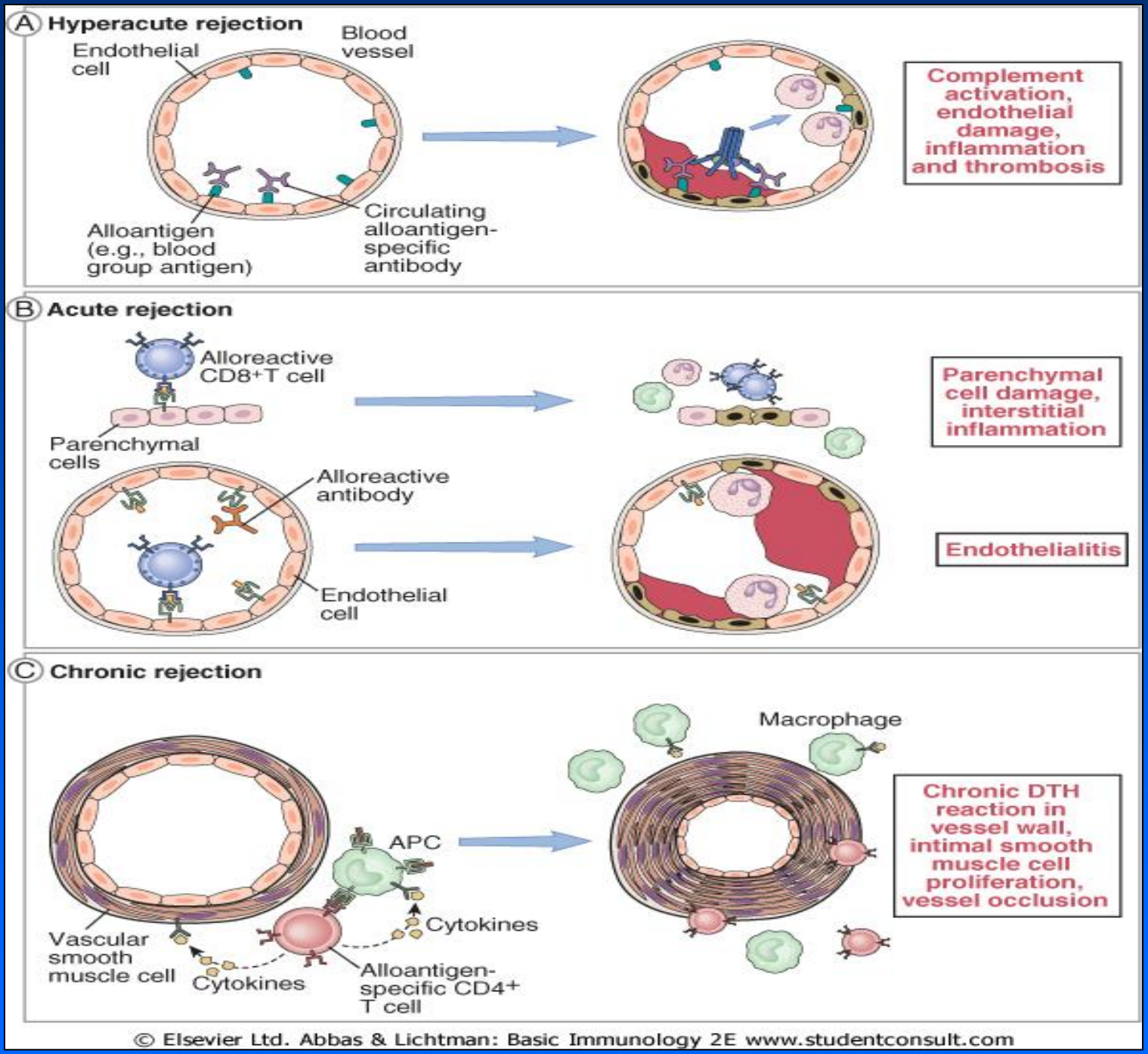


Interactions among Innate and Adaptive Immune Responses, Organ Regeneration, and Graft Function in IRI

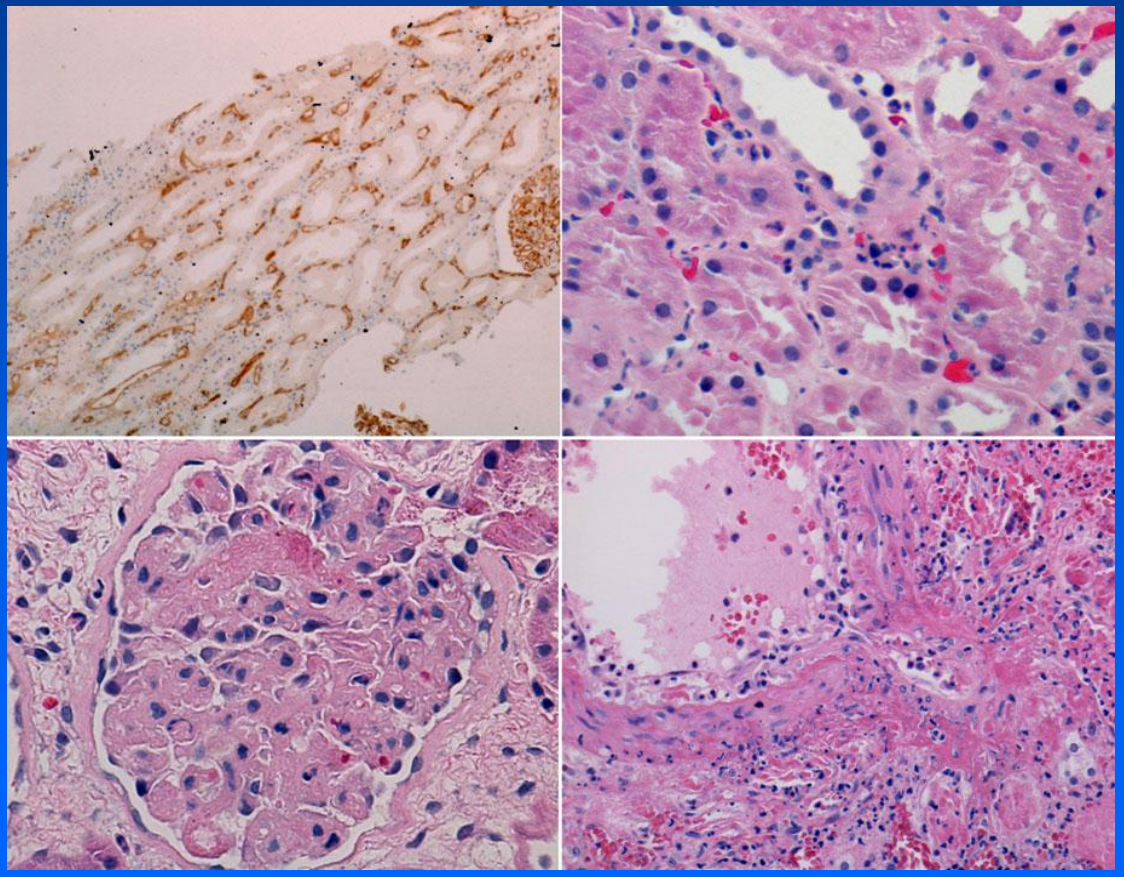


Types of Allograft Rejection

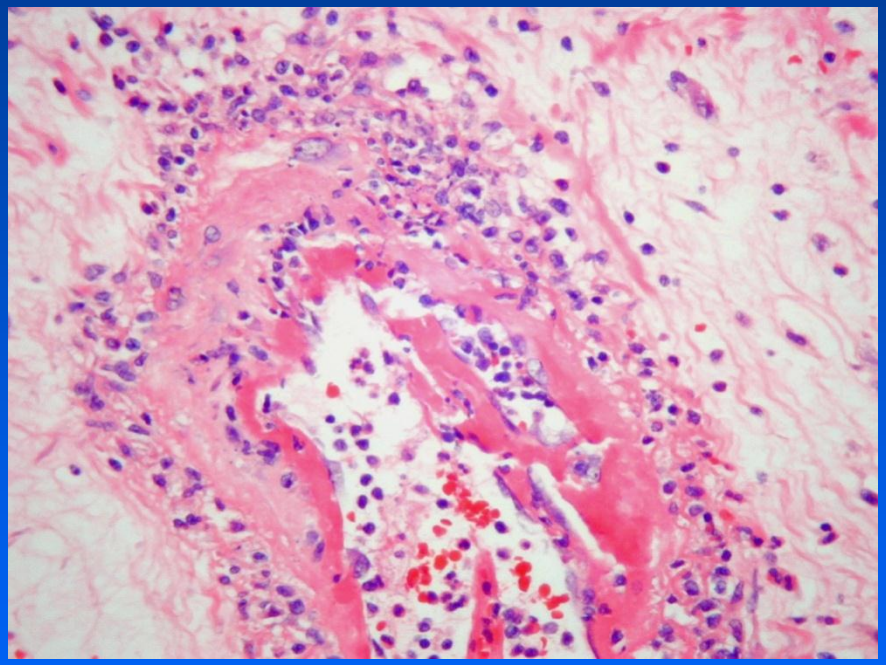
- **Hyperacute** **Avoidable**
  - **Antibody-, Complement-mediated**
- **Acute** **Treatable**
  - **T Cell mediated (macrophages)**
  - **Antibody mediated: “humoral”**
- **Chronic** **Not fully understood**
  - **T cell-driven anti-donor antibody**
  - ***Late consequence of initial injury?***

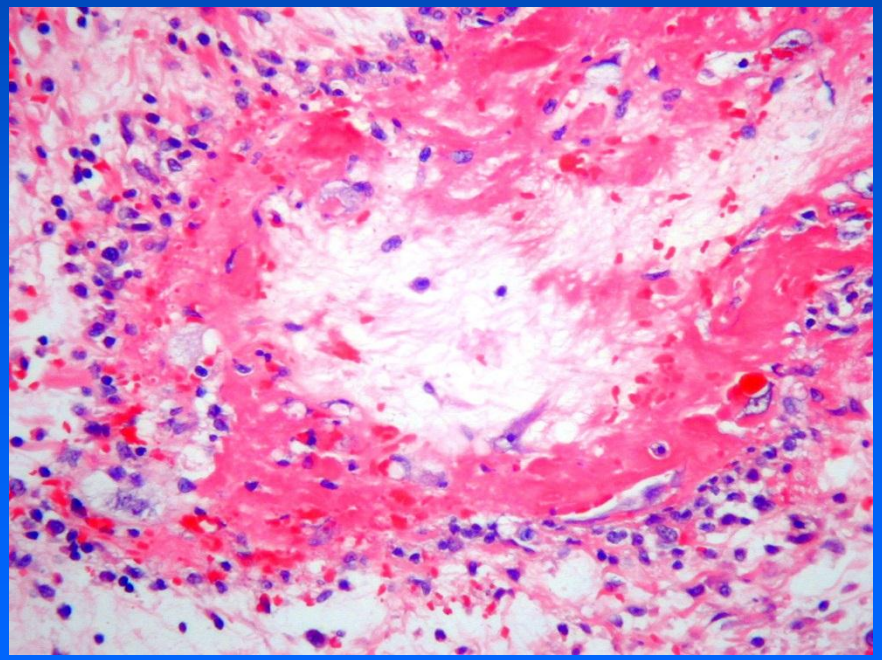


Acute Antibody Mediated Rejection

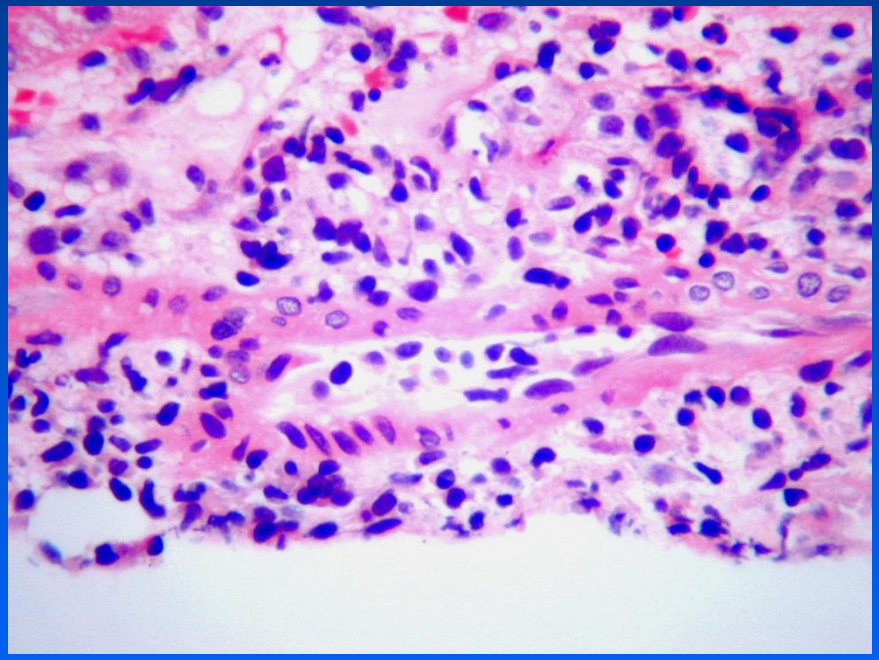


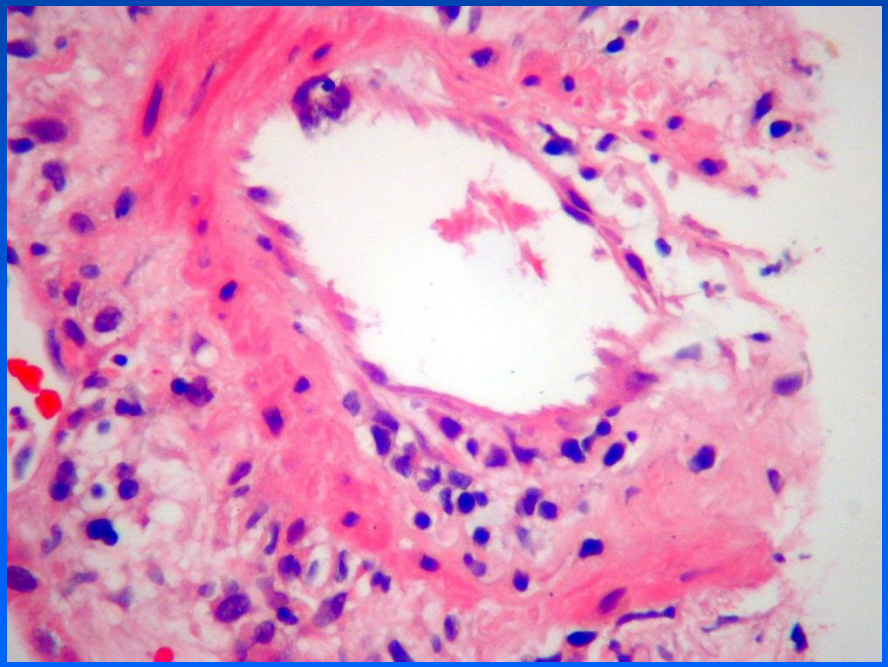
Acute Vascular Rejection



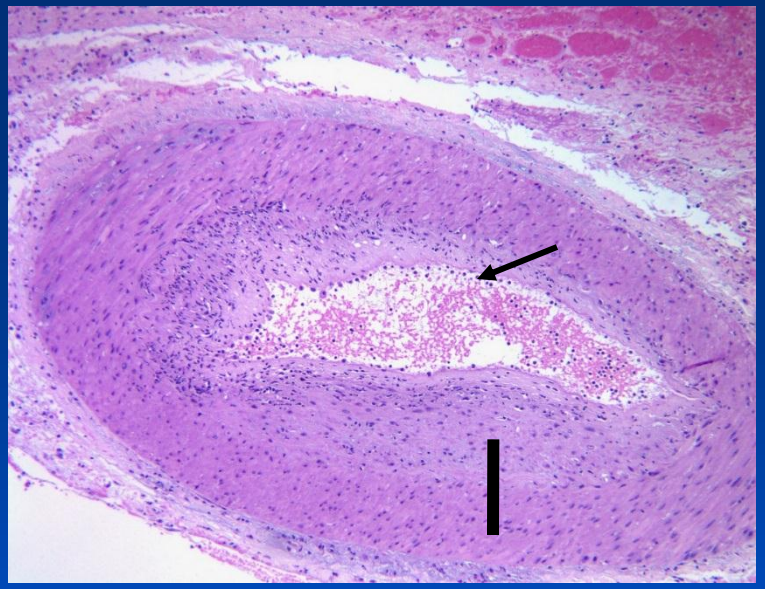


Acute Cellular Rejection

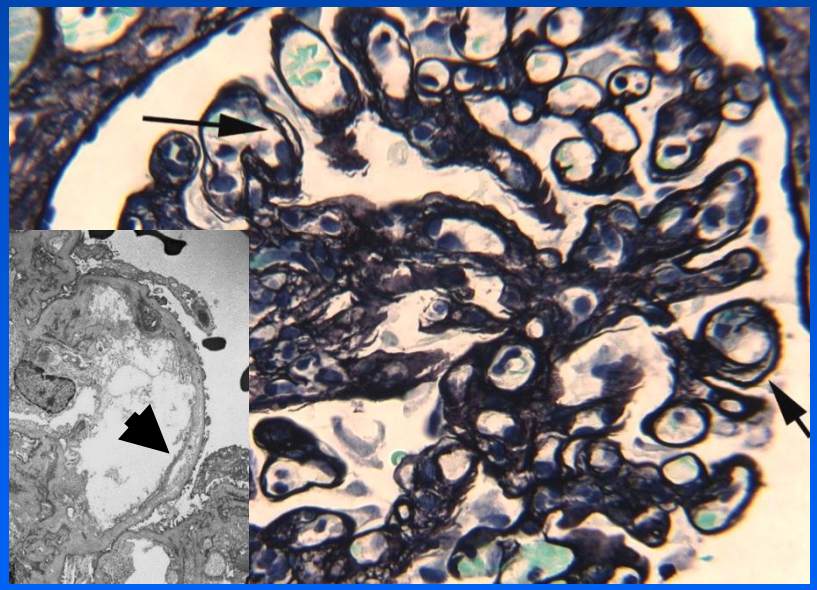




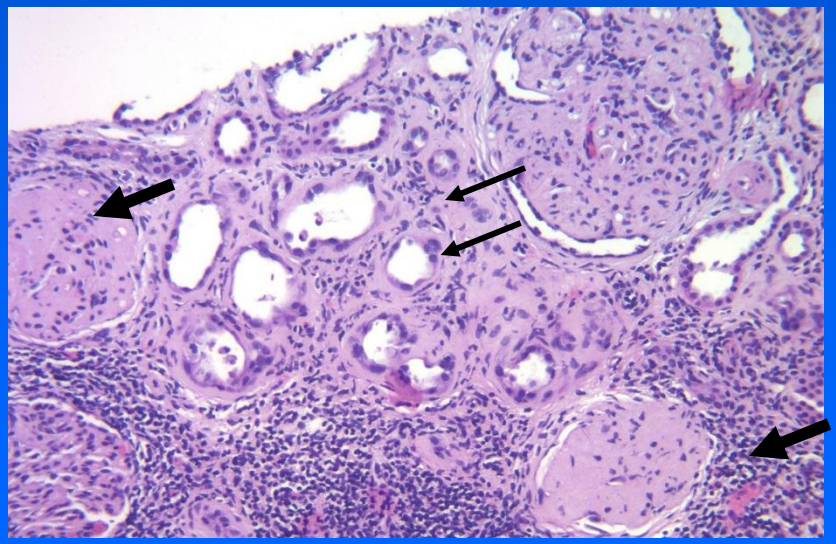
Chronic Allograft Nephropathy



Transplant arteriopathy with intimal proliferation (arrow), subintimal/medial smooth muscle proliferation and fibrosis (bar);Progressive luminal narrowing

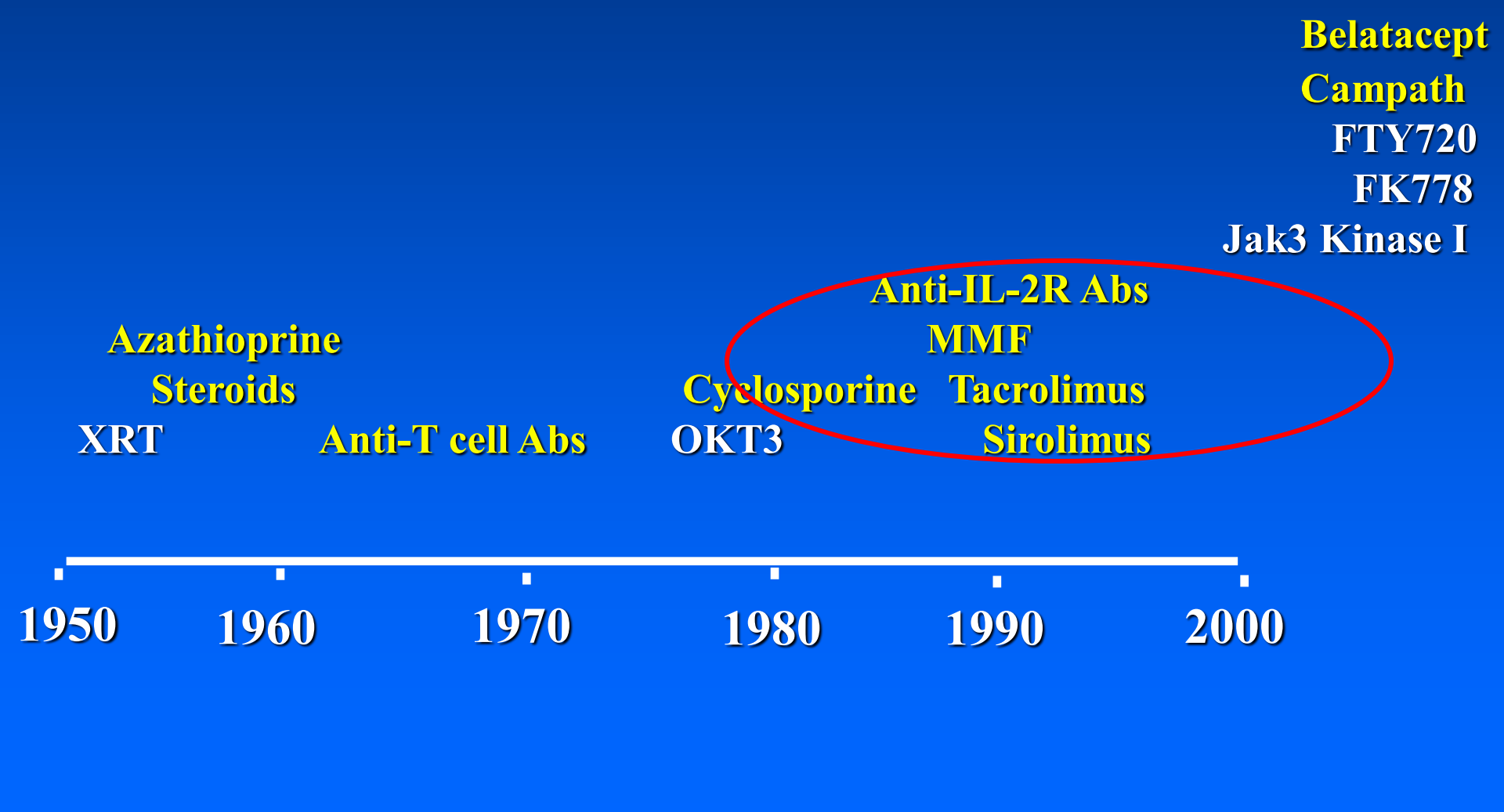


Transplant glomerulopathy with reduplication of the glomerular basement membranes (arrows), a lesion typical of chronic antibody-mediated rejection (chronic AMR)



Global glomerular sclerosis (heavy arrows) and interstitial fibrosis/tubular atrophy (IF/TA; light arrows)

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**

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

Individualizing Immunosuppression Based on Immunologic Risk

PRE-TRANSPLANT
IMMUNOMODULATION

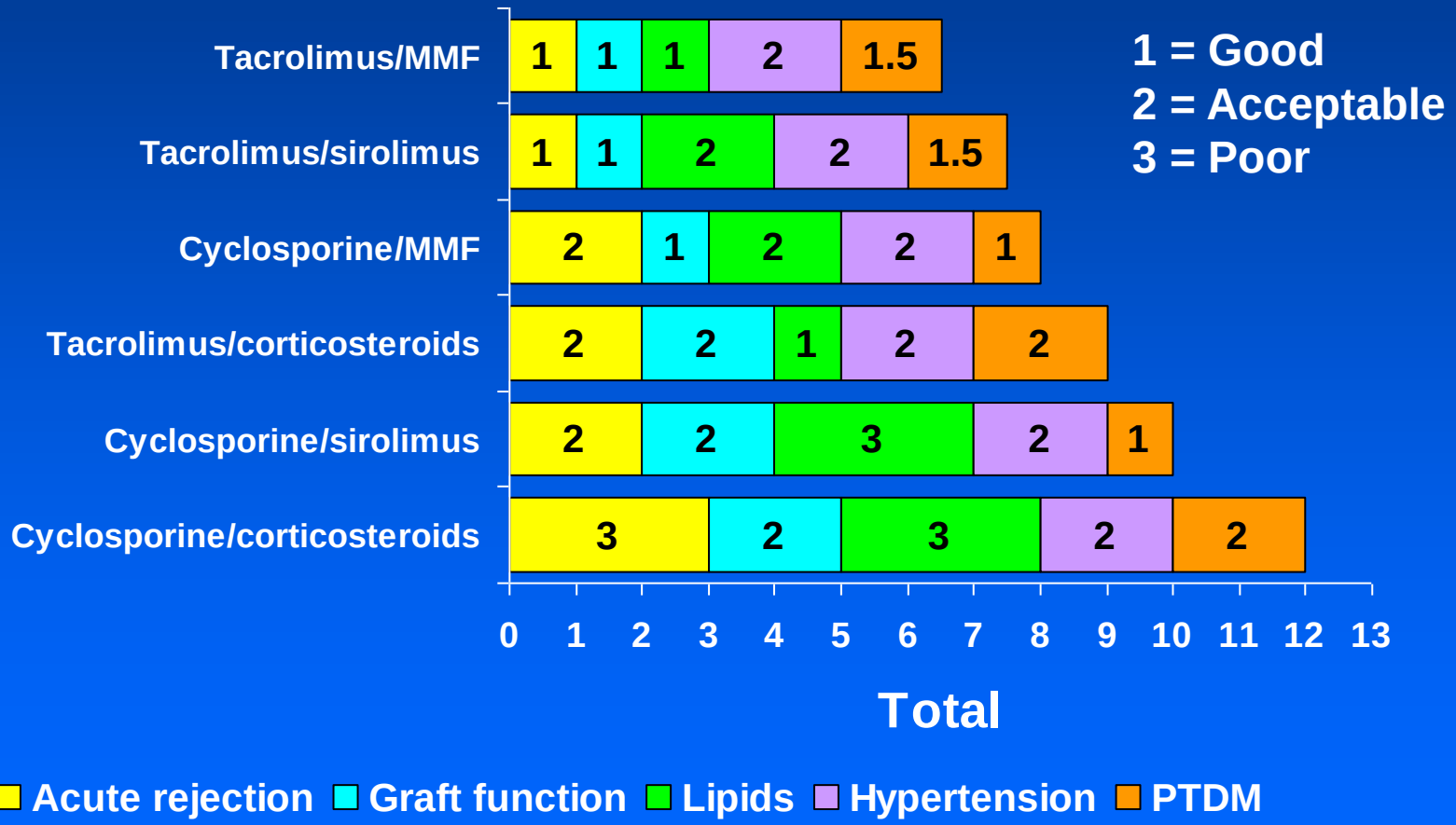
INDUCTION
ANTIBODY THERAPY

TRIPLE THERAPY
MAINTENANCE

MINIMIZATION
PROTOCOLS

HIGH RISK	LOW RISK
HIGHLY SENSITIZED	NONSENSITIZED
NON-PRIMARY TRANSPLANT	ASIAN/CAUCASIAN ETHNICITY
AFRICAN AMERICAN/HISPANIC ETHNICITY	THE ELDERLY
CADAVERIC DONOR SOURCE	LIVING DONOR SOURCE
POOR HLA MATCH	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 Withdrawal

- **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 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

Novel Combinations

- Thymoglobulin + belatacept + steroids + MMF → rapamycin

Clinical Transplantation

Clinical Scenario

26 yo F crescentic glomerulosclerosis

Impending dialysis for renal failure

BUN 70 (nl< 20), Creat 4.5 (nl <1.4)

Mother, two brothers ABO compatible (O-A, A-A, A-A)

Patient	A2,52	B7,14	DR2,4
Mother	A2,8	B7,32	DR2,7
Brother 1	A1,8	B6,32	DR4,7
Brother 2	A2,52	B7,14	DR2,4

Clinical Transplantation

Clinical Scenario

26 yo F crescentic glomerulosclerosis, ABO=A

Impending dialysis for renal failure

BUN 70 (nl< 20), Creat 4.5 (nl <1.4)

Mother (O), two brothers (A,A) ABO compatible

Patient	**A2,52**	**B7,14**	**DR2,4**	
Mother	**A2,8**	**B7,32**	**DR2,7**	**3 Ag match**
Brother 1	**A1,8**	**B6,32**	**DR4,7**	
Brother 1	**A2,52**	**B7,14**	**DR2,4**	

"Haploidentical"

Clinical Transplantation

Clinical Scenario

26 yo F crescentic glomerulosclerosis

Impending dialysis for renal failure

BUN 70 (nl< 20), Creat 4.5 (nl <1.4)

Mother (O), two brothers (A,A) ABO compatible

Patient	**A2,52**	**B7,14**	**DR2,4**	
Mother	**A2,8**	**B7,32**	**DR2,7**	
Brother 1	**A1,8**	**B6,32**	**DR4,7**	**0 Ag Match**
Brother 2	**A2,52**	**B7,14**	**DR2,4**	

(fully mismatched)

Clinical Transplantation

Clinical Scenario

26 yo F crescentic glomerulosclerosis

Impending dialysis for renal failure

BUN 70 (nl< 20), Creat 4.5 (nl <1.4)

Mother (O), two brothers (A,A) ABO compatible

Patient	A2,52	B7,14	DR2,4	
Mother	A2,8	B7,32	DR2,7	
Brother 1	A1,8	B6,32	DR4,7	
Brother 2	A2,52	B7,14	DR2,4	0 Ag mismatch

"HLA identical" (Minor Ag mismatch: 'Y')

Clinical Transplantation

Clinical Scenario

26 yo F crescentic glomerulosclerosis

Impending dialysis for renal failure

BUN 70 (nl< 20), Creat 4.5 (nl <1.4)

Mother (O), two brothers (A,A) ABO compatible

Patient	A2,52	B7,14	DR2,4
Mother	A2,8	B7,32	DR2,7
Brother 1	A1,8	B6,32	DR4,7
Brother 2	A2,52	B7,14	DR2,4

Pre-emptive living related renal allograft

technical success, 90 minute ischemic interval

Discharged home POD 3

Clinical Transplantation

Clinical Scenario

Our patient is now 34 yo

Inconsistent early compliance, better recently
Multiple episodes of acute rejection early
BUN 86 (nl< 20), Creat 3.8 (nl <1.4)
refractory to increased immunoRx

What has happened to renal allograft from her mother?

Clinical Transplantation

Clinical Scenario

Our patient is now 34 yo with two children

- **Inconsistent early compliance, better recently**
- **Multiple episodes of acute rejection, now with CR**
- **BUN 86 (nl< 20), Creat 3.8 (nl <1.4)**

Mother, two brothers ABO compatible (~~O-A~~, A-A, A-A)

Patient	**A2,52**	**B7,14**	**DR2,4**	**αA8,B32,DR7 Ab**
~~**Mother**~~	~~**A2,8**~~	~~**B7,32**~~	~~**DR2,7**~~	
Brother 1	**A1,8**	**B6,32**	**DR4,7**	
Brother 2	**A2,52**	**B7,14**	**DR2,4**	

What would happen with renal allograft from Brother 1?

Clinical Transplantation

Clinical Scenario

Our patient is now 34 yo

- **Inconsistent early compliance, better recently**
- **Multiple episodes of acute rejection, now with CR**
- **BUN 86 (nl< 20), Creat 3.8 (nl <1.4)**

Mother, one brother ABO compatible (~~O-A, A-A~~,A-A)

Patient	**A2,52**	**B7,14**	**DR2,4**	**αA8,B32,DR7 Ab**
~~**Mother**~~	~~**A2,8**~~	~~**B7,32**~~	~~**DR2,7**~~	
~~**Brother 1**~~	~~**A1,8**~~	~~**B6,32**~~	~~**DR4,7**~~	
Brother 2	**A2,52**	**B7,14**	**DR2,4**	**"Cross-match neg"**

What would happen with renal allograft from Brother 2?

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

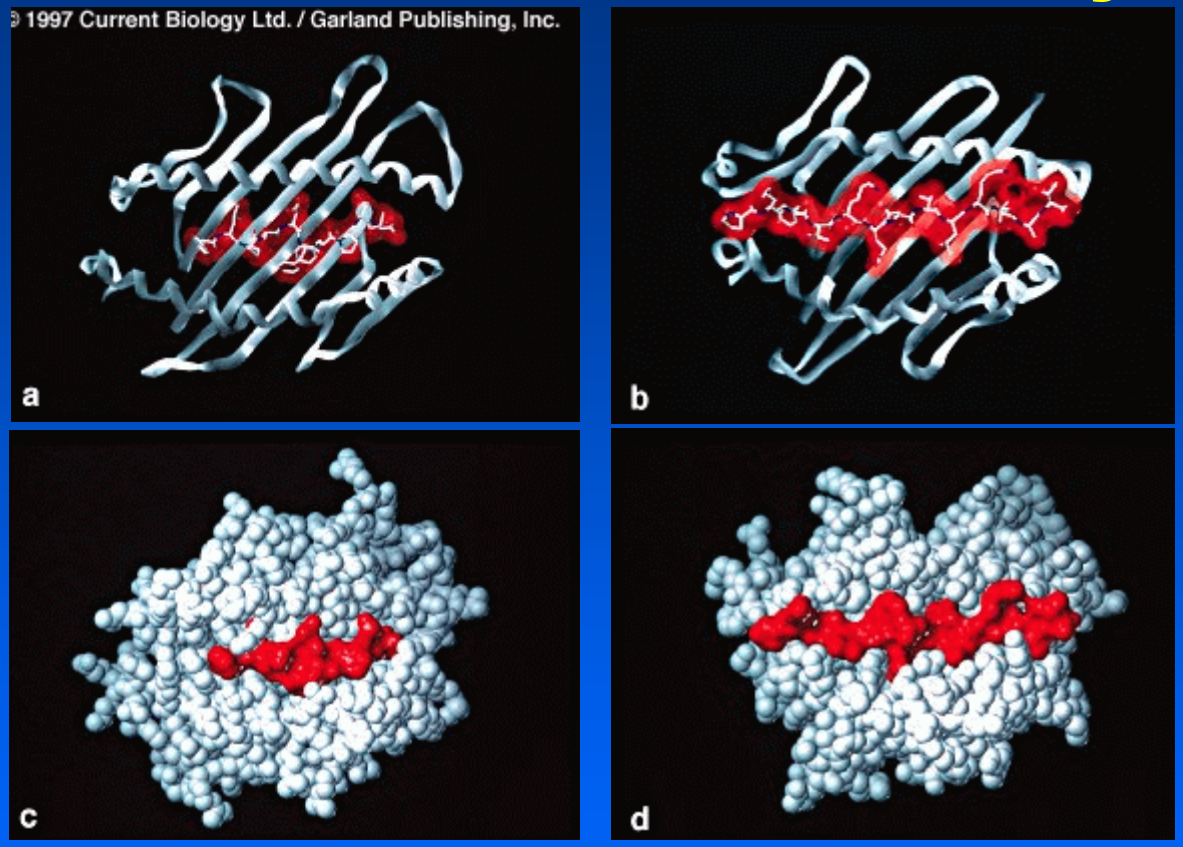
MHC Molecules

		Class I	Class II
Human	HLA	A B C	DQ DR DP
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)

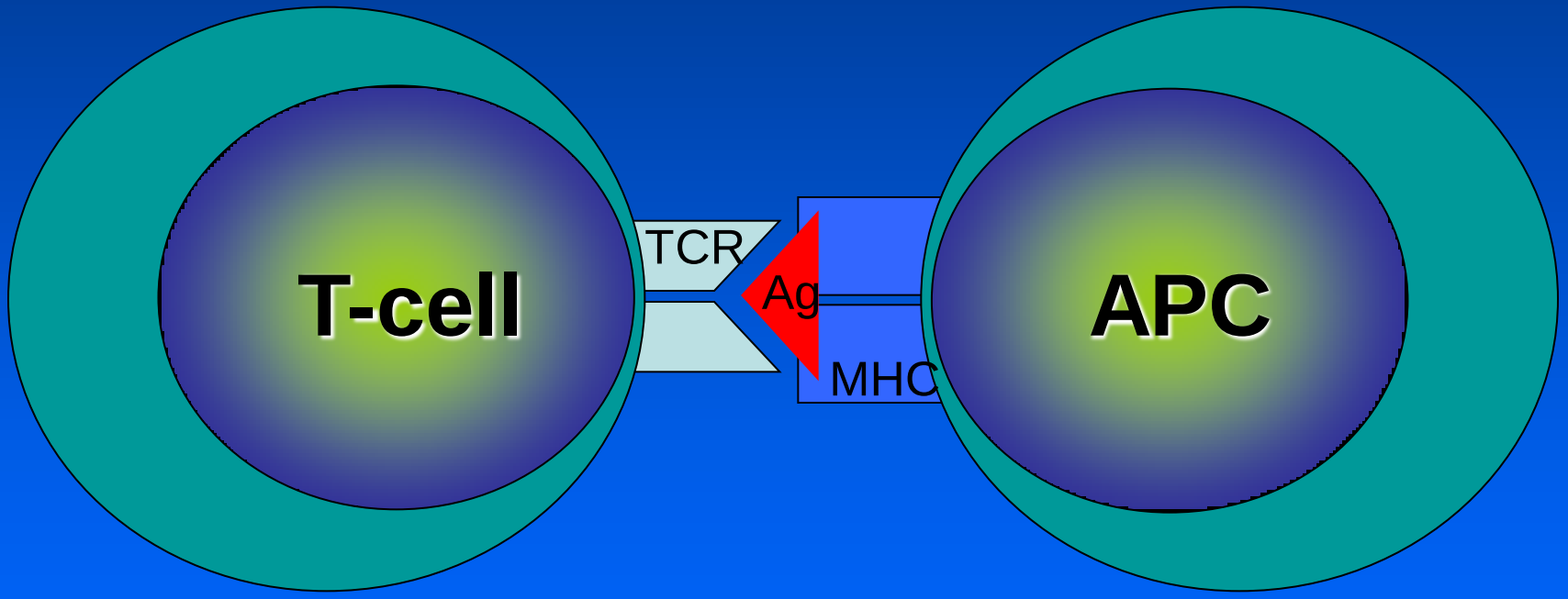
Peptides Fit into MHC I and II Molecules Differently



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

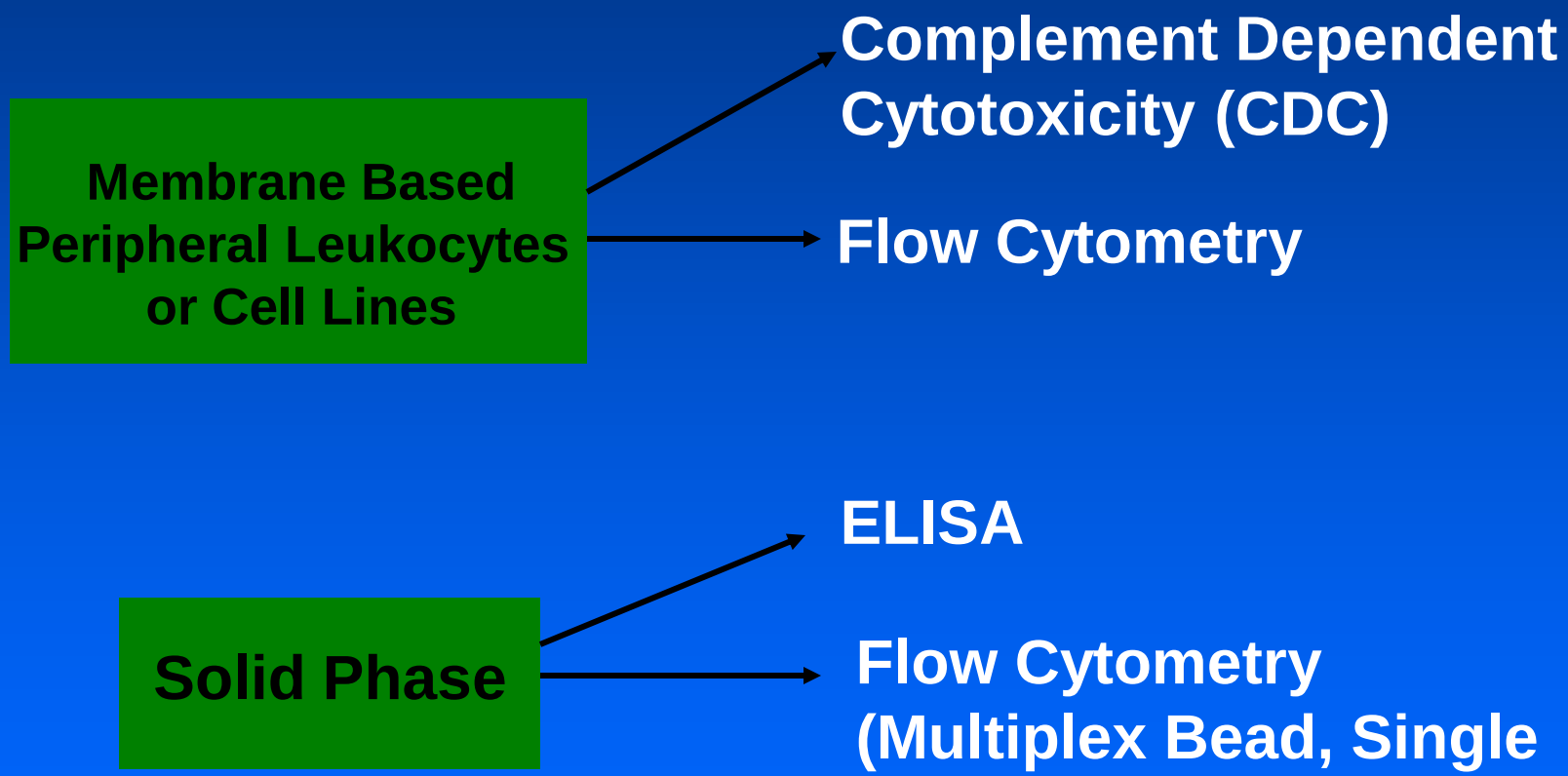
Goals in Antibody Detection

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**

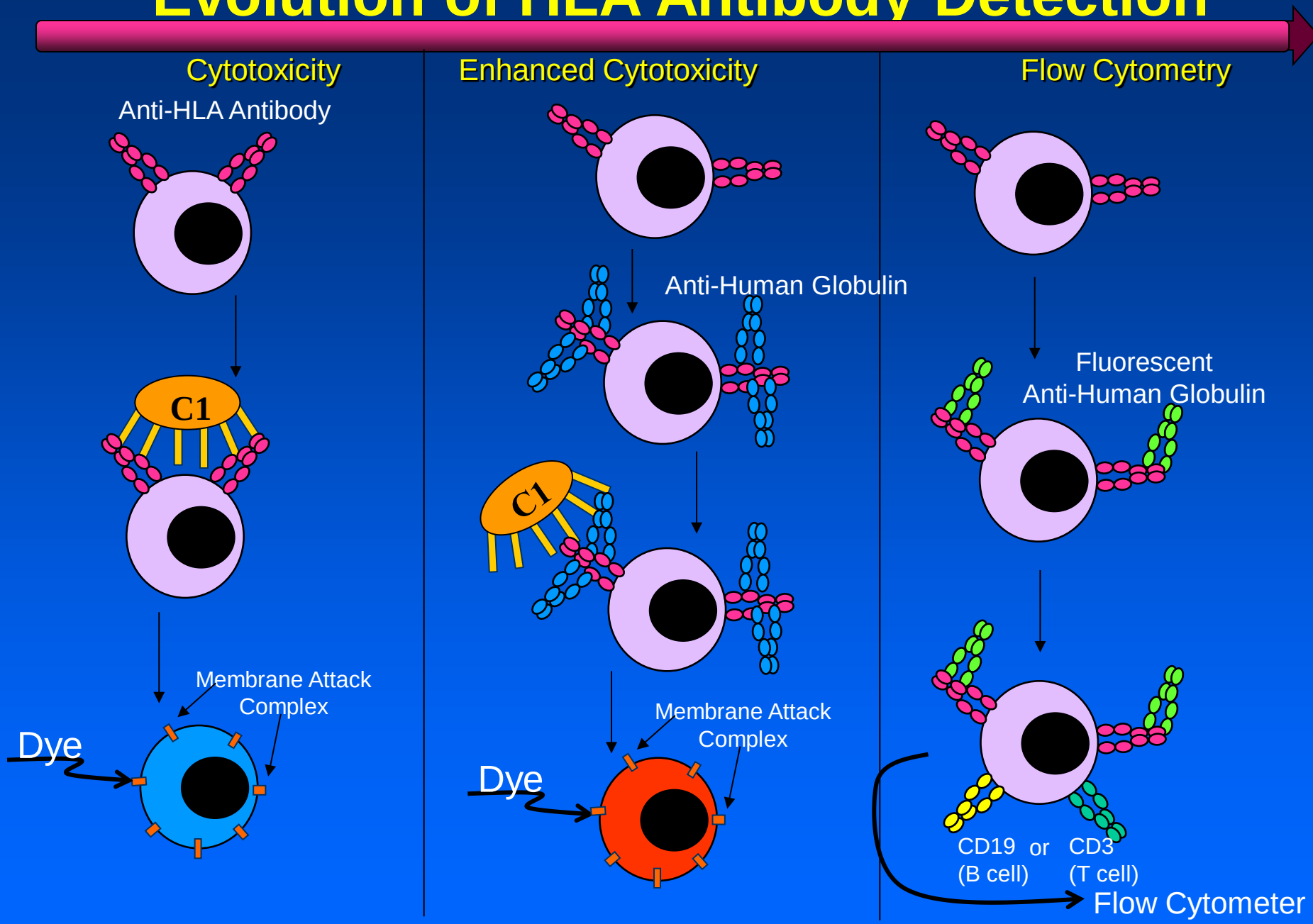
   Quantitative assessment
   - **Titer**

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

Antibody Detection Methods

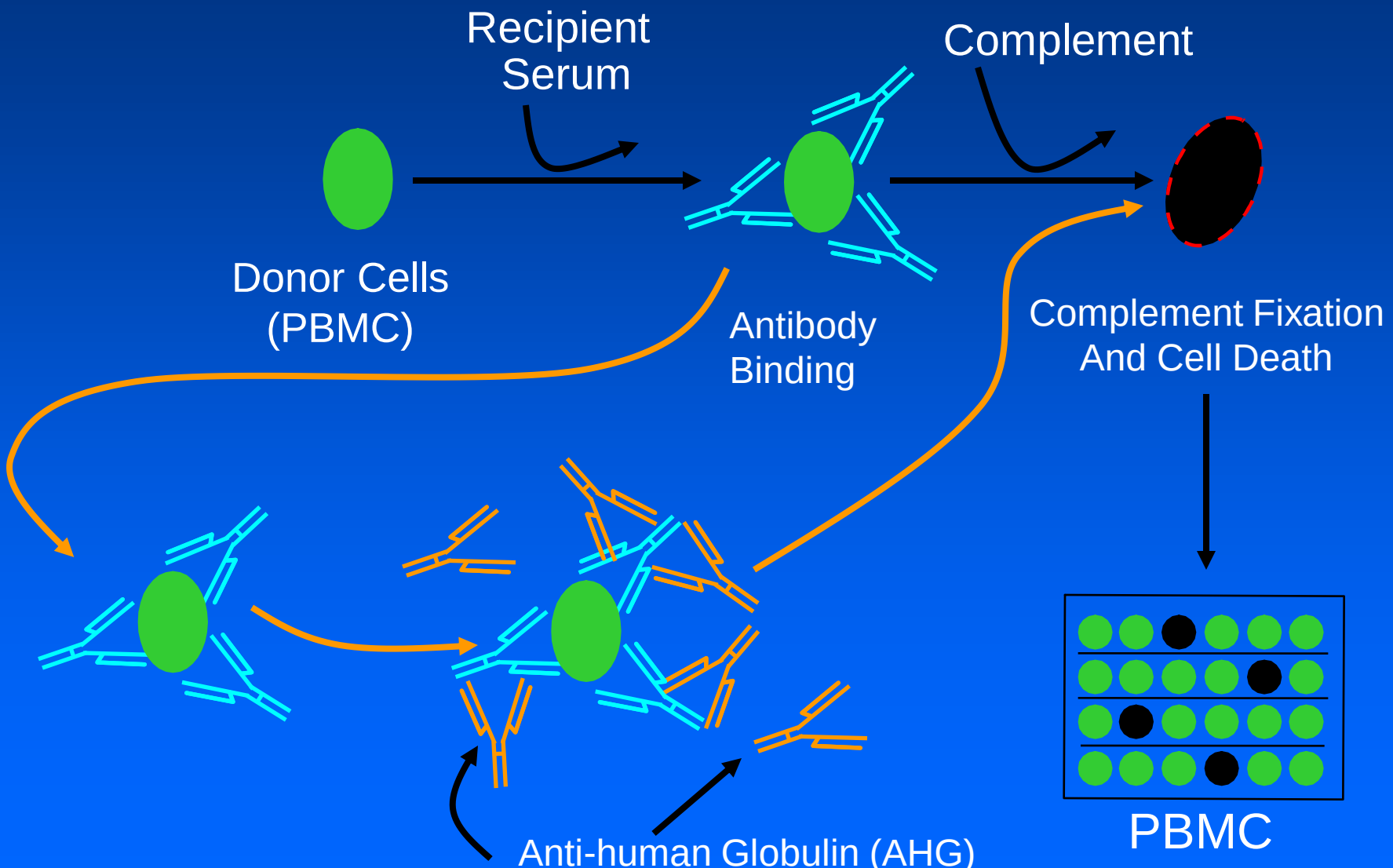


Evolution of HLA Antibody Detection

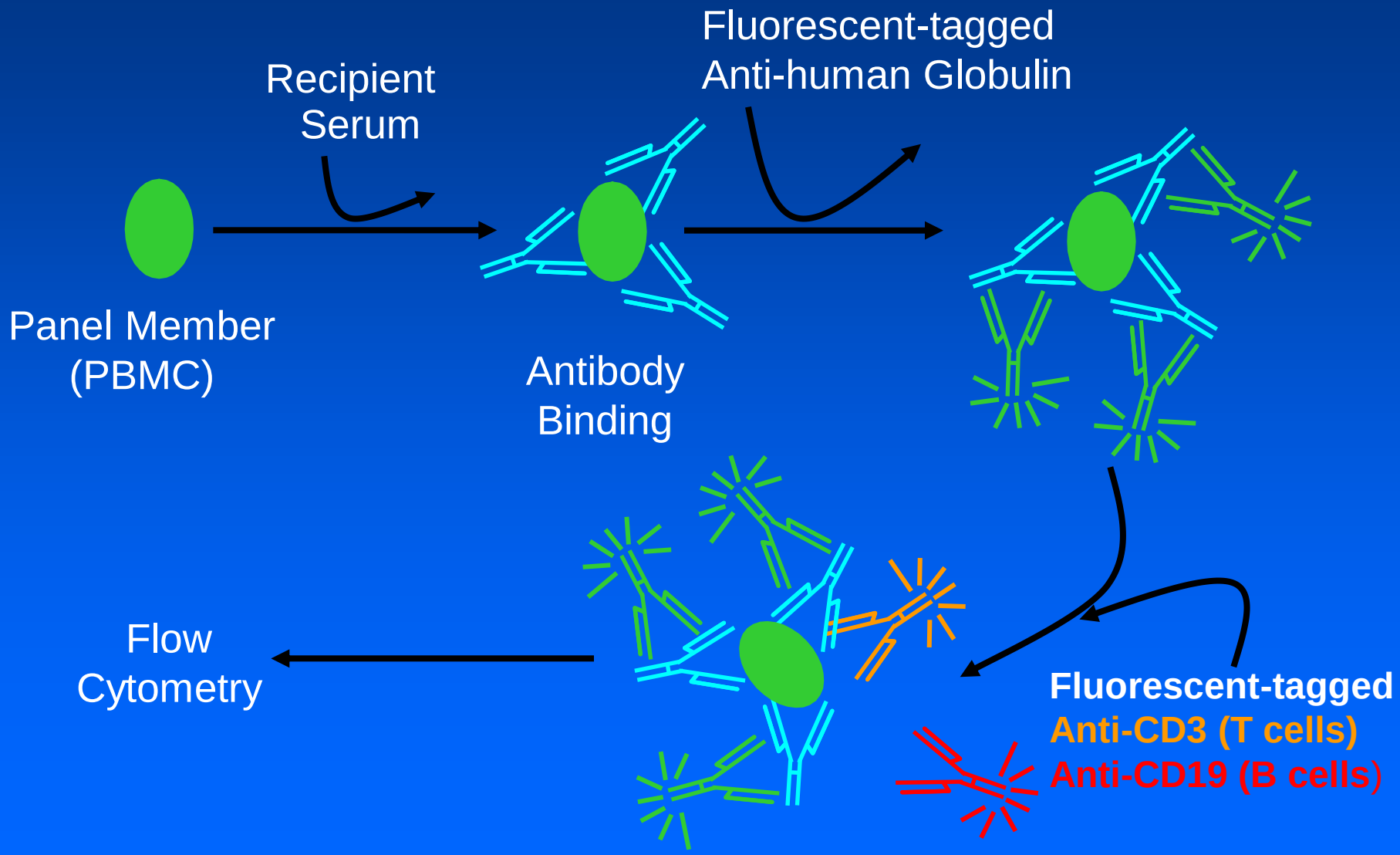


Bray et al Immunol Res. 29:41, 2004

Complement Dependent Cytotoxicity

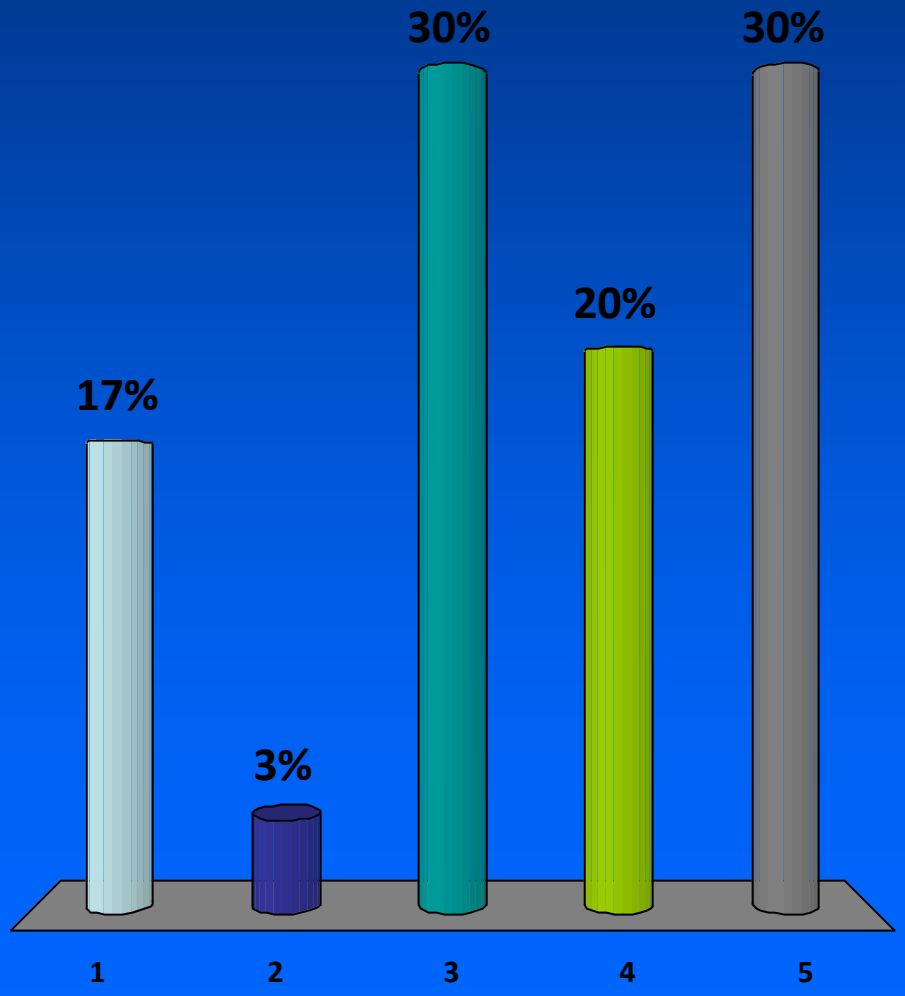


Flow Cytometric Antibody Detection

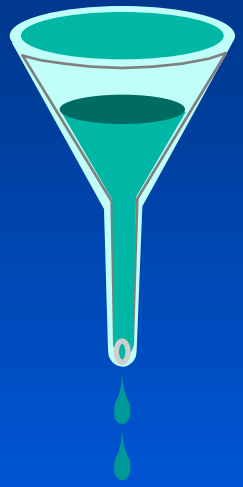


Solid phase single antigen beads or single antigen testing of anti-HLA antibodies:

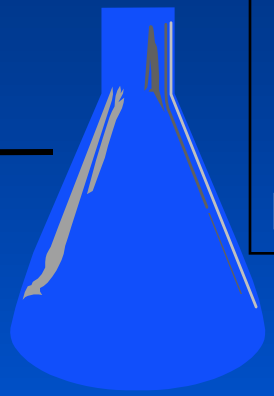
1. Is not quantitative
2. Is not functional
3. Is overly sensitive
4. Has a lot of variation
5. Has a lot of technical variability



Solid Phase, Antigen-Specific Assays



Extract and Purify
HLA Antigens

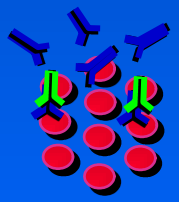


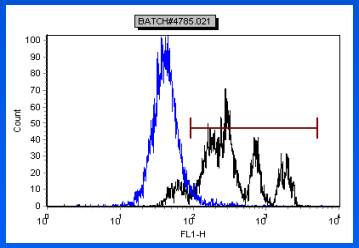
B cells + EBV =
B cell line
or
Peptide Synthesis

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

Purified HLA

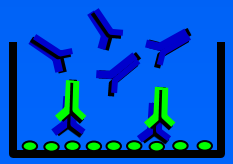
Microparticles





Flow Cytometry

Plate



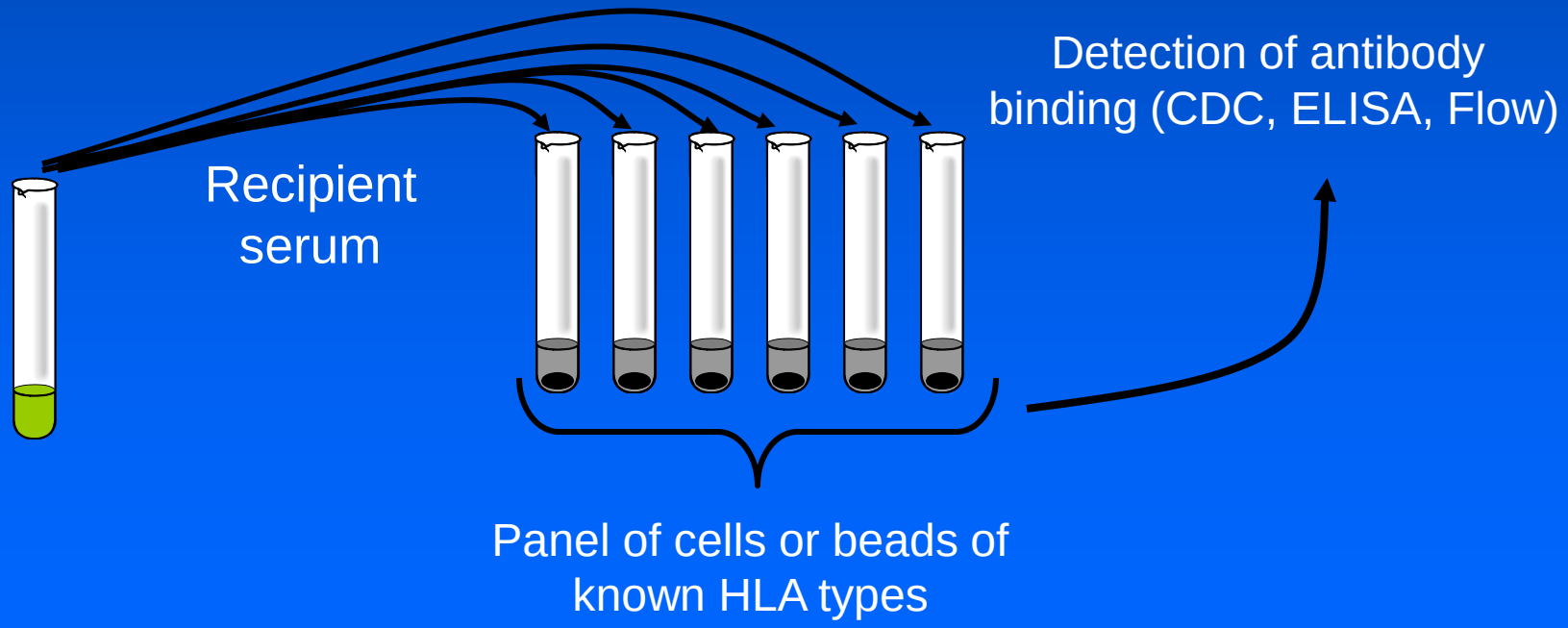
ELISA

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

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.

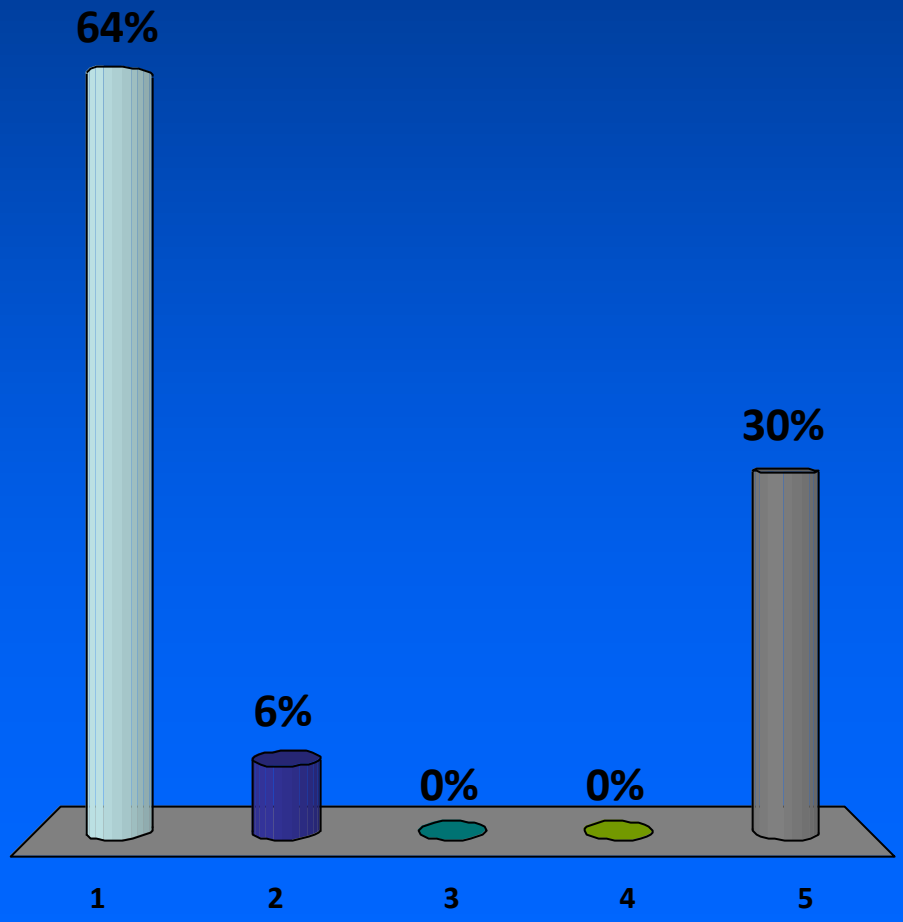


Consequence of HLA typing, antibody identification, and knowledge of population distribution of HLA types:

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

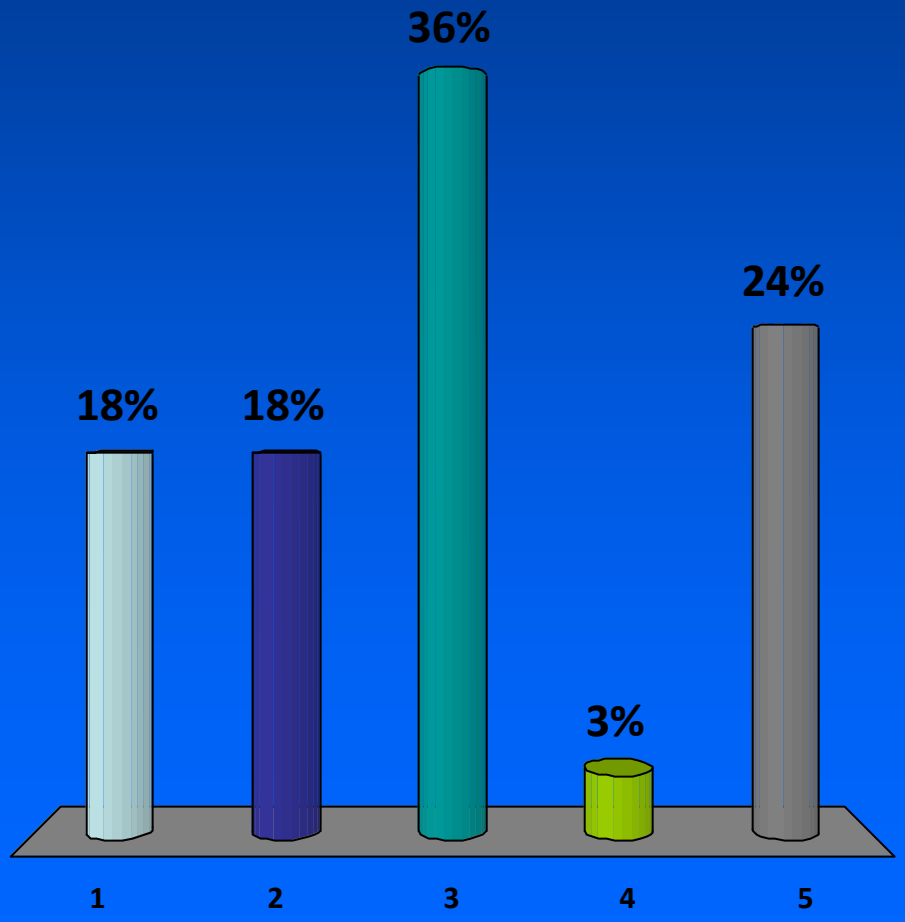


Target Antigens

- 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

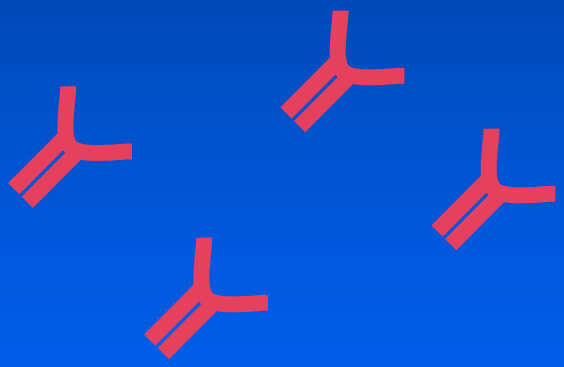
Flow Single Antigen Beads

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

Areas of Uncertainty

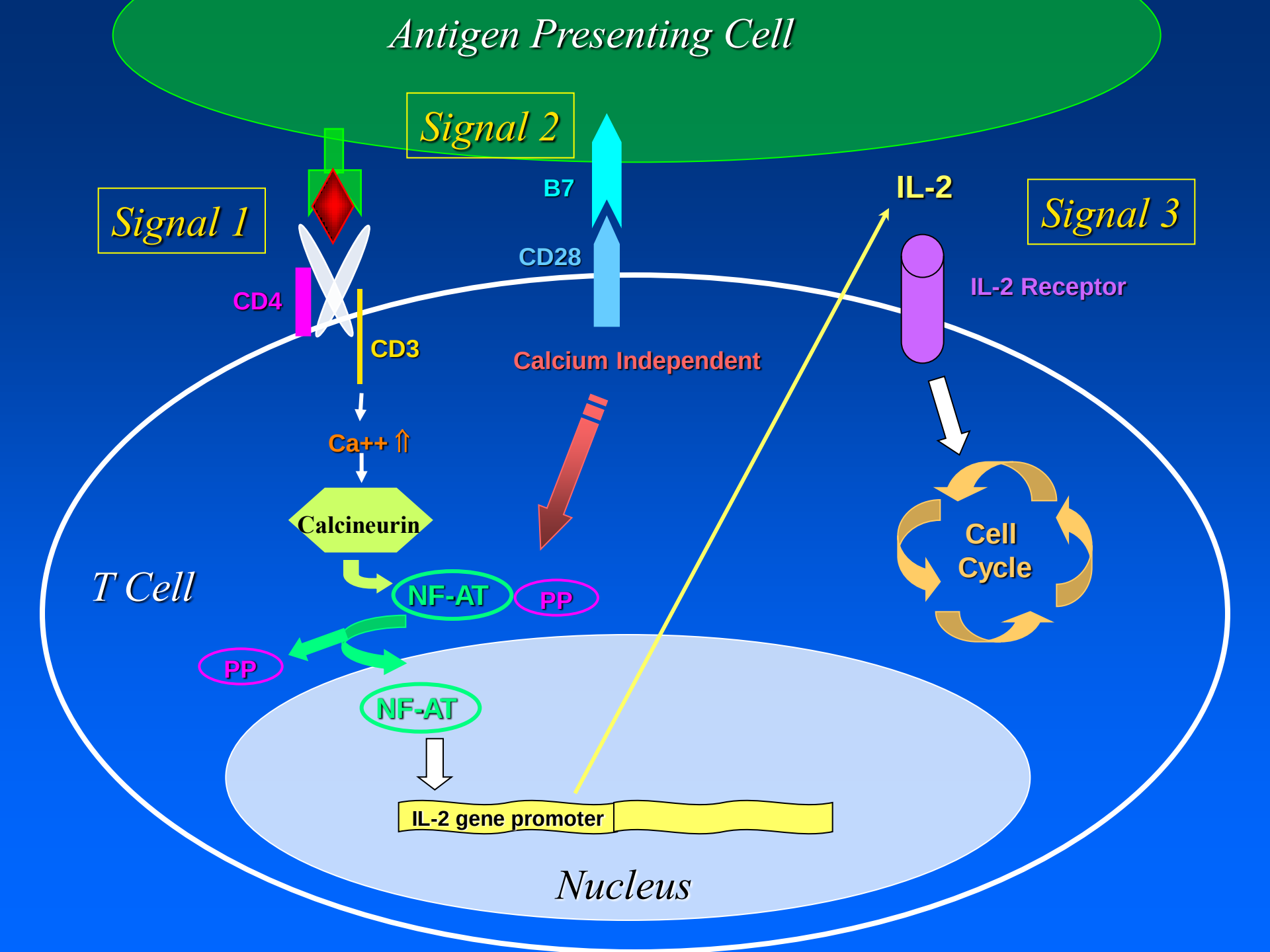
- Sensitivity
- Specificity
- Function
- Pathogenicity

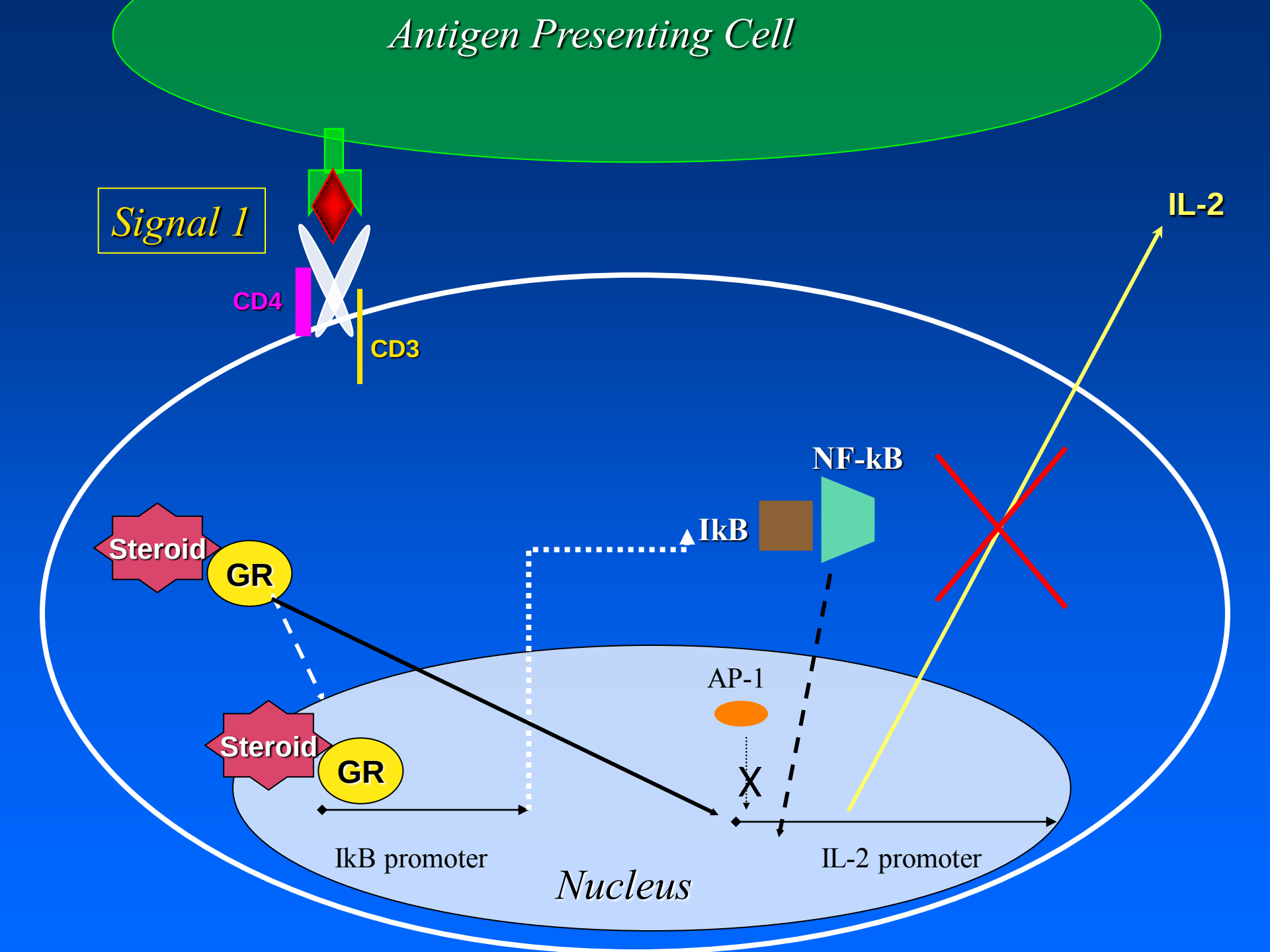
Bottom Line

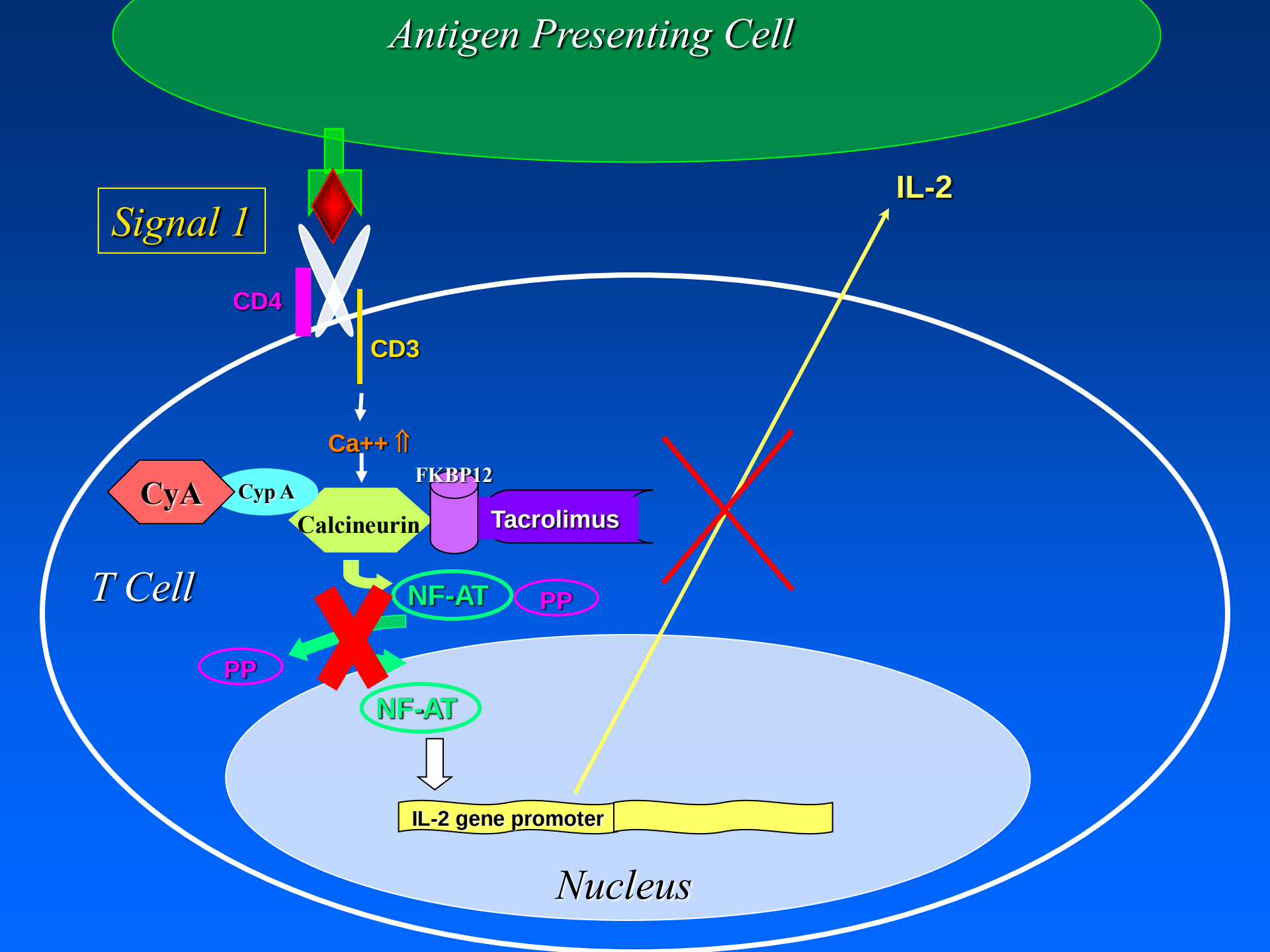
- Negative CDC XM is good
- Positive CDC XM is very bad
- Positive flow XM with high titre DSA is probably very bad
- Positive flow XM with medium titre DSA may be bad, or not. Low titre DSA?
- Negative flow XM with DSA may be ok, or not
- Some positive tests plus some negative tests??

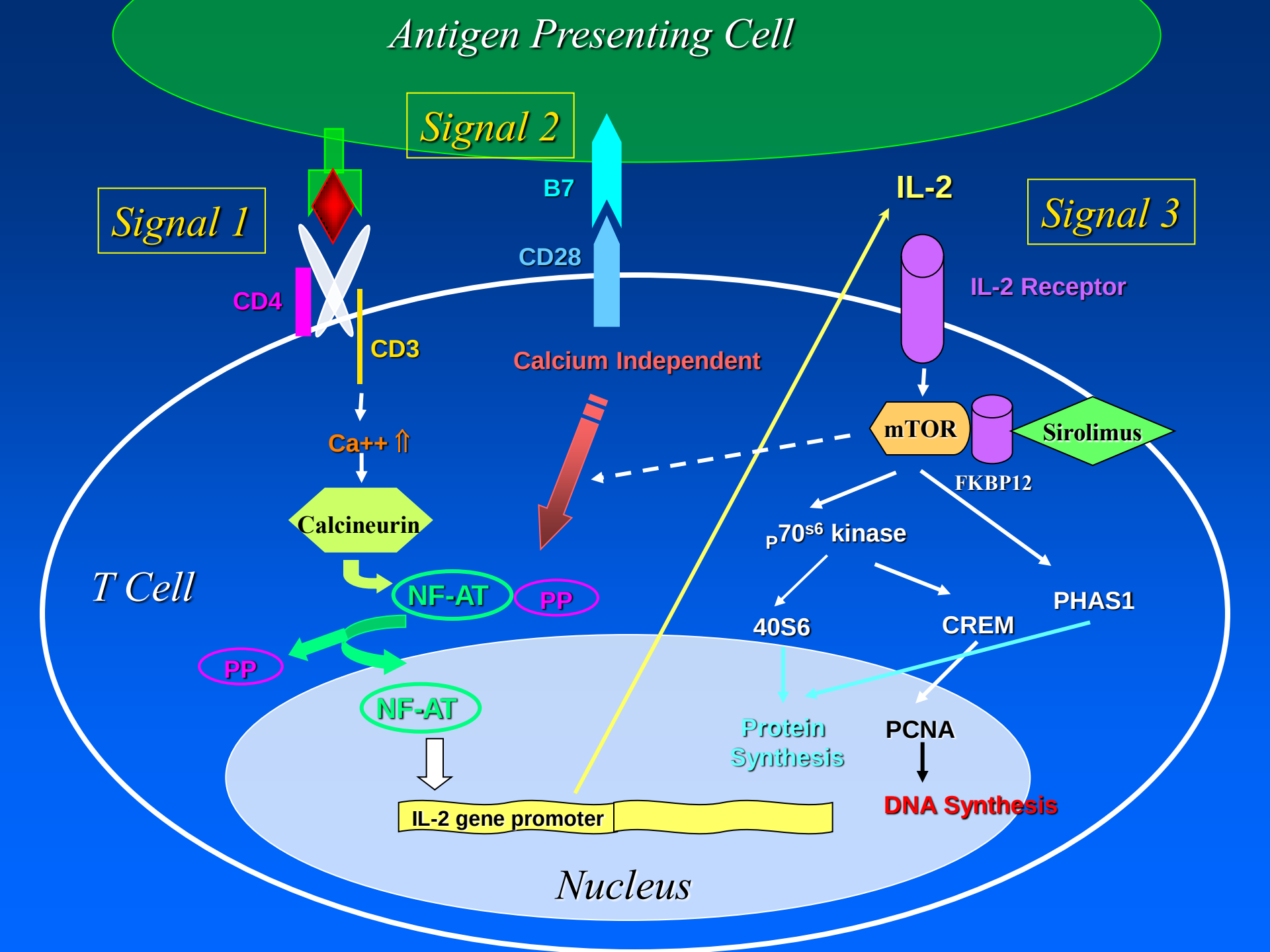
Causes of Allosensitization

- 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

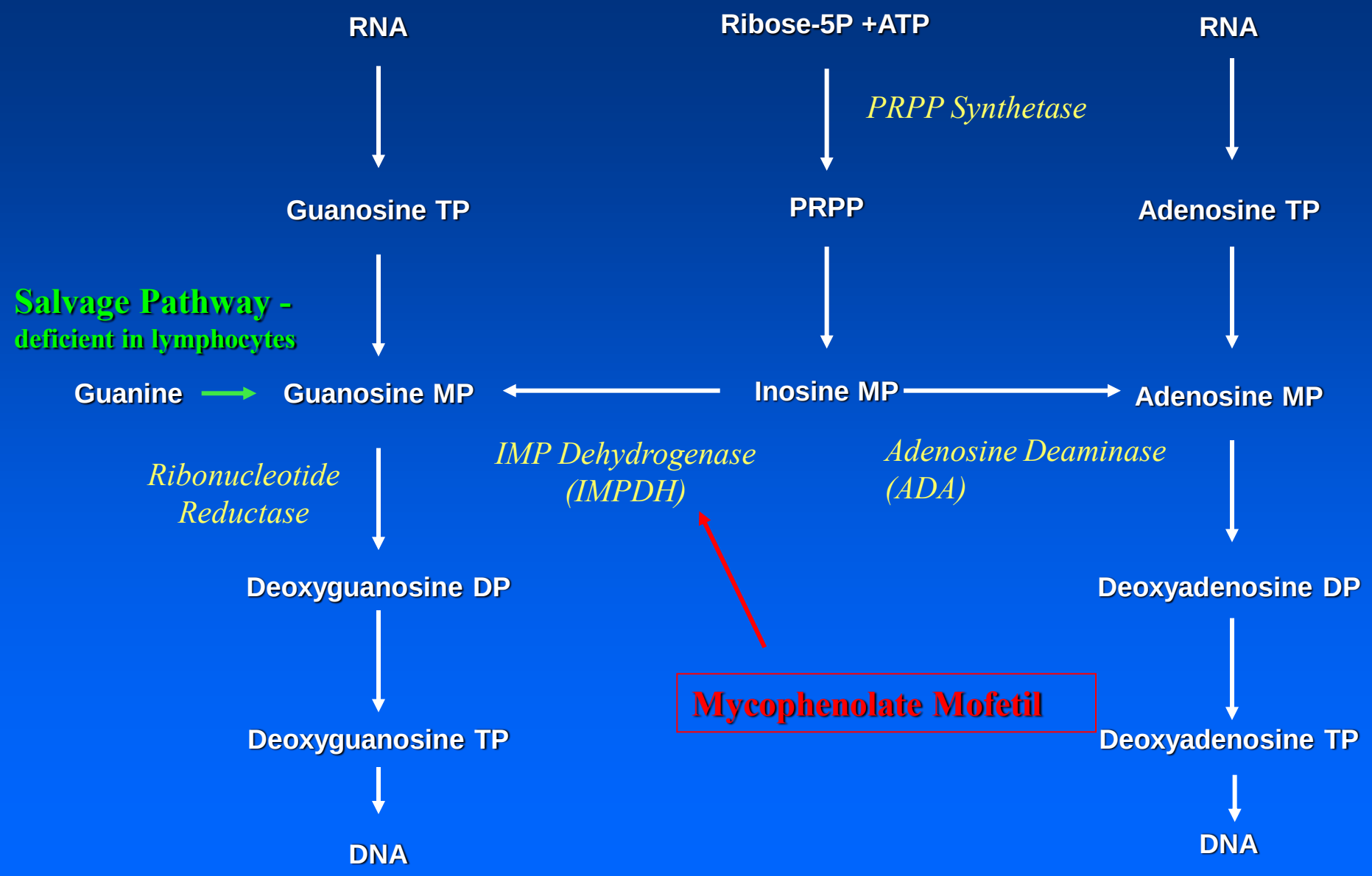

Antigen Presenting Cell
Signal 2
Signal 1
B7
CD28
IL-2
Signal 3
CD4
CD3
IL-2 Receptor
Calcium Independent
Ca++ ⇑
Calcineurin
T Cell
NF-AT
PP
PP
Cell
Cycle
NF-AT
IL-2 gene promoter
Nucleus


Antigen Presenting Cell
Signal 1
CD4
CD3
IL-2
NF-kB
IkB
Steroid
GR
Steroid
GR
AP-1
X
IkB promoter
IL-2 promoter
Nucleus


Antigen Presenting Cell
Signal 1
CD4
CD3
IL-2
Ca++ ⇑
CyA
Cyp A
FKBP12
Calcineurin
Tacrolimus
T Cell
NF-AT
PP
PP
NF-AT
IL-2 gene promoter
Nucleus

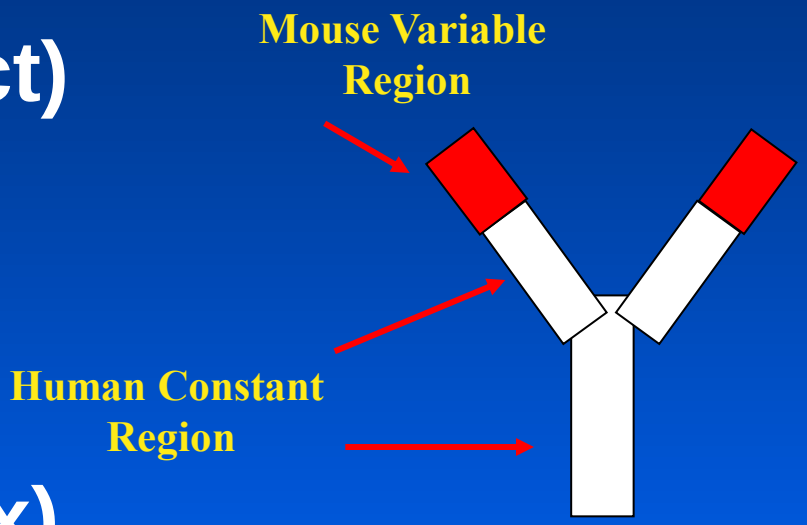

Antigen Presenting Cell
Signal 2
Signal 1
Signal 3
B7
CD28
IL-2
IL-2 Receptor
CD4
CD3
Calcium Independent
Ca++ ⇑
mTOR
Sirolimus
FKBP12
Calcineurin
P70S6 kinase
T Cell
NF-AT
PP
PP
40S6
CREM
PHAS1
NF-AT
Protein Synthesis
PCNA
DNA Synthesis
IL-2 gene promoter
Nucleus

De Novo Pathway of Purine Synthesis

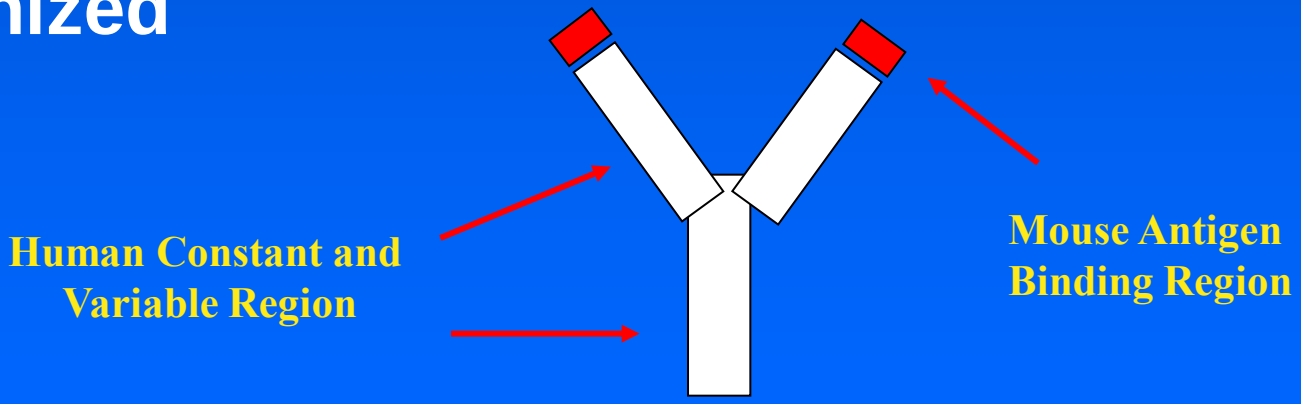


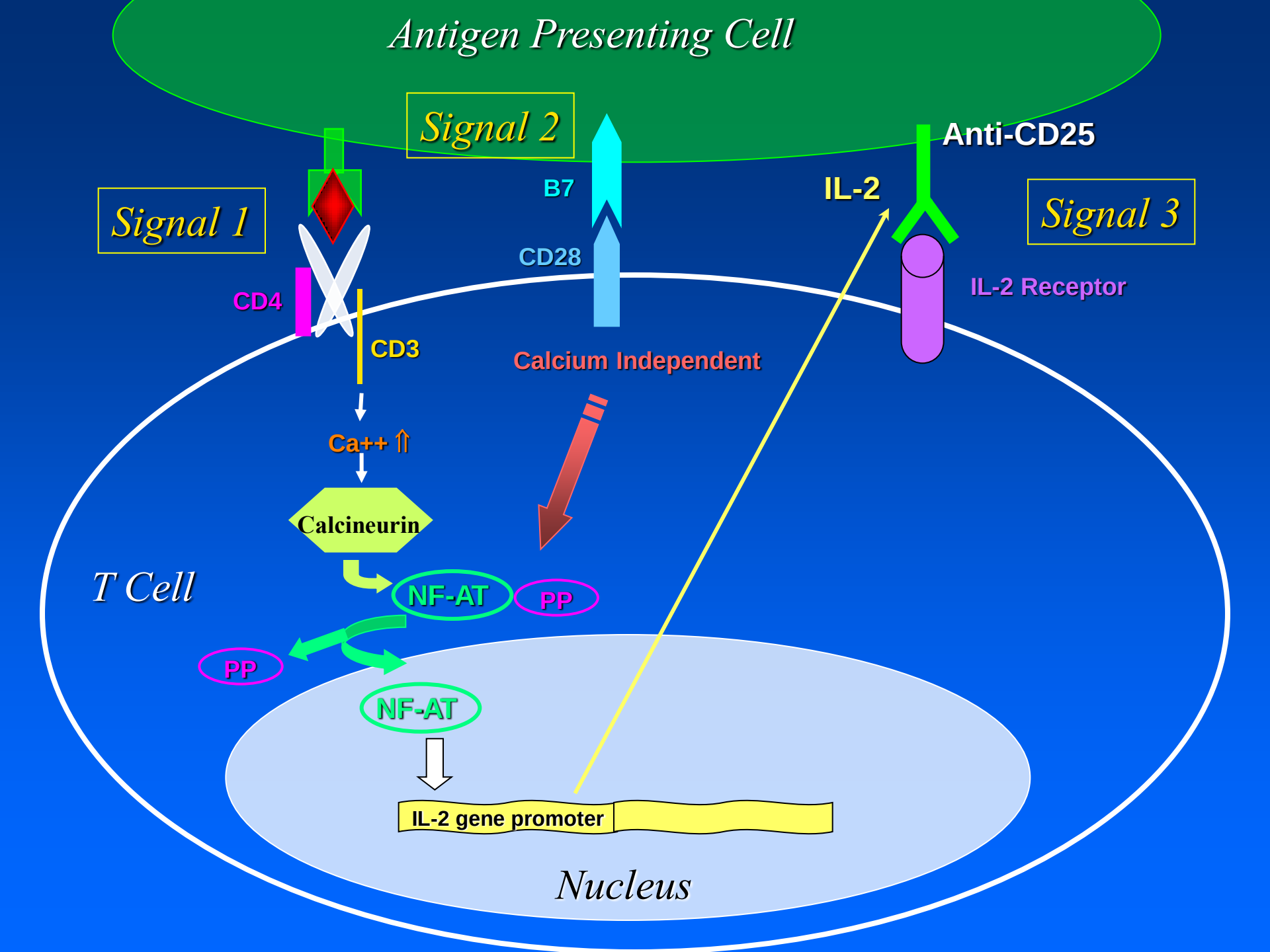
Anti-CD25 Monoclonal Antibodies

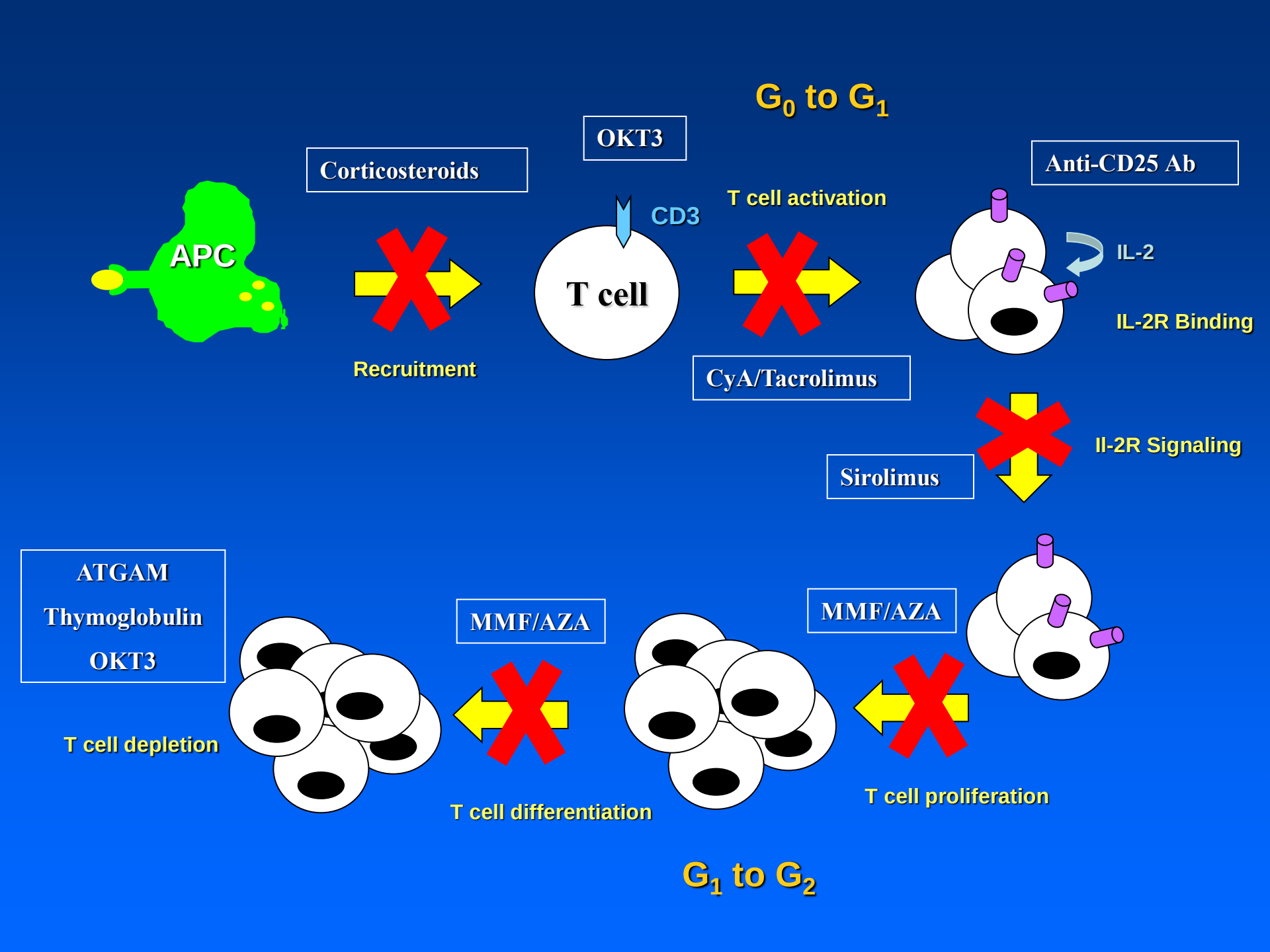
- **Basiliximab (Simulect)**
  - **chimeric**

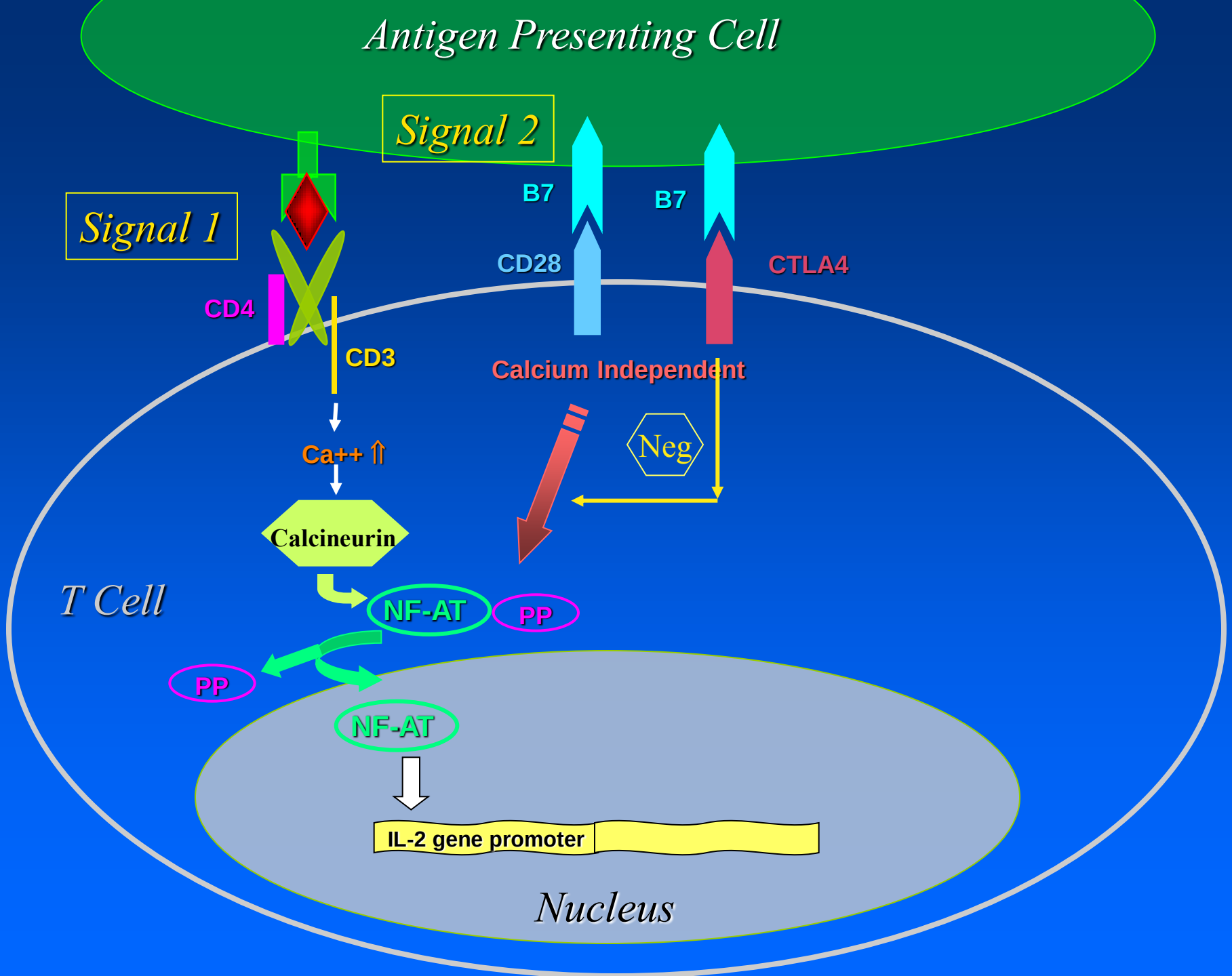


- **Daclizumab (Zenapax)**
  - **humanized**

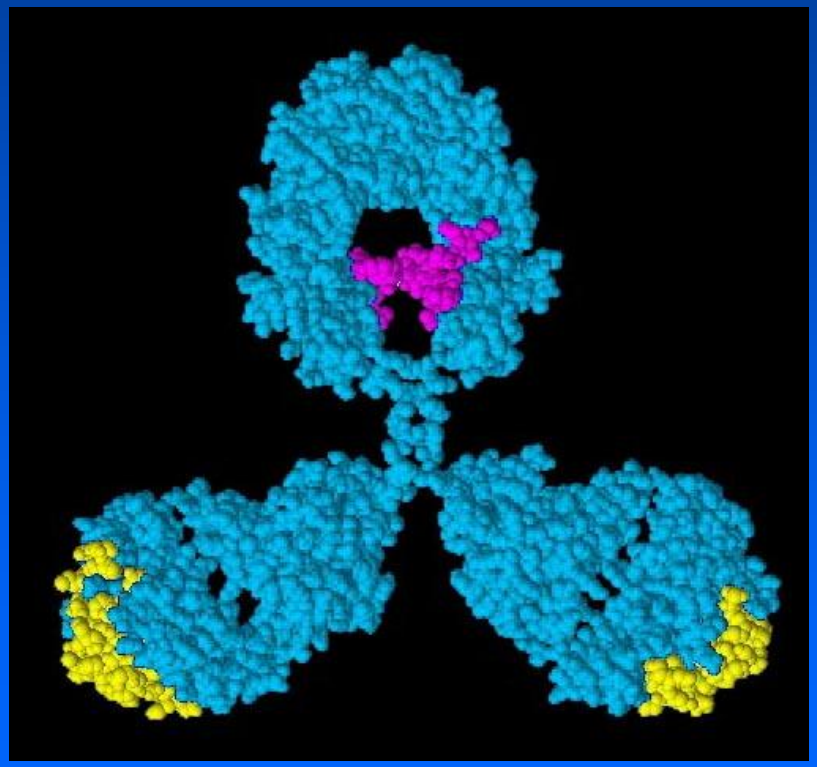



Antigen Presenting Cell
Signal 2
Signal 1
B7
CD28
Anti-CD25
IL-2
Signal 3
IL-2 Receptor
CD4
CD3
Calcium Independent
Ca++ ⇑
Calcineurin
T Cell
NF-AT
PP
PP
NF-AT
IL-2 gene promoter
Nucleus


G0 to G1
OKT3
Corticosteroids
Anti-CD25 Ab
APC
CD3
T cell activation
T cell
IL-2
IL-2R Binding
Recruitment
CyA/Tacrolimus
Sirolimus
Il-2R Signaling
ATGAM
Thymoglobulin
OKT3
MMF/AZA
MMF/AZA
T cell depletion
T cell differentiation
T cell proliferation
G1 to G2

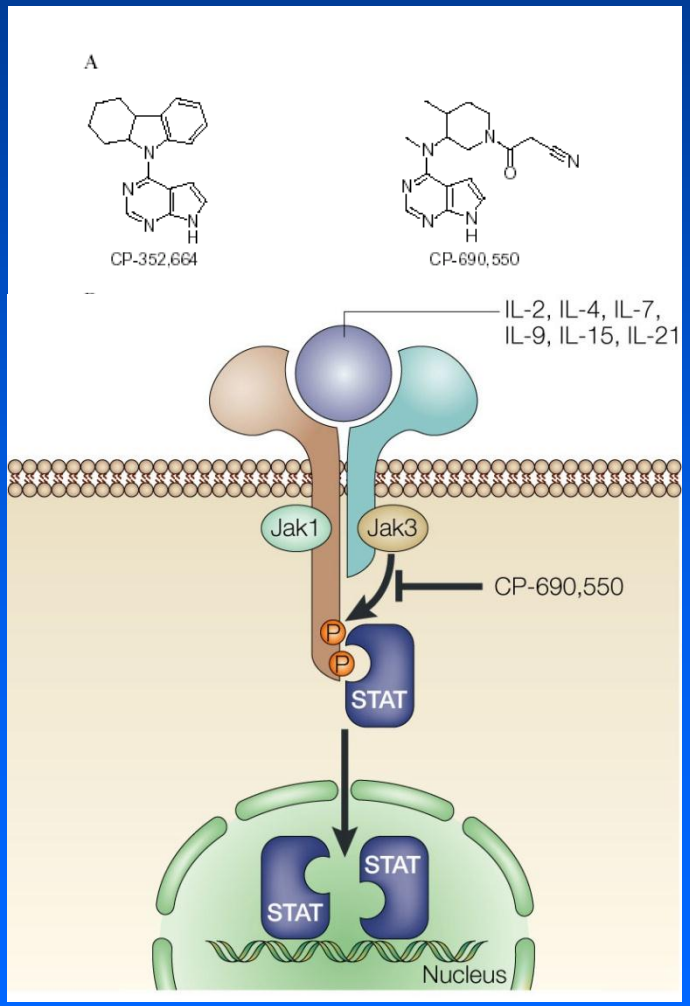

Antigen Presenting Cell
Signal 2
Signal 1
B7
B7
CD28
CTLA4
CD4
CD3
Calcium Independent
Neg
Ca++ ⇑
Calcineurin
T Cell
NF-AT
PP
PP
NF-AT
IL-2 gene promoter
Nucleus

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.

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

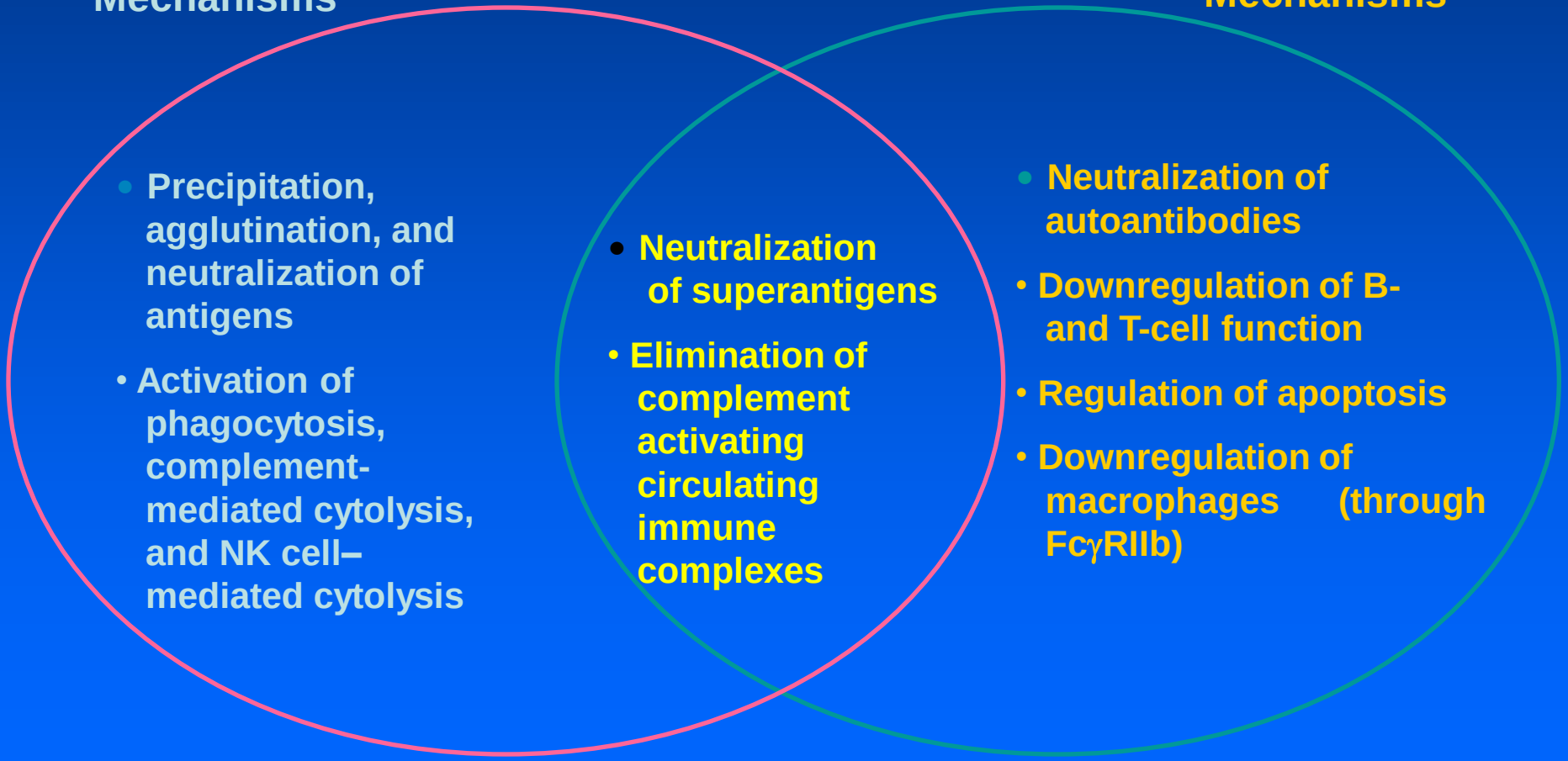
- Splenectomy
- Plasmapheresis
- Rituximab (anti-CD20 mAb)
- Intravenous Immunoglobulin (IVIG)
- Bortezomib

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

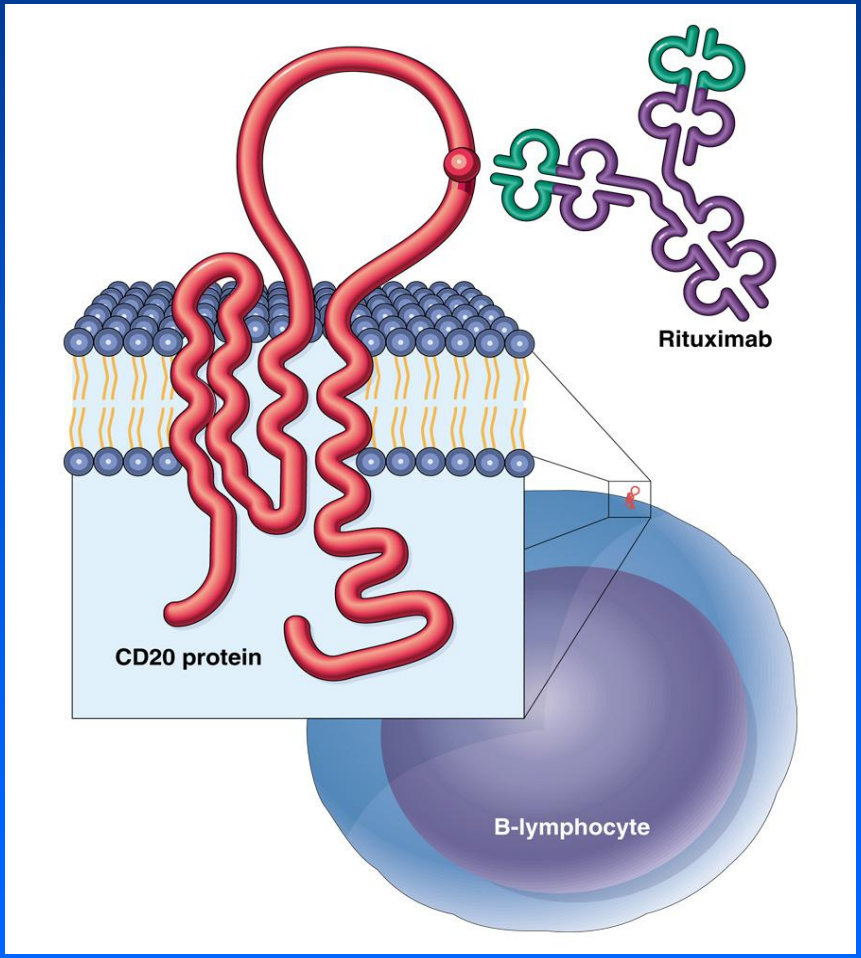
Mechanisms of action may overlap



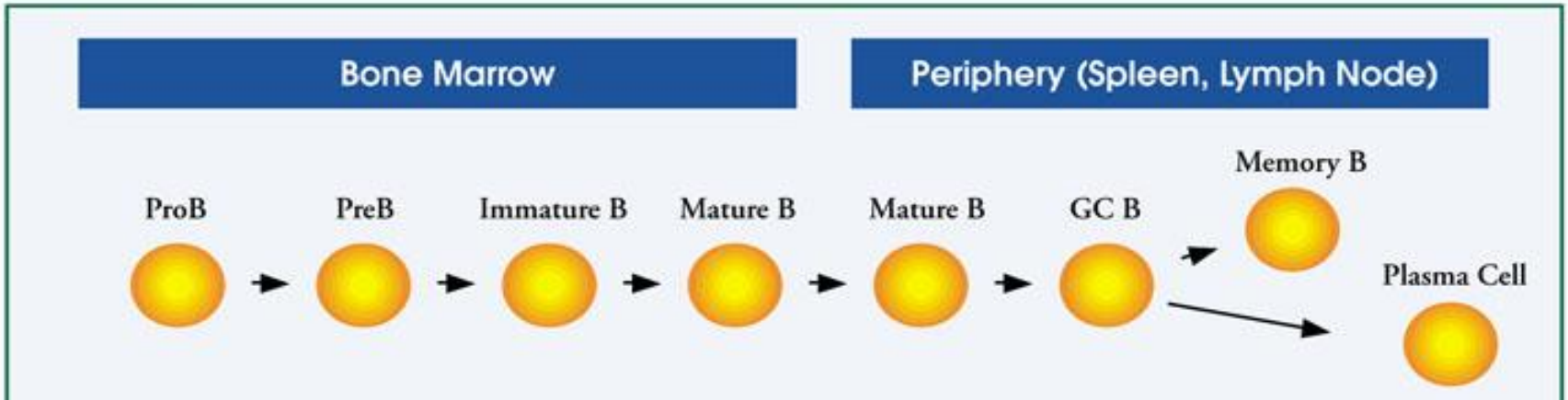
Kazatchkine MD, Kaveri S. N Engl J Med. 2001;345:747-755.

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



	ProB	PreB	Immature B	Mature B	Mature B	GC B	Memory B	Plasma Cell
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

Additional References

More History
http://nobelprize.org/nobel_prizes/medicine/laureates/1990/murray-lecture.html

Evolving understanding of antigen presentation in transplantation
L. A. Smyth, B. Afzali, J. Tsang, G. Lombardi, R. I. Lechler
Intercellular Transfer of MHC and Immunological Molecules:
Molecular Mechanisms and Biological Significance
American Journal of Transplantation 2007; 7 (6): 1442–1449.
doi:10.1111/j.1600-6143.2007.01816.x

Tolerance induction: why, and where are we?
Pierson RN 3rd. Tolerance in heart transplantation: the Holy Grail, or an attainable goal?
Heart Fail Clin. 2007; 3(1): 17-29. Review.

Current Status of Xenotransplantation
Pierson RN 3rd. Current Status of Xenotransplantation.
JAMA. 2009; 301 (9): 967-9.
Pierson RN 3rd, Dorling A, Ayares D, Rees MA, Seebach JD, Fishman JA, Hering BJ, Cooper DK. Current status of xenotransplantation and prospects for clinical application.
Xenotransplantation. 2009; 16(5): 263-80.

The History of Transplantation

Alexis Carrel, 1908 (1912 Nobel prize)
Technique of anastamosis

Sporadic clinical and exptl. efforts, 1910-1950
Isografts functioned indefinitely
Allografts functioned for days or weeks
exhibited "rejection"
Xenografts failed in minutes/hours/days

Clinical Transplantation

"Antibodies" defined (1930's -60's)

Skin graft recipients; multiparous women

Agglutination, lysis of donor cells

Predicted immediate/early graft failure

Donor-Recipient "Cross-match"

Clinical Transplantation

"Antibodies" defined (1930's -60's)

Skin graft recipients; multiparous women

Agglutination, lysis of donor cells

Predicted immediate/early graft failure

Donor-Recipient "Crossmatch"

"tissue type"

Inherited "antigens", one from each parent

Major Histo-Compatibility antigens: "MHC"

# of mismatches predicted strength of anti-donor immune response

Clinical Transplantation

Cellular acceptance / rejection (1940's -60's)

Owen: RBC chimerism in twin cattle if placental link

Medawar, Burnett –

chimeric cattle: accept donor skin

Neonatal "Tolerance" 1953 (1962 Nobel)

Skin grafts to treat burn wounds, or in animals

first set 1-2 weeks

second set more rapid

Clinical Transplantation

Clinical context (1950's)

Primitive support for renal failure

Dialysis (Wilhelm Kolf): temporary

ethically fraught

Joseph Murray (Hume, Merrill)

Identical twin kidney txp 1954: dramatic,

life-saving; reproducible (1990 Nobel)

Allografts: technical success

recipient deaths

Acute rejection, infection

Clinical Transplantation

1960's "Birth of clinical txp"

Calne, others

Azathioprine (6MP): 20-40% 1 yr survival

Elion and Hitchings (1988 Nobel)

Reduced dependence on steroids, radiation

Starzl, Najarian, Russell/Monaco

Anti-lymphocyte, Anti-thymocyte Globulin

Reemtsma, Starzl, Najarian Xenografts

Chimpanzee, monkey kidneys "Heterografts"

Institute of Medicine: Brain Death definition

The History of Transplantation

1970's Improved techniques, new treatments

"Transfusion effect" (Intravenous donor antigen)

Sensitization vs improved acceptance

Immune monitoring

Caves, Shumway: heart biopsies

Renal biopsy

Starzl/Calne

Liver transplant technique

Borrel, Calne/White

Cyclosporine A: from test tube into patients

Results: 20-40% to 70% 1 year survival

The History of Transplantation

1980's "Balanced Immunosuppression"

Improved safety, efficacy

- **Explosion of activity**
- **Dramatic survival improvement**

Immune monitoring

- **Drug levels, echo, science, biopsies**

Cooper, Reitz **Lung, HL techniques**

Results: Nearly 90% 1 yr survival!

The History of Transplantation

1990's "Maturation"

Infection control

- Viral, bacterial: treatable!

Variety of drugs expands:

- FK 506, MMF

donor supply, older/sicker recipients

- Seat belt, MADD laws
- LVADs

Results: Plateau of survival, activity

The History of Transplantation

2000's "Continued Maturation"

Chronic rejection: cause, cure?

Tolerance trials

More new drugs:

mTOR inhibitors, αCD52 (CAMPATH)

Donor supply shrinking!

Alternatives

LVAD destination therapy

Stem cell, tissue engineering

(Xenografts)

Contact information

Your feedback is most welcome!

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