TRANSPLANTATION IMMUNOBIOLOGY FOR THE NEPHROLOGIST

Ιωάννης Γ. Γριβέας,MD,PhD

athens-NEPHROLOGY

DEFINITIONS

- Isograft (syngeneic)-identical to self
  - Identical twins
  - Inbred mice
- Allograft-between individuals of the same species
- Xenograft-between individuals of different species

STEPS IN ALLOGRAFT REJECTION

1-Recognition of alloantigen:

- T cell-APC interaction (importance of peripheral lymphoid organs for naïve T cell recognition)
- T cell-endothelial cell interaction

2-T cell activation:

- T cell costimulation
- Cytokine and chemokine production
- Proliferation and clonal expansion
- T helper cell function (CD4+ T cells)

STEPS IN ALLOGRAFT REJECTION

3-Effector mechanisms leading to graft destruction:

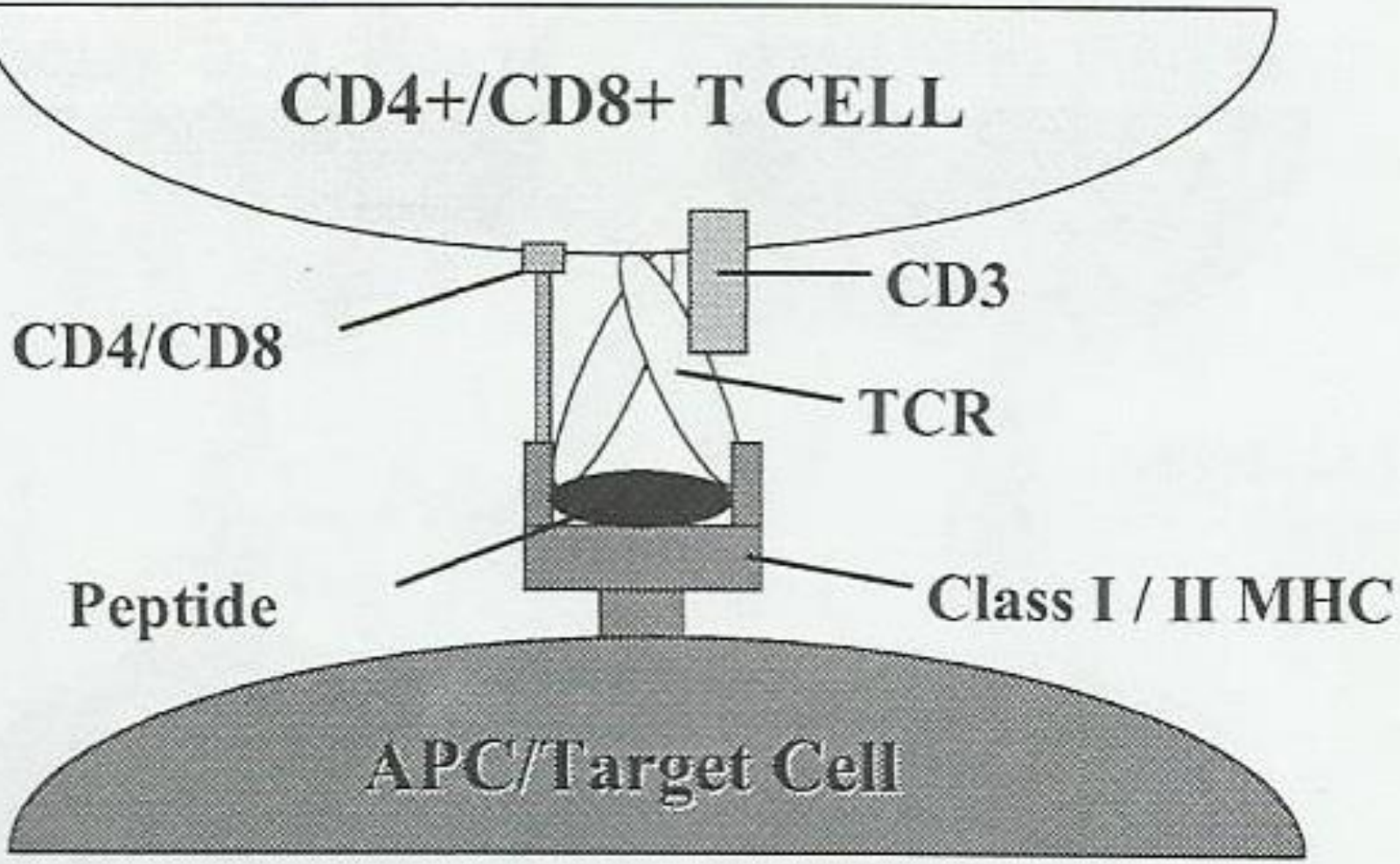
- Lymphocyte mediated cytotoxicity (CD8+ T cells)
- Alloantibodies: complement and cell mediated cytotoxicity
- Delayed type hypersensitivity (macrophages)

4-Resolution of the response with residual memory

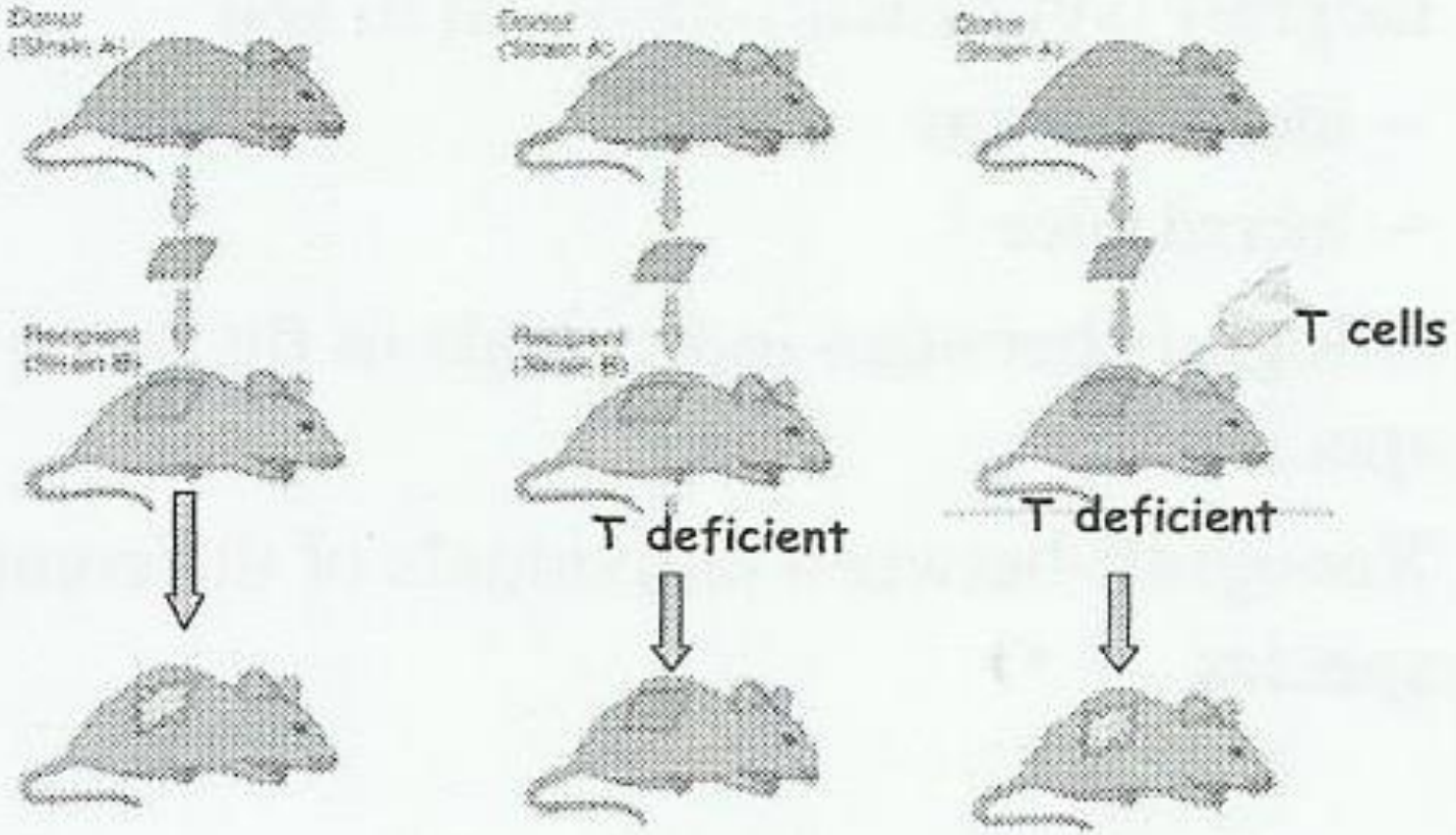
STEP ONE

Recognition of Alloantigen

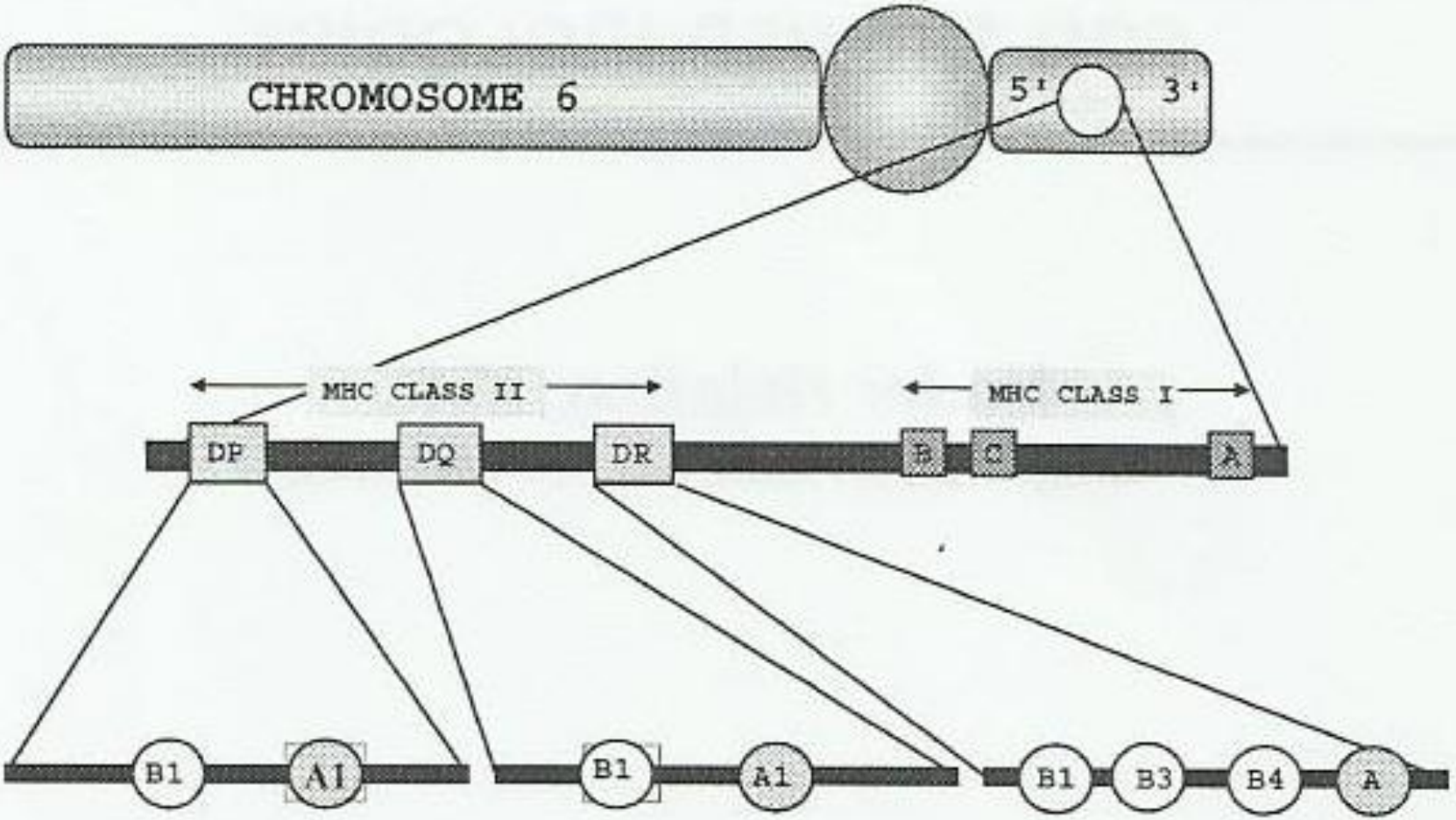
ANTIGEN RECOGNITION



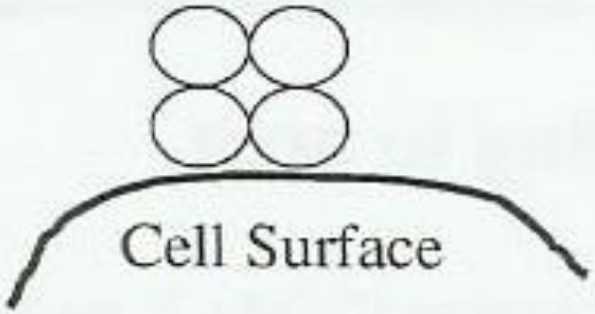
T Cells Play A Key Role in Rejection



HLA Complex


CHROMOSOME 6
5'
3'
MHC CLASS II
MHC CLASS I
DP
DQ
DR
B
C
A
B1
A1
B1
A1
B1
B3
B4
A

MHC (HLA) Antigens



Two Types

Class I

Class II

Several Loci

A,B,C

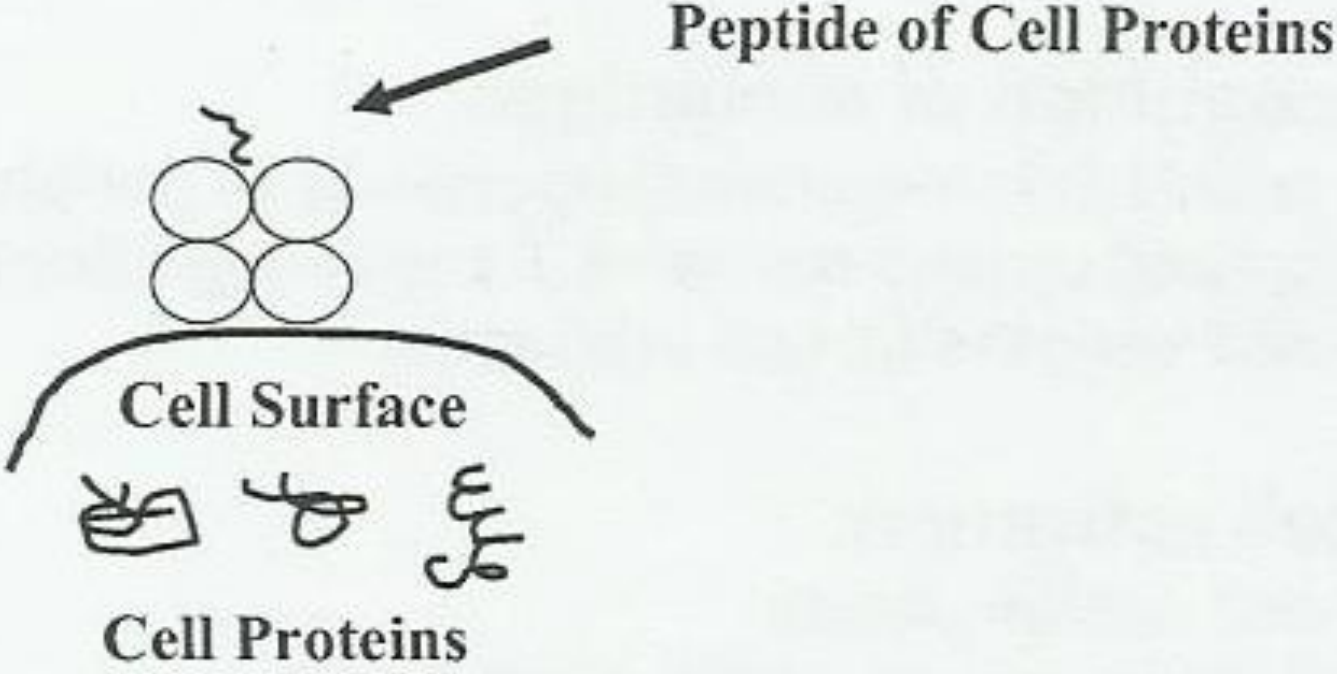
DP,DQ,DR

Many Alleles

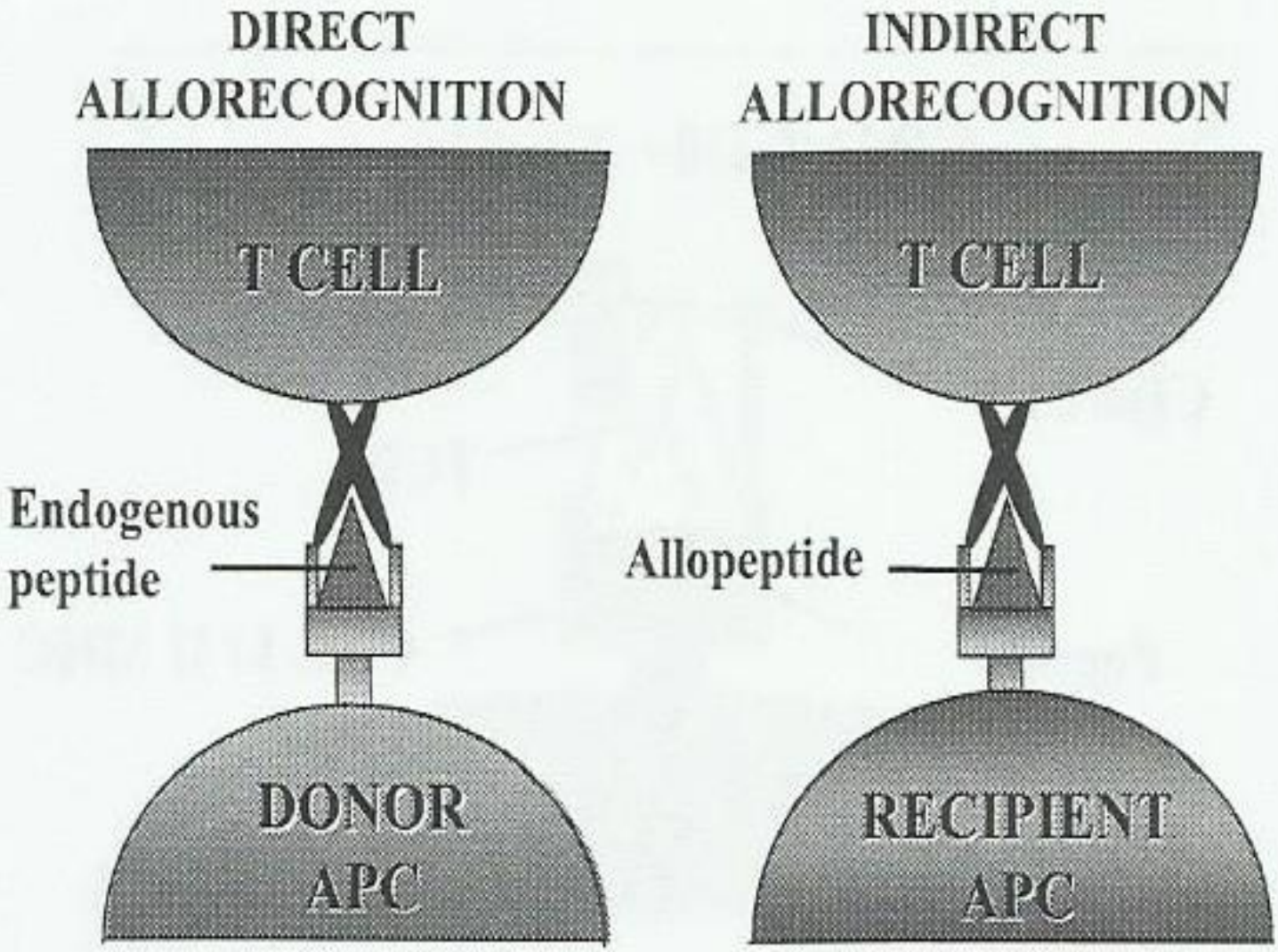
Polymorphism

Co-dominant Expression

The Function of MHC Molecules



ALLORECOGNITION

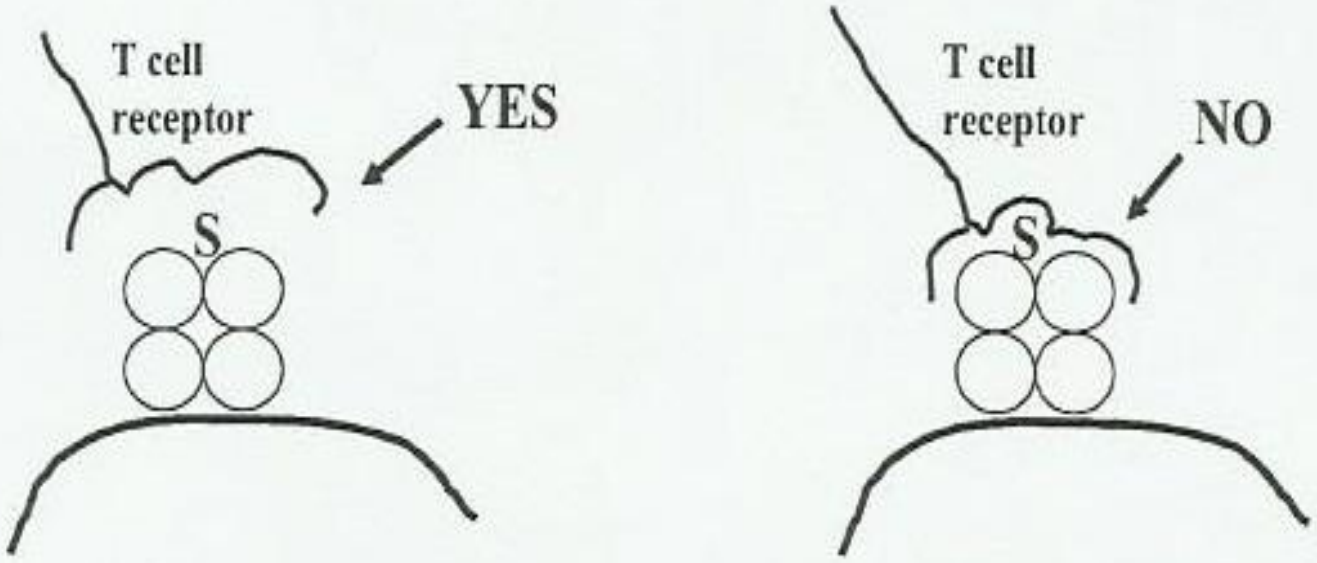


T Cell Selection in the Thymus

The repertoire of mature T cells is selected by two processes:

Positive Selection: only T cells with receptors that recognize something that looks like a self MHC molecule

Negative Selection: no T cells with receptors that recognize self MHC molecules too well (protects against autoimmunity)



Allogeneic MHC Antigens

Fundamental principle in immunology: T cells don't recognize foreign proteins directly, they recognize peptides of foreign proteins presented by self MHC molecules on antigen presenting cells

Allogeneic MHC molecules have the unique property that they look so much like self MHC molecules, but aren't, that T cells can recognize them "directly" as foreign

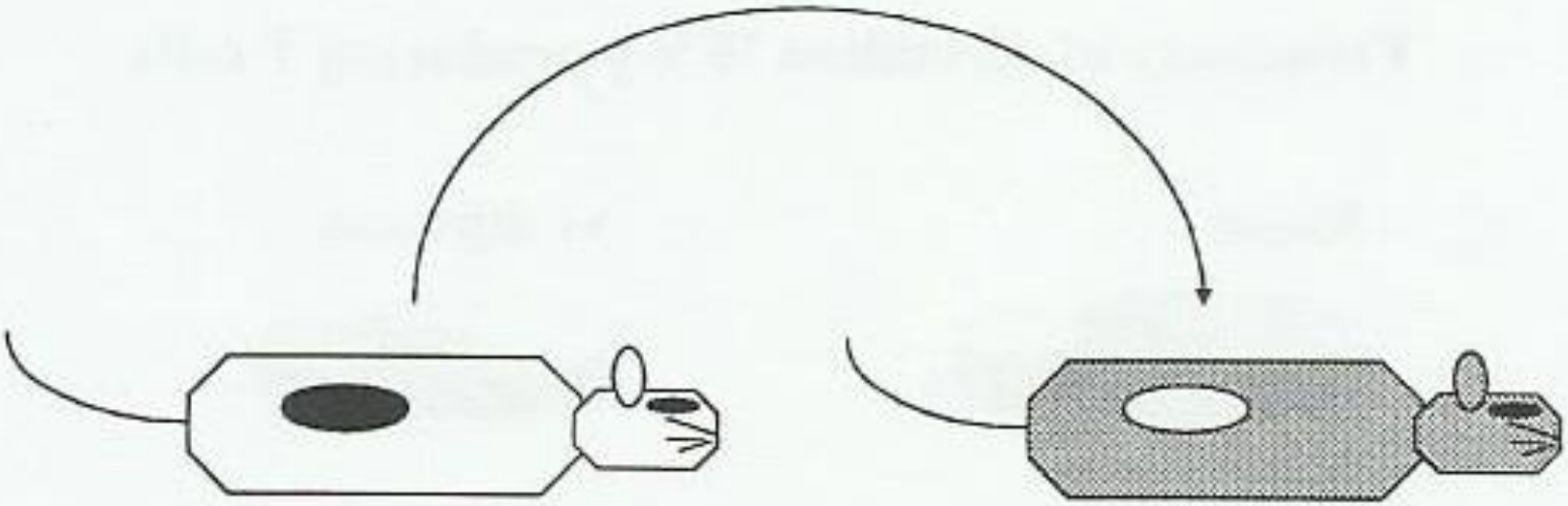
T Cell Precursor Frequency

The precursor frequency for T cells that recognize environmental pathogens: 1/10,000

The precursor frequency for T cells that recognize allogeneic MHC antigens: 1/100-1/1000

Because allogeneic MHC antigens can be recognized "directly", they give rise to a T cell response that is 100x more powerful than an ordinary T cell response!

MODEL -REJECTION BY INDIRECT ALLORECOGNITION

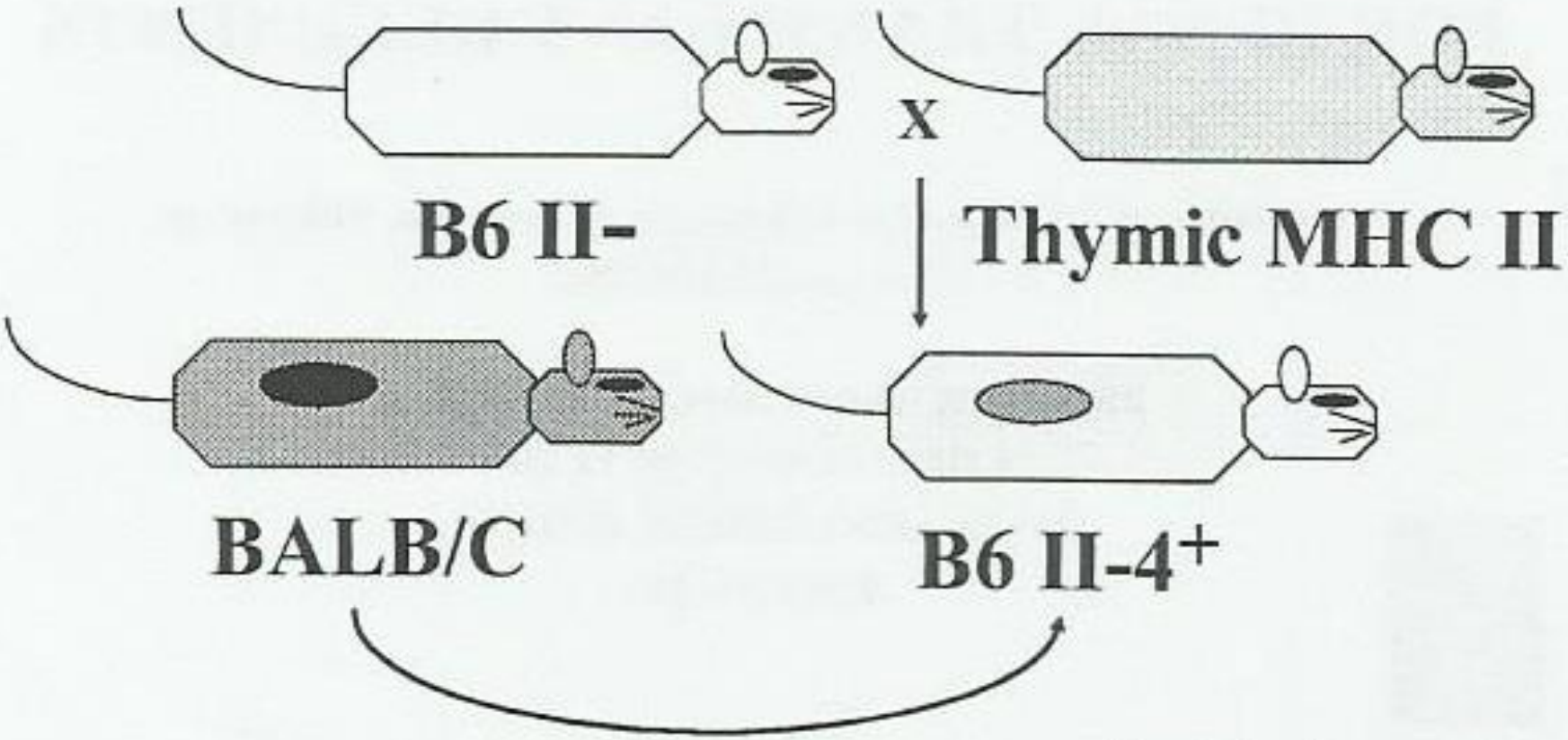


B6 II-
(Lack MHC Class II)

BALB/C

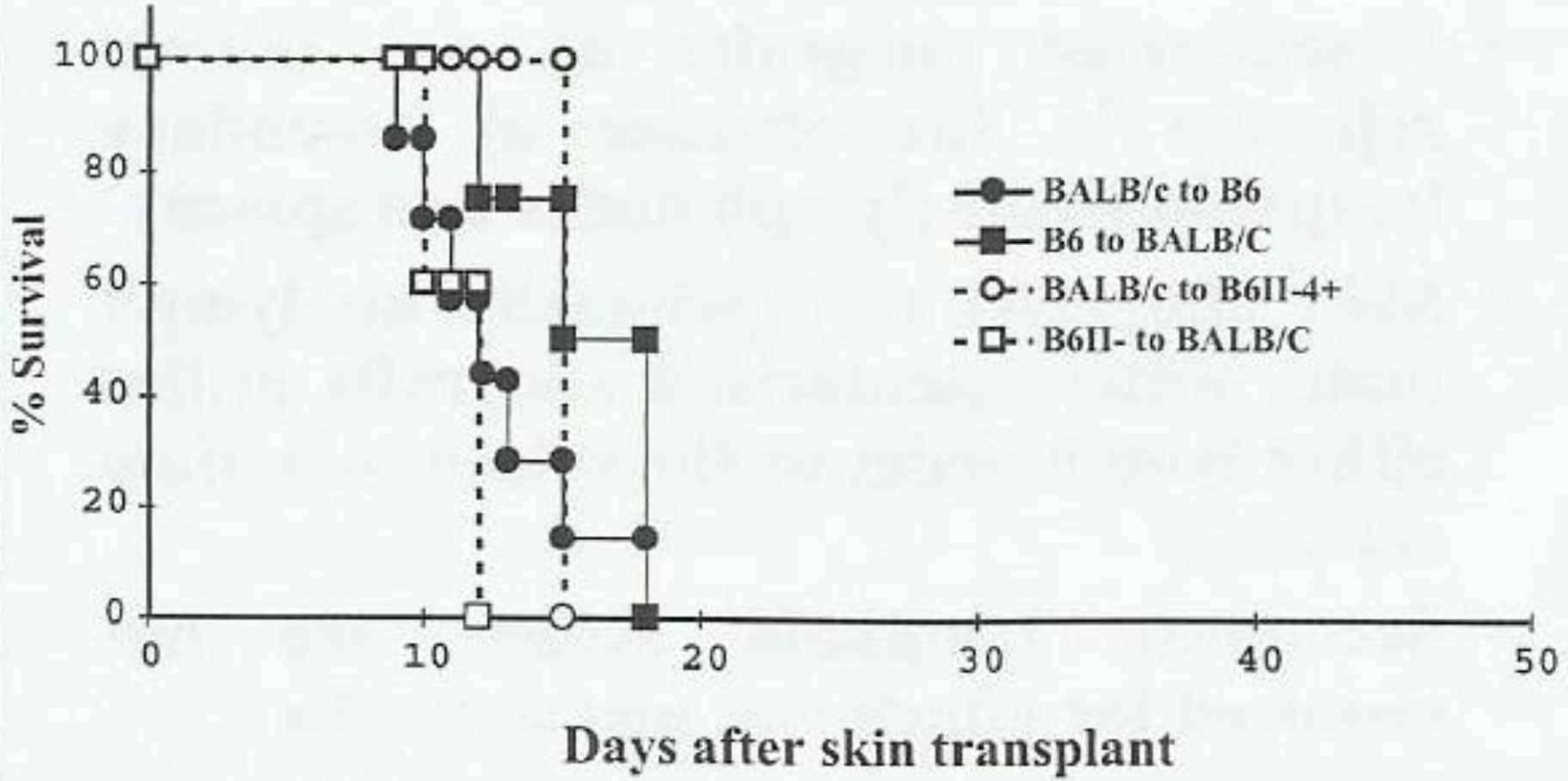
Auchincloss et al.

MODEL - REJECTION BY DIRECT ALLORECOGNITION

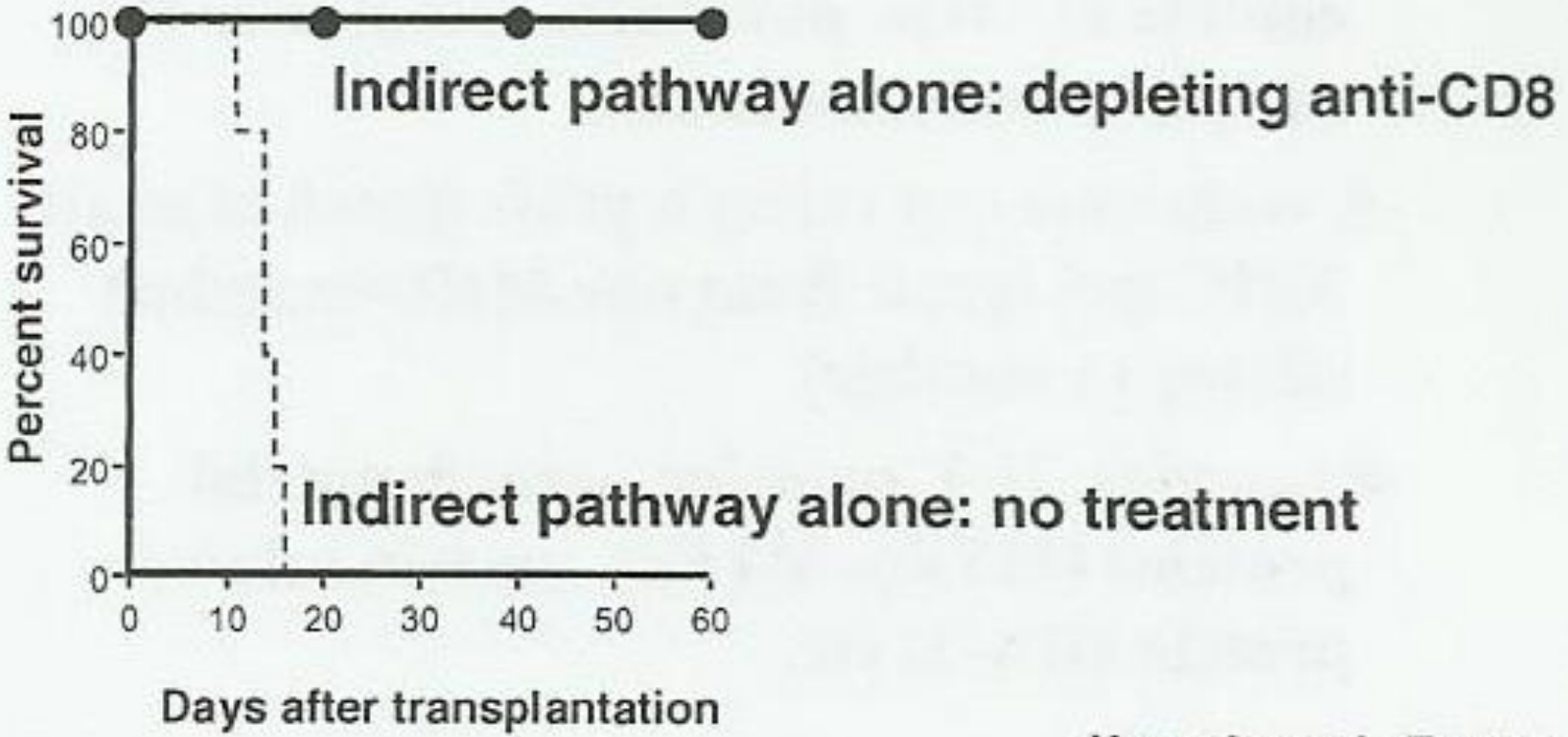


Auchincloss et al.

DIRECT & INDIRECT ALLORECOIGNITION MEDIATE GRAFT REJECTION



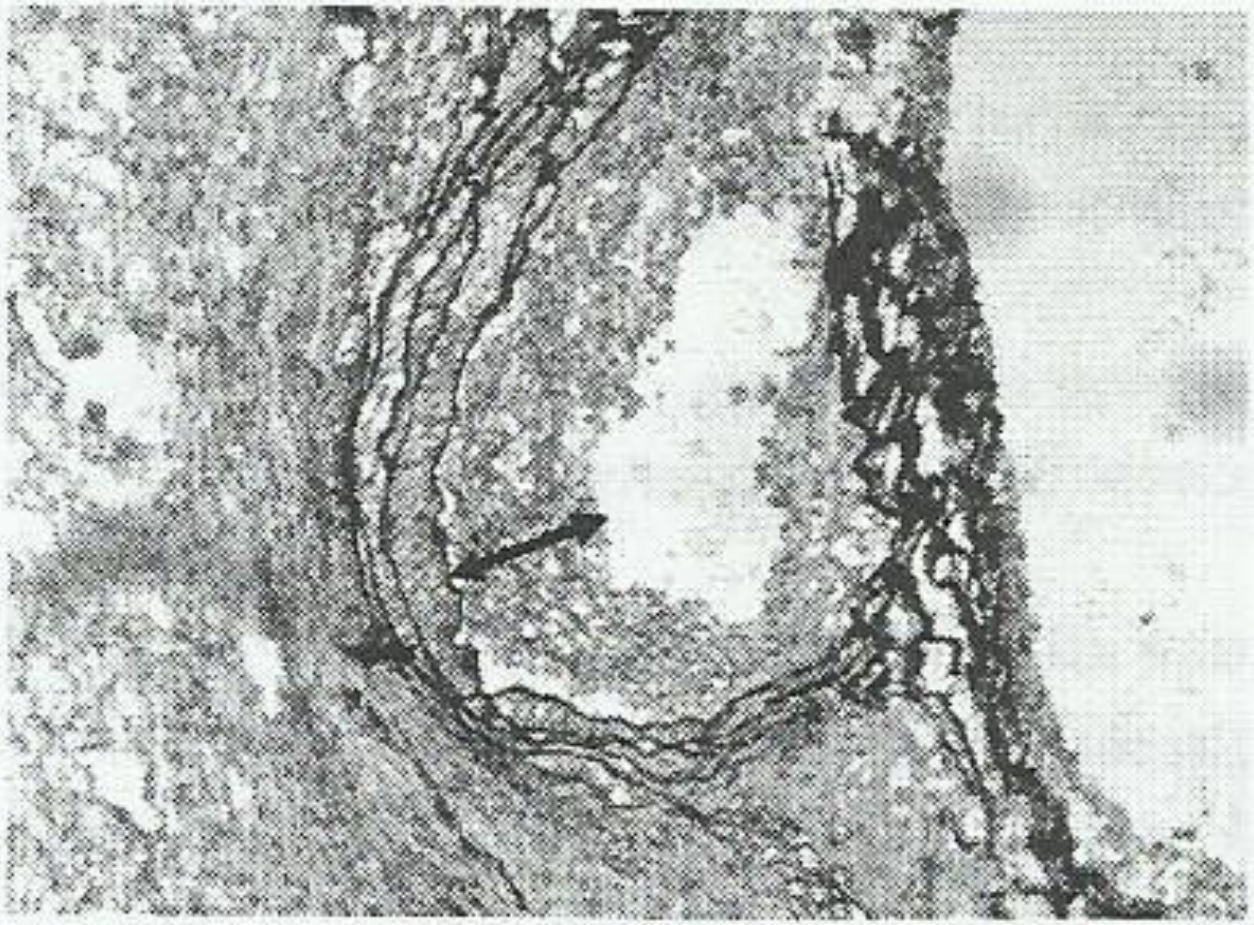
VASCULARIZED CARDIAC ALLOGRAFT REFECTION BY CD4+ T CELLS
- Indirect pathway alone -



Yamada et al., Tx 2001

CHRONIC ALLOGRAFT VASCULOPATHY

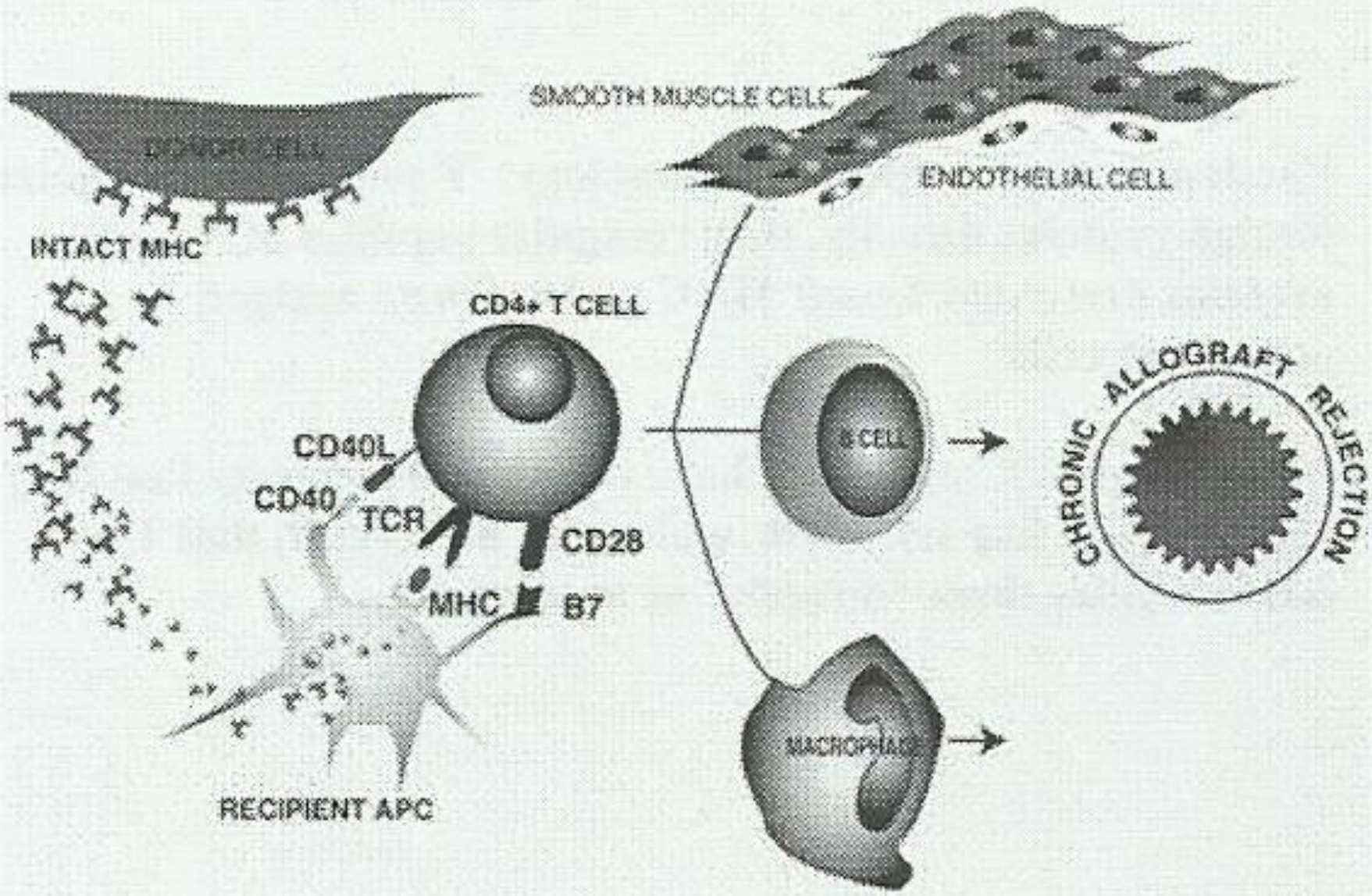
- Indirect pathway alone: CD4+ T cells -



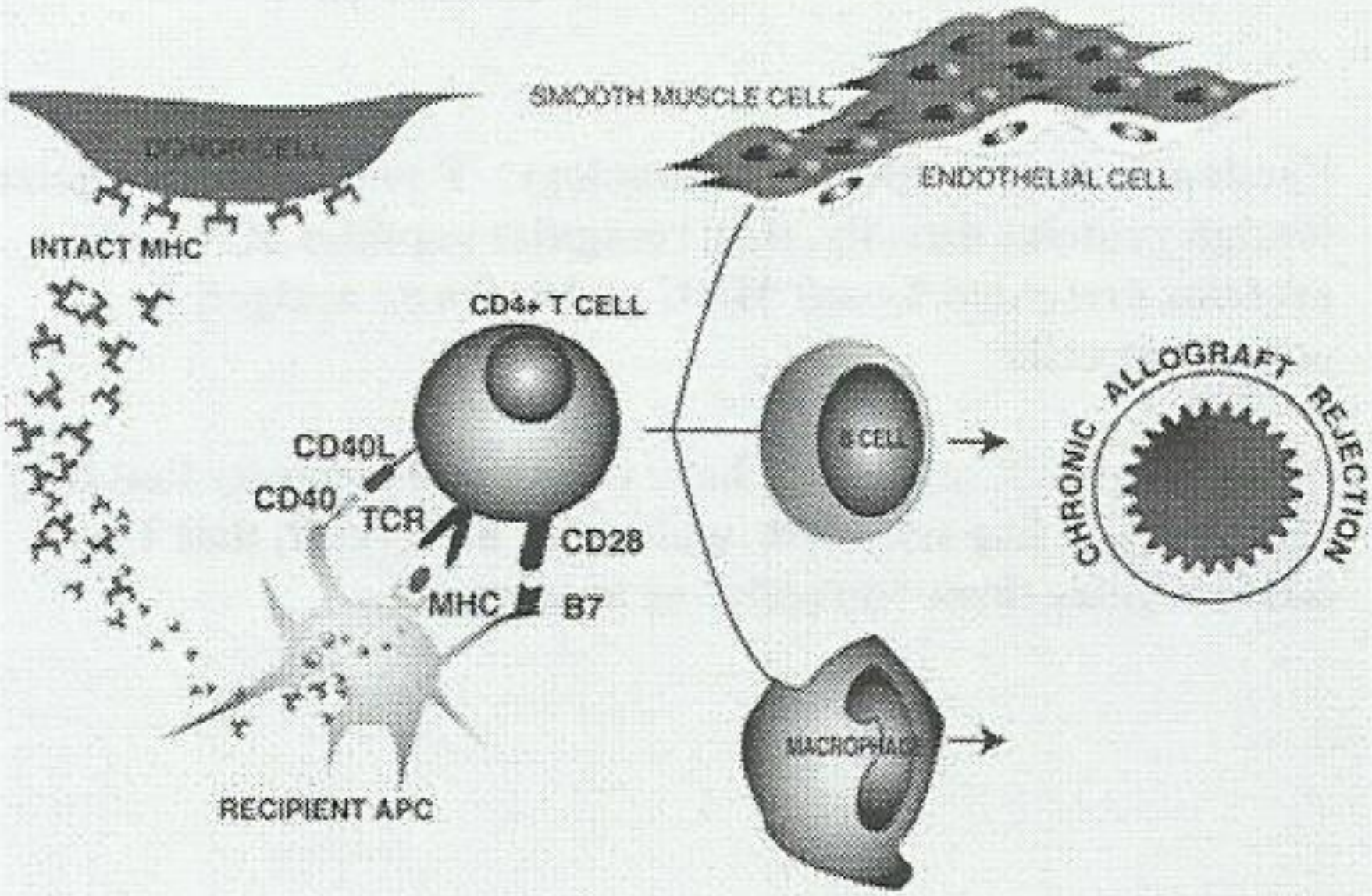
#860, day 236

Yamada et al., Tx 2001

INDIRECT ALLORECOGNITION AND CHRONIC REJECTION



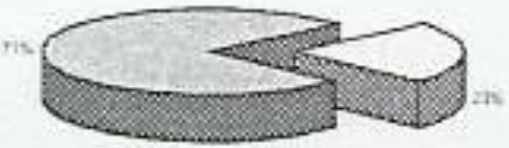
INDIRECT ALLORECOGNITION AND CHRONIC REJECTION



IMMUNE REACTIVITY IN STABLE AND HIGH RISK PATIENTS

Frequency of 60/million IFN-γ producing T cells

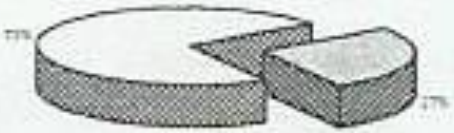
Stable



< 60 spots/million: 77%

> 60 spots/million: 23%

>1 rejection



< 60 spots/million: 27%

> 60 spots/million: 73%

p=0.02 by two tailed Chi squared test

Najafian et al JASN 2002

MINOR TRANSPLANTATION ANTIGENS

-Minor antigens are non-MHC, donor-derived peptide determinants expressed in the context of MHC molecules common to the recipient and the donor

-A recipients can reject a graft matched at all MHC loci (graft from one MHC-matched sibling to another)

-Examples: H-Y proteins, mitochondrial proteins (MTFα, MTFβ), myosin related protein (HA-2) etc.

ROLE OF LYMPHOID ORGANS IN GRAFT REJECTION

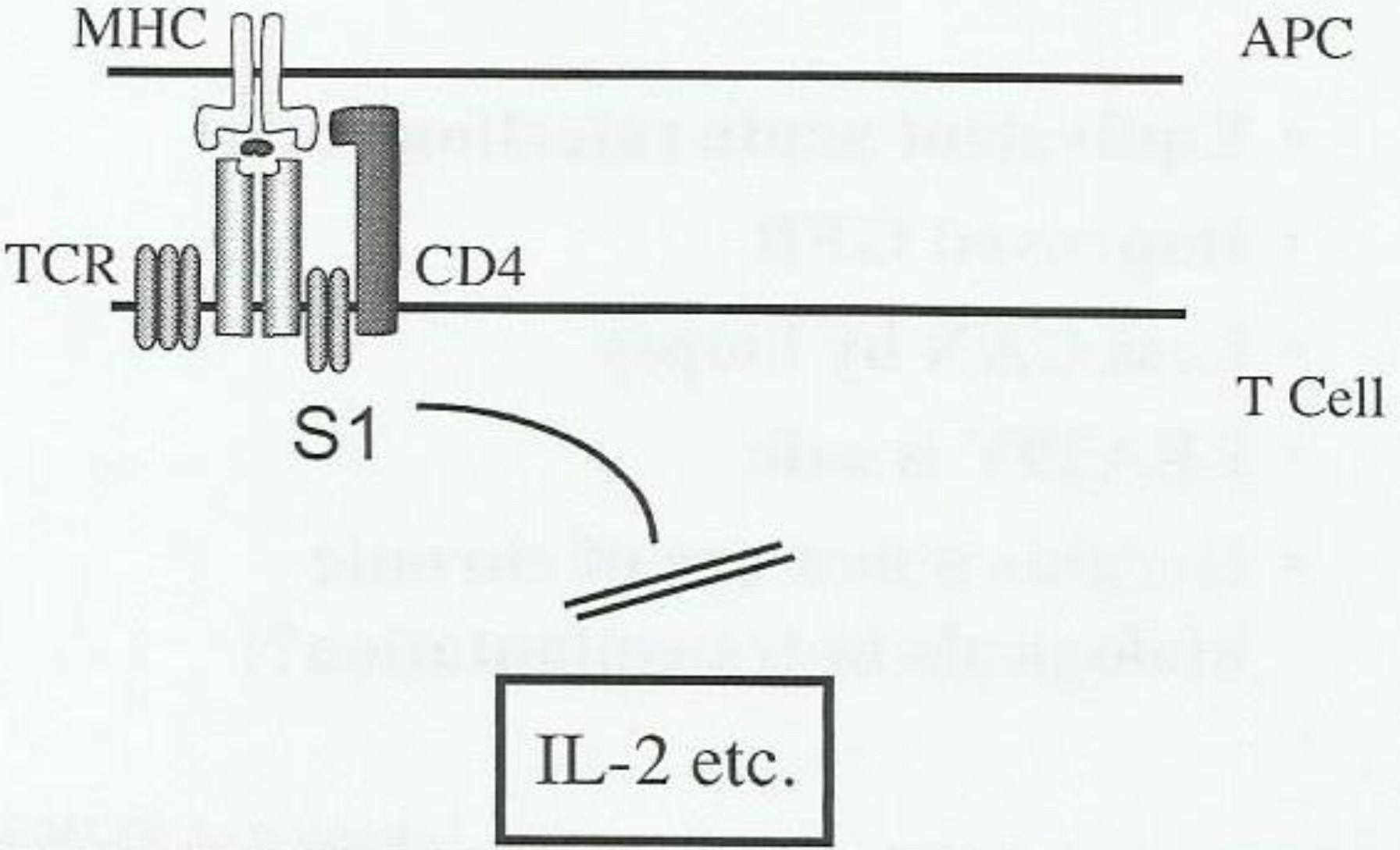
- Vascularized allografts do not initiate rejection in the absence of secondary lymphoid tissue (lymph nodes and spleen)
- Skin allografts rely primarily on lymph nodes while vascularized allografts utilize either lymph nodes or the spleen to initiate rejection
- Secondary lymphoid organs are not required for effector or memory cells

Lakkis et al. Nature Medicine 2000

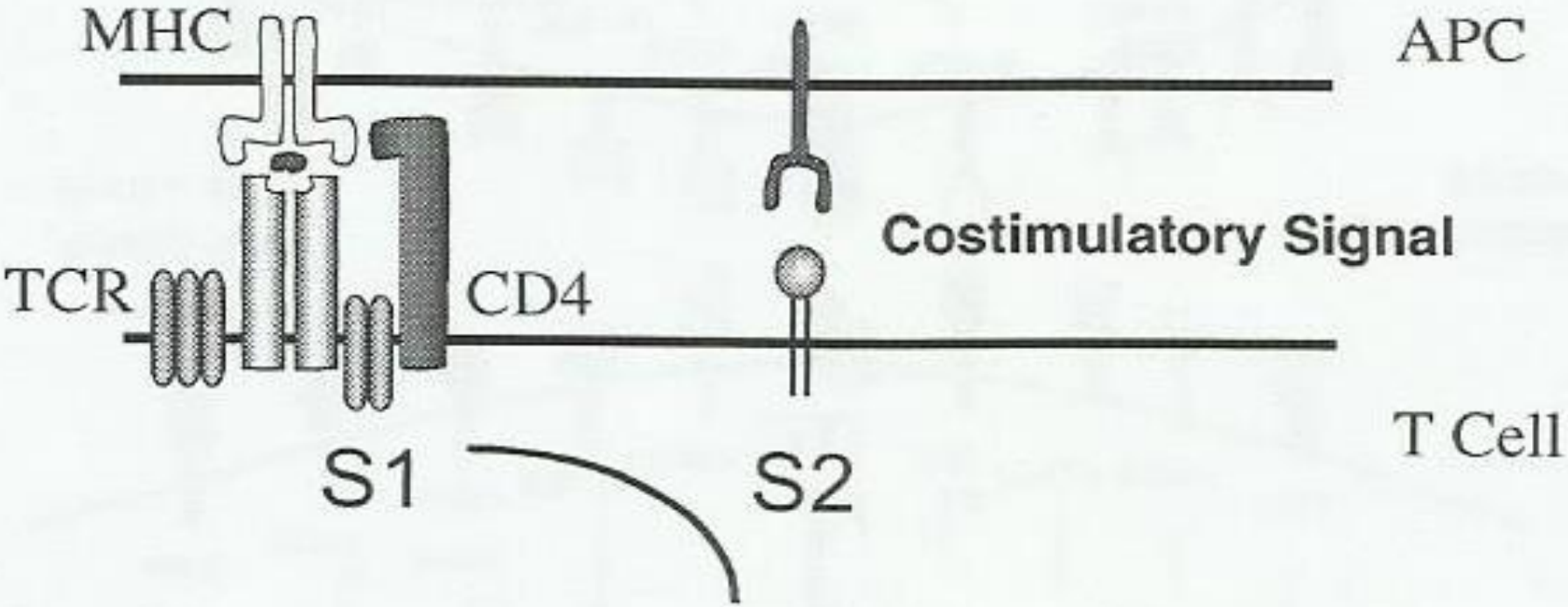
STEP TWO

T Cell Activation

T Cells Require 2 Signals



T Cells Require 2 Signals

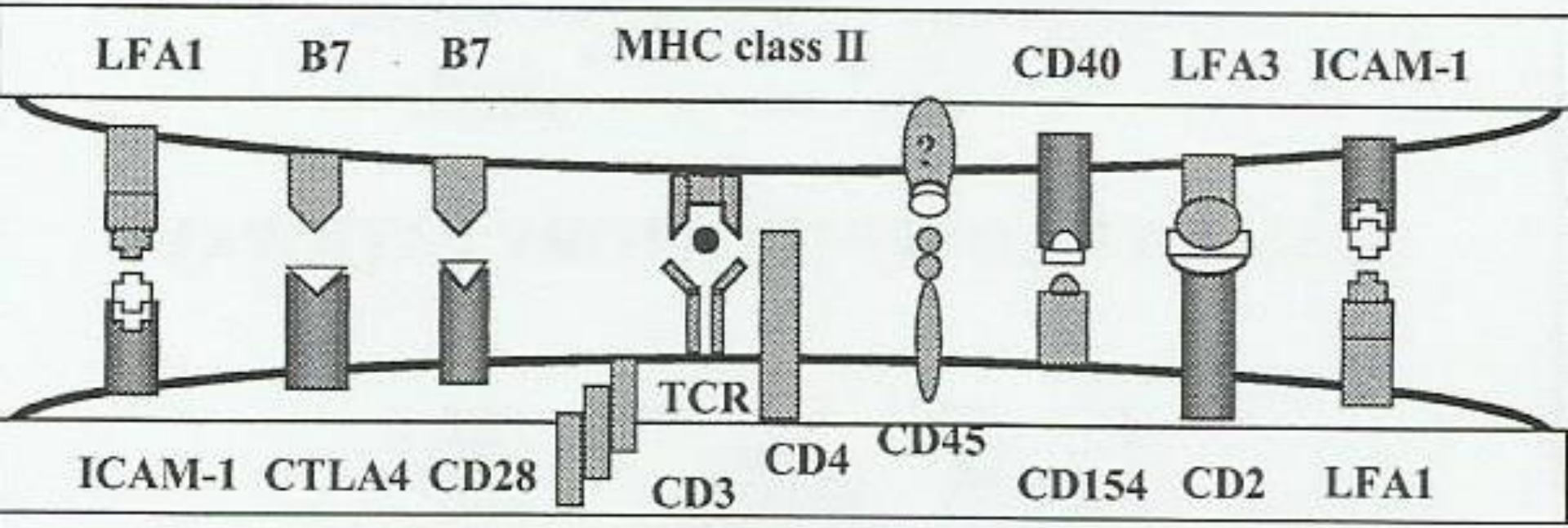


IL-2 etc.

T CELL COSTIMULATION

"The Happy days"

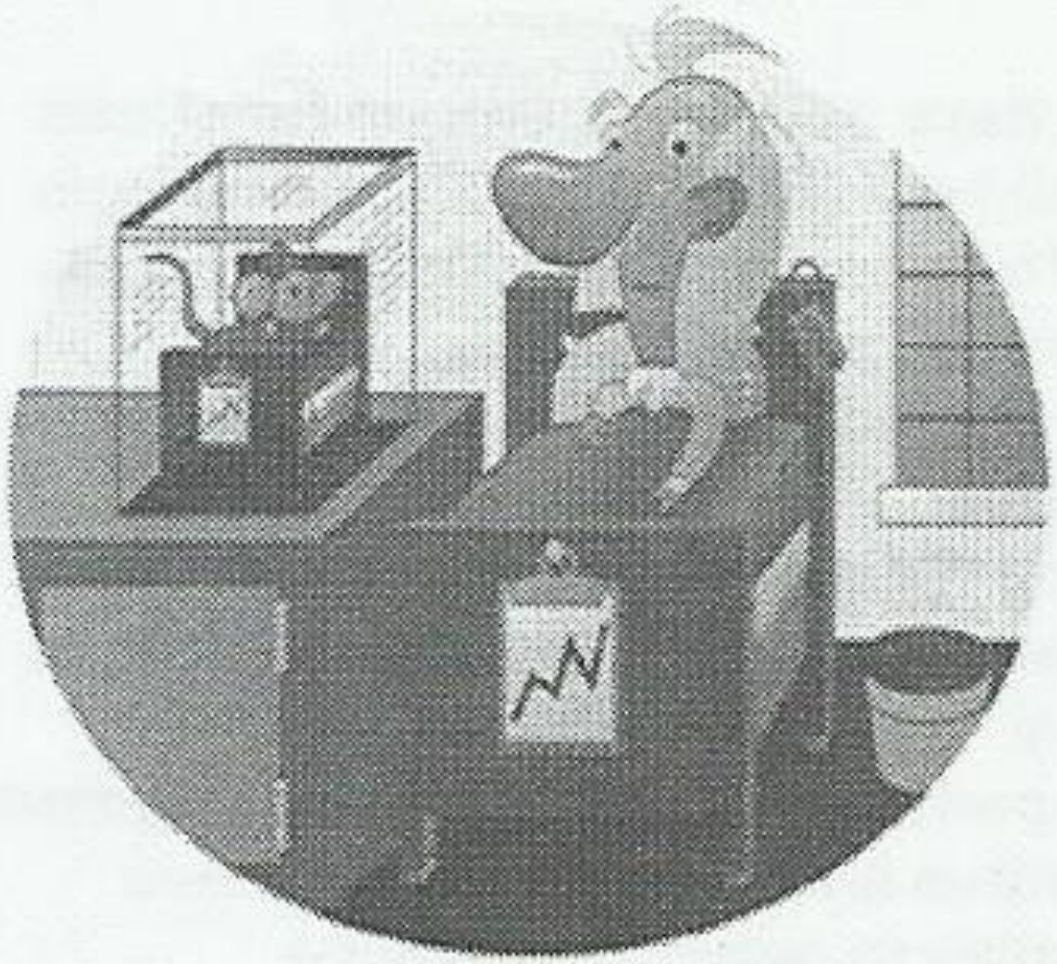
APC



T Cell

Denton et al., Lancet 1999

Clinical Development of T Cell Costimulatory Blockade



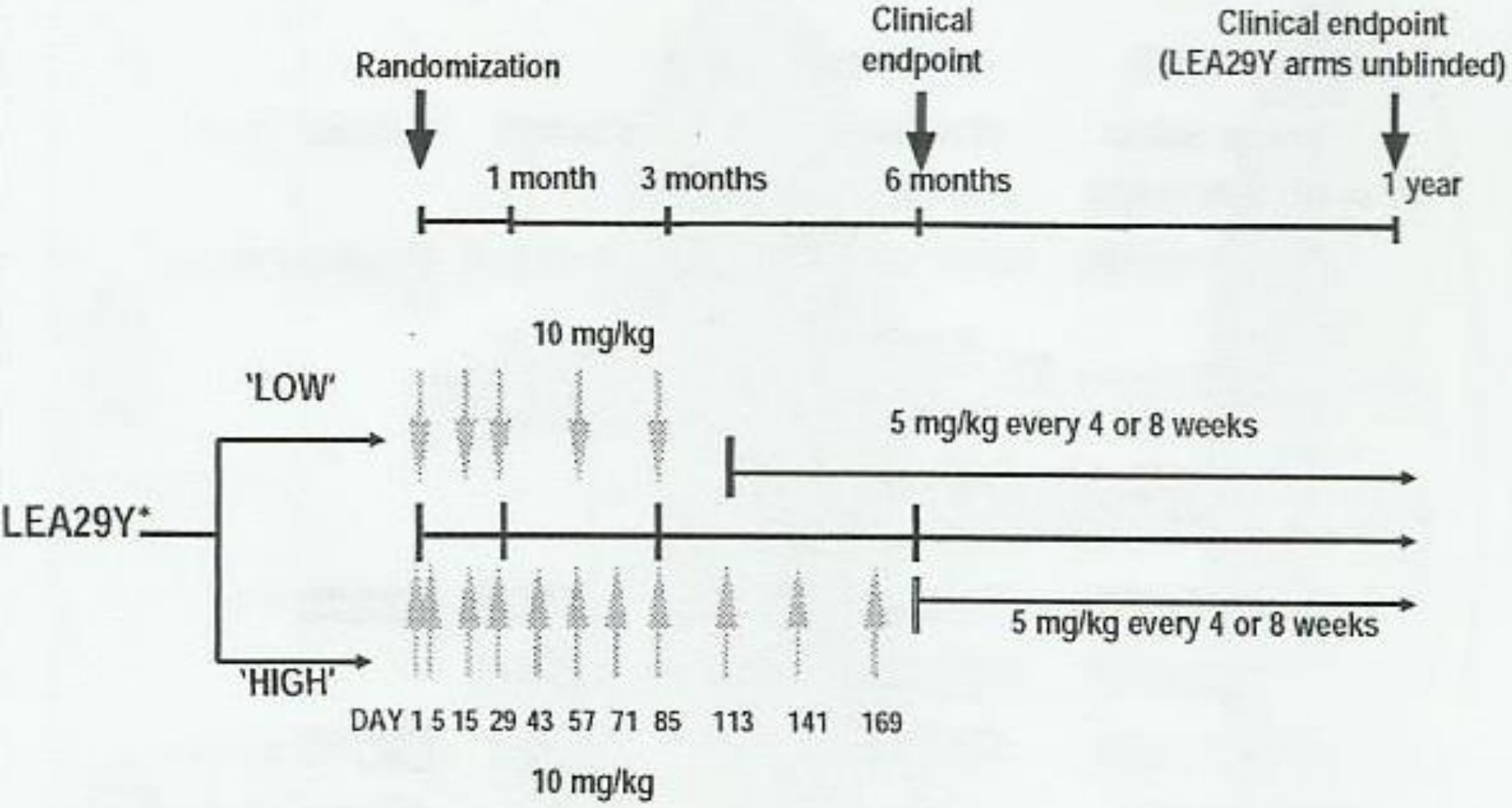
B7: Renal Tx
Lupus
MS
RA
?

CD154: ? Future

CD40: Pre-clinical

Others: ?

LEA29Y Phase II Dose-Finding Study Design



*All patients received MMF, basiliximab, and corticosteroid-tapering regimen

LEA29Y Phase II Trial: Results

- **Equivalent acute rejection rates**
- **Improved GFR**
- **Less CAN by biopsy**
- **LEA29Y is safe**
- **Heralds a new era of chronic biologicals in transplantation?!**

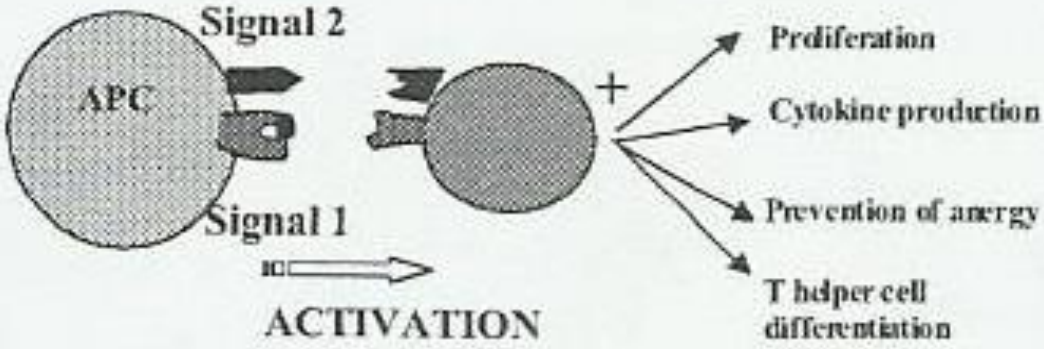
Vincenti et al. NEJM 2005

T Cell Costimulatory Pathways: Emerging Concepts

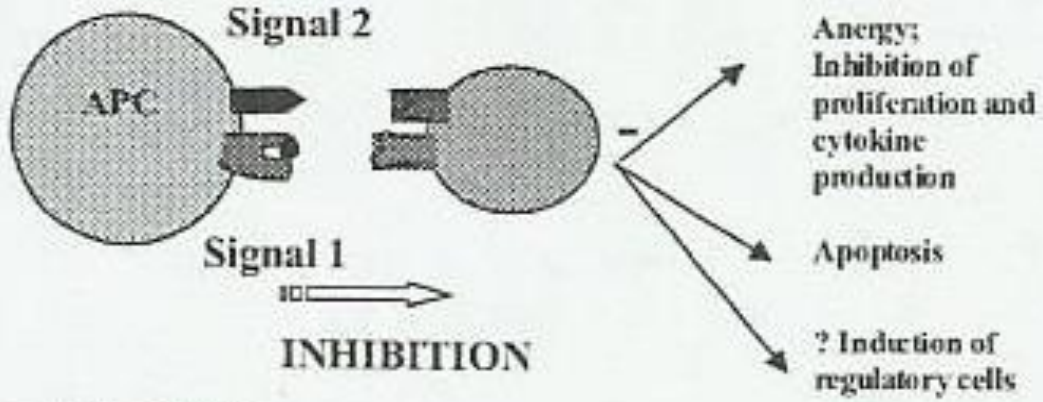
- Negative and positive signaling pathways
- Complex interactions among, and between, the positive and negative pathways
- Regulation of naive and effector/memory cells
- Molecules expressed on bone marrow derived cells and parenchymal cells

Rothstein & Sayegh, Imm. Rev 2003

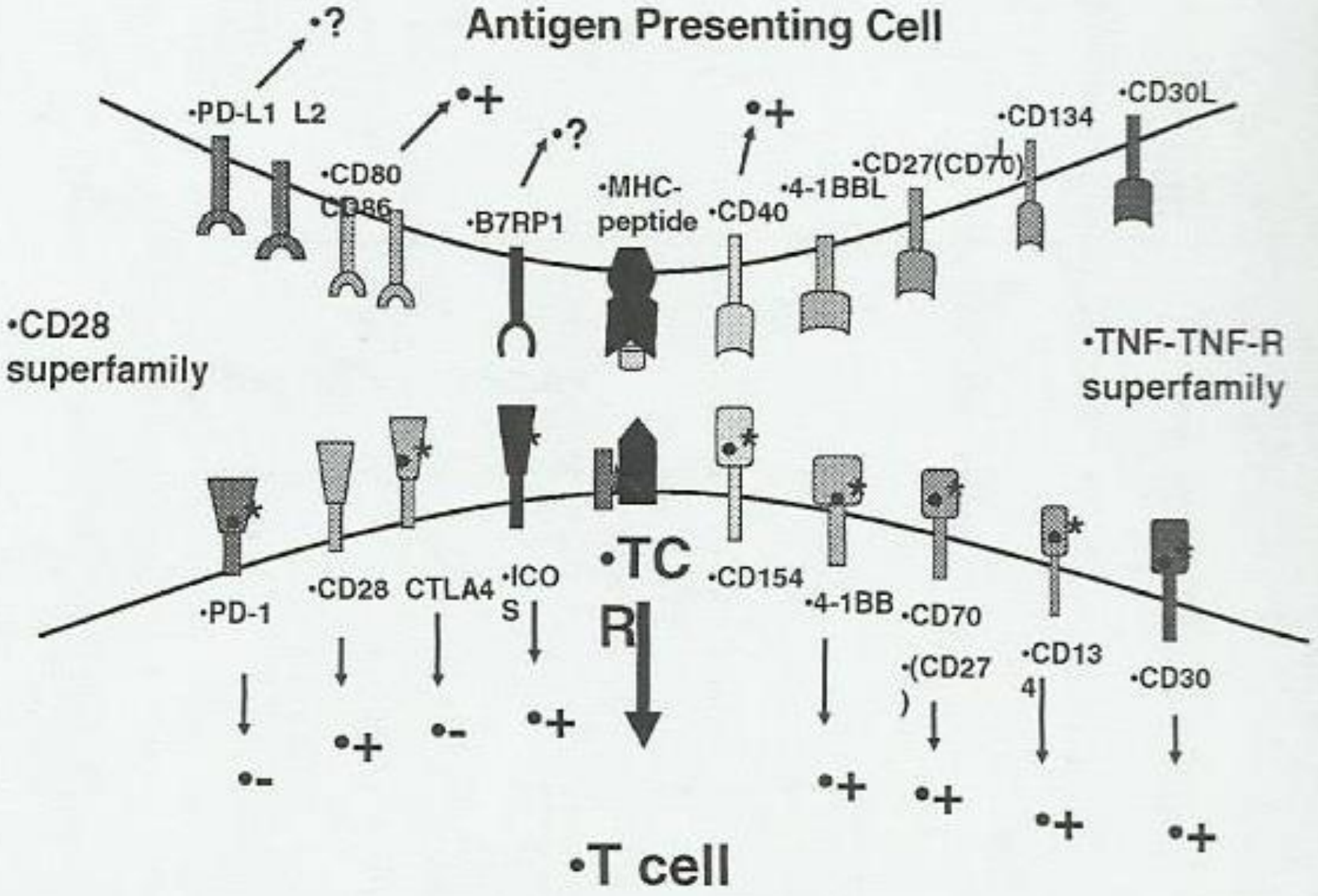
POSITIVE COSTIMULATORY PATHWAYS



NEGATIVE COSTIMULATORY PATHWAYS



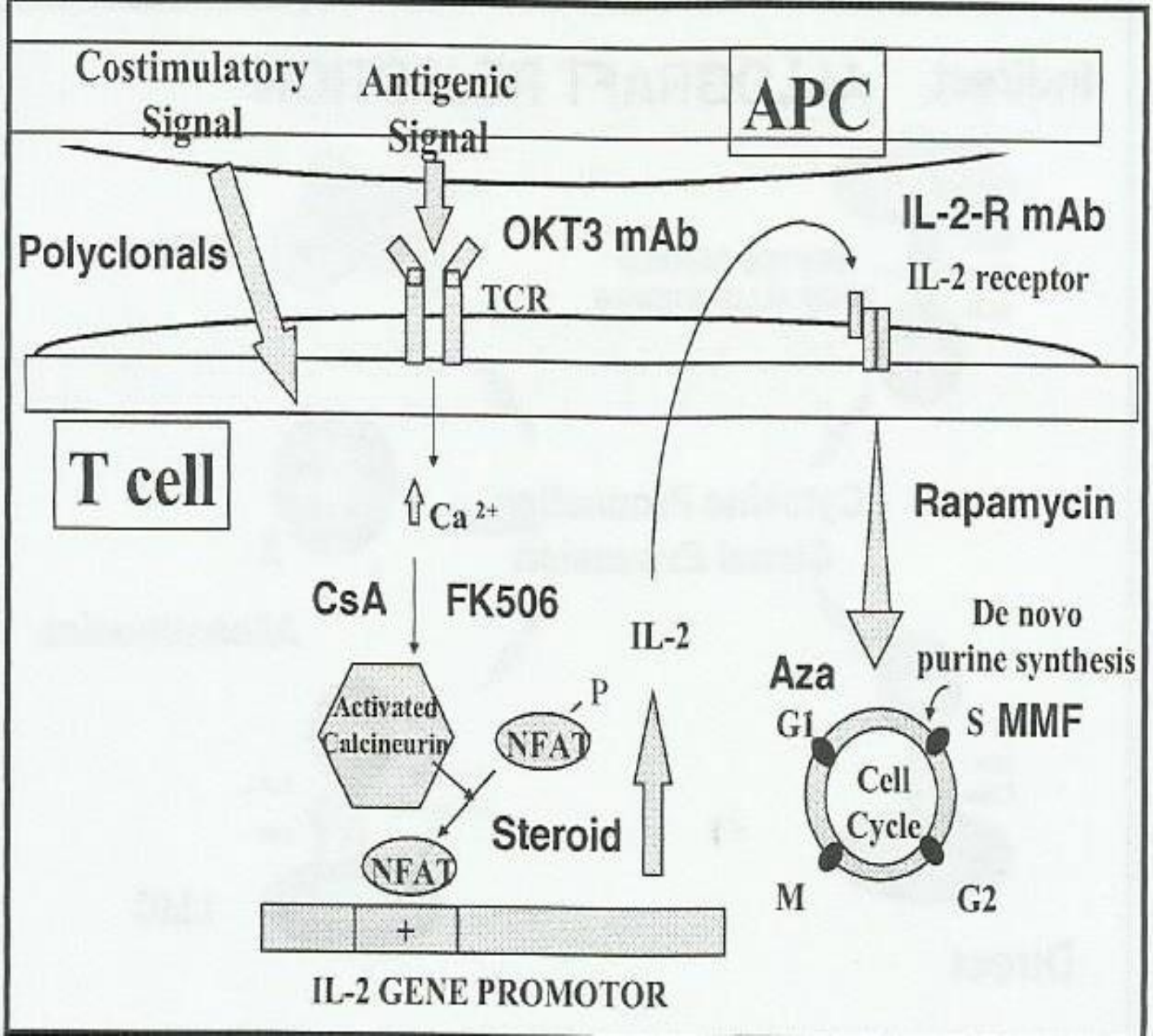
Clarkson and Sayegh Tx 2005


Antigen Presenting Cell
•?
•PD-L1 L2
•+
•CD80
CD86
•?
•B7RP1
•MHC-
peptide
•+
•CD40
•4-1BBL
•CD27(CD70)
•CD134
•CD30L
•CD28
superfamily
•TNF-TNF-R
superfamily
•TC
R
•PD-1
•CD28
CTLA4
•ICO
S
•CD154
•4-1BB
•CD70
•(CD27
)
•CD13
4
•CD30
•-
•+
•-
•+
•+
•+
•+
•+
•T cell

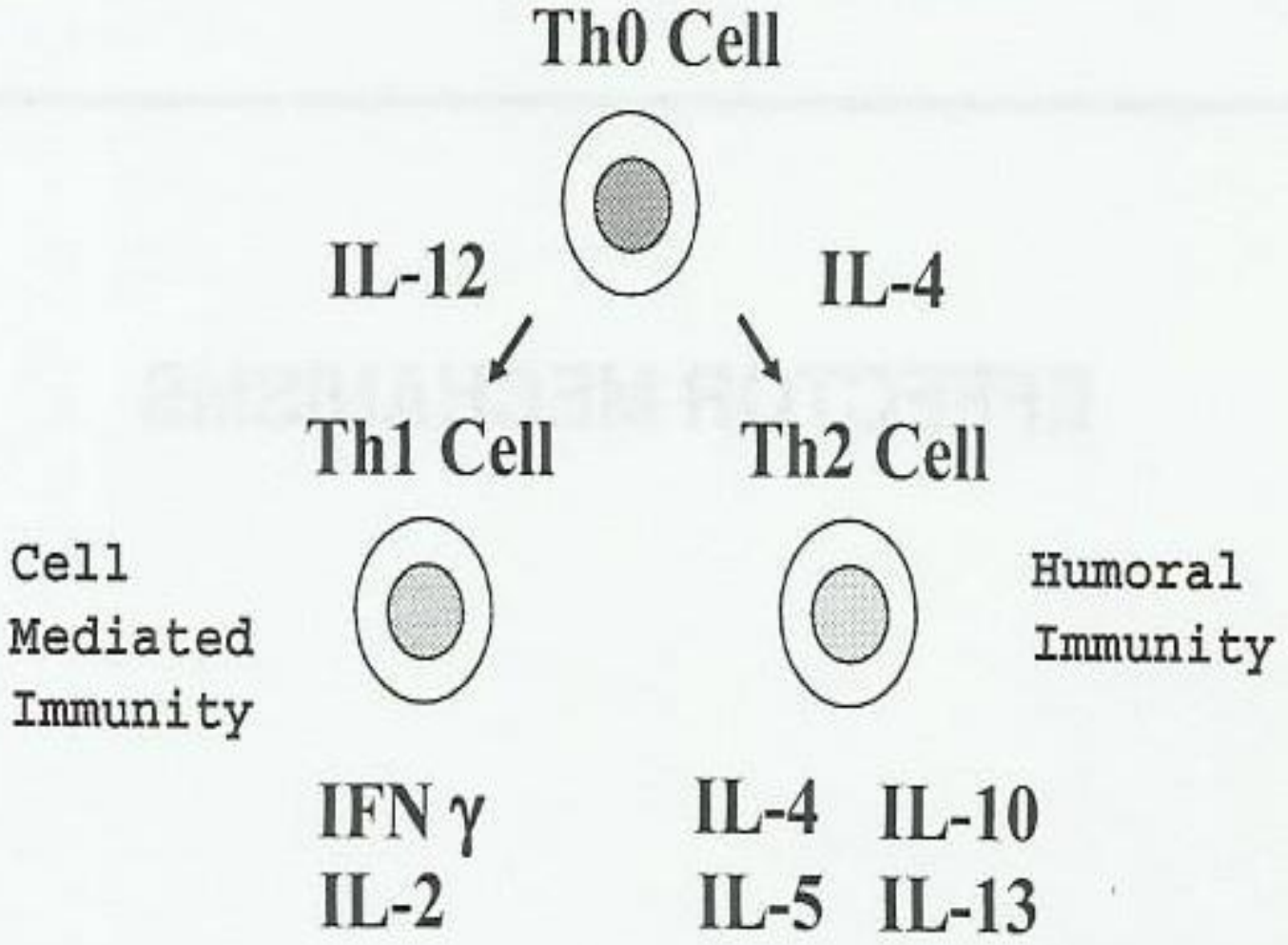
New T Cell Costimulatory Pathways

- Redundancy insures continuation of immune responses
- Each pathway may have additional unique functions based on specific cell type (CD4/CD8/NK), phenotypic differentiation (Th1/Th2), and/or timing of the immune response (naïve, effector, memory)
- There are complex interactions among the various costimulatory pathways
- Interactions between "positive" and "negative" costimulatory pathways determine the ultimate fate of the immune response
- Ultimately, multiple pathways may have to be targeted with blockade of positive and signaling of negative costimulatory molecules to achieve tolerance

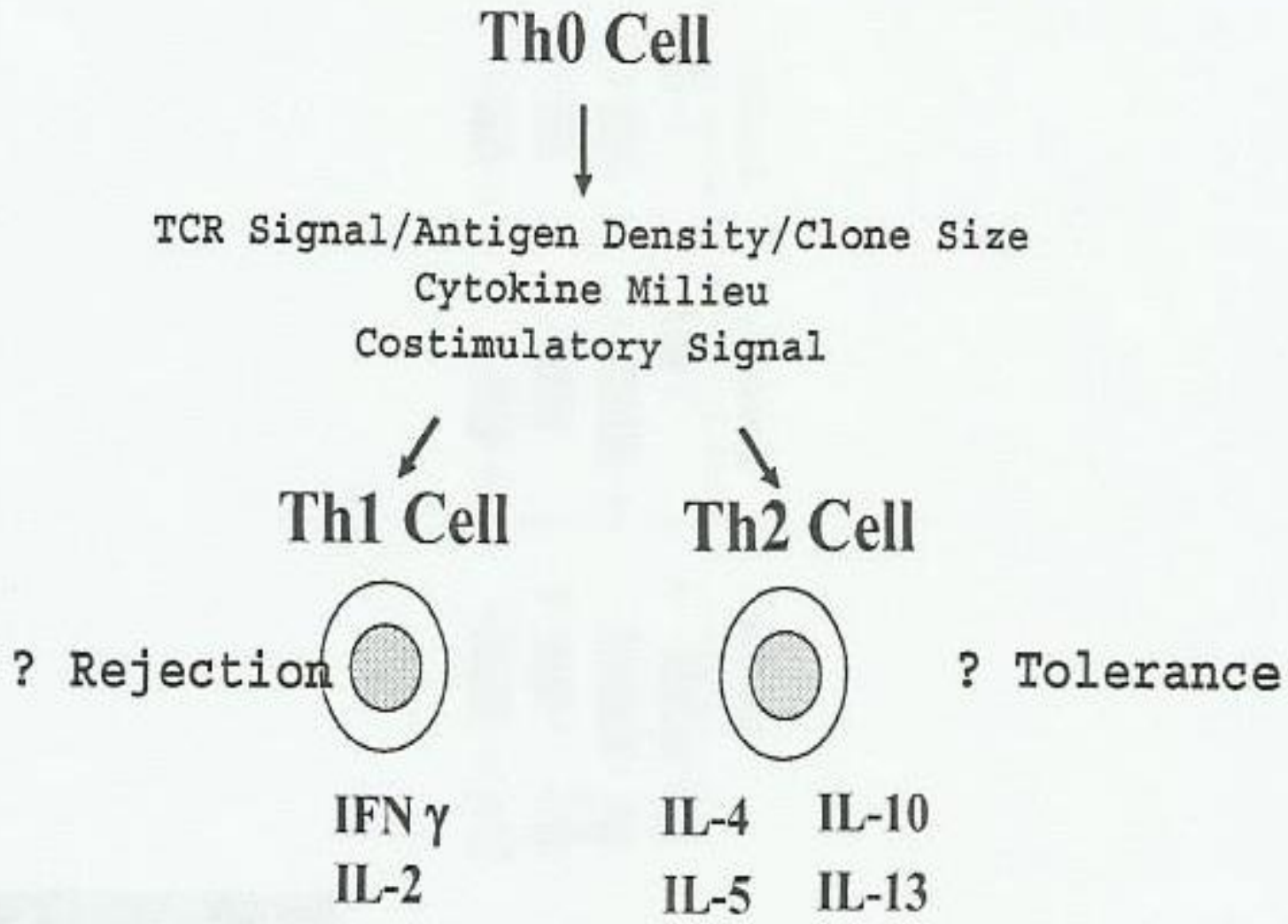
Rothstein & Sayegh, Imm. Rev 2003


Costimulatory Signal
Antigenic Signal
APC
Polyclonals
OKT3 mAb
TCR
IL-2-R mAb
IL-2 receptor
T cell
Ca 2+
Rapamycin
CsA
FK506
IL-2
De novo purine synthesis
Aza
S MMF
G1
Activated Calcineurin
P
NFAT
Cell Cycle
Steroid
NFAT
M
G2
+
IL-2 GENE PROMOTOR

TH1/TH2 PARADIGM



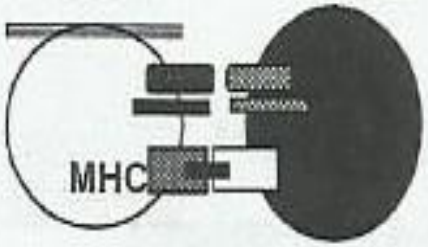
TH1/TH2 PARADIGM



CHALLENGES TO THE TH1/TH2 PARADIGM

- Th1 cytokine knockout recipients can reject allografts
- Th1 cytokine knockout recipients show resistance to tolerance
- Th2 cytokine knockout recipients can be tolerized in some models
- Th2 cytokines permissive but do not cause tolerance in fully MHC mismatched models

DUAL ROLE OF TH1 CYTOKINES IN GRAFT REJECTION



Efferent Phase

T cell proliferation
CTL generation
DTH response
Alloantibody
NK cell activation

Regulatory Phase

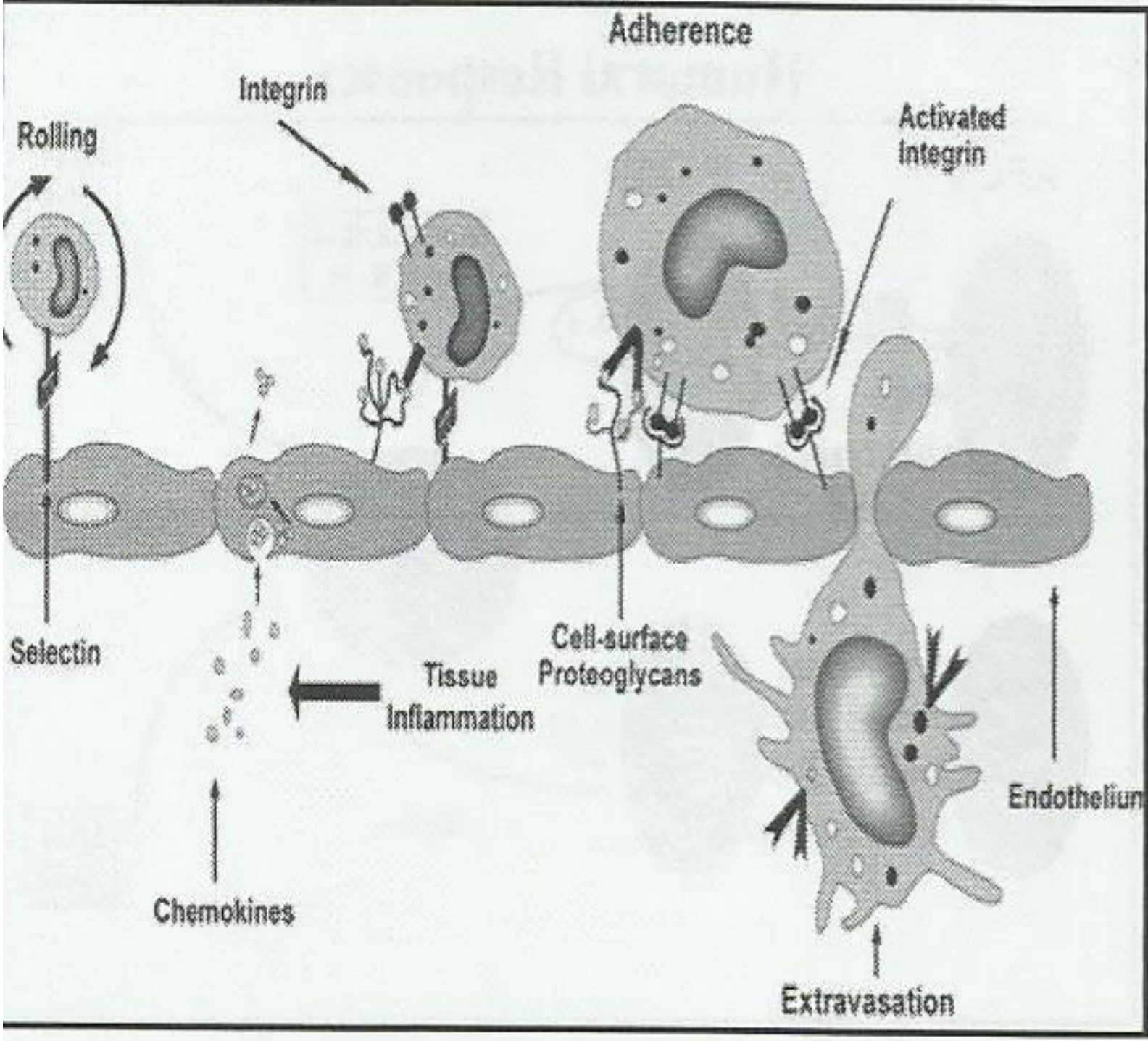
AICD (apoptosis)
Proliferation arrest
Anergy

redundant

essential

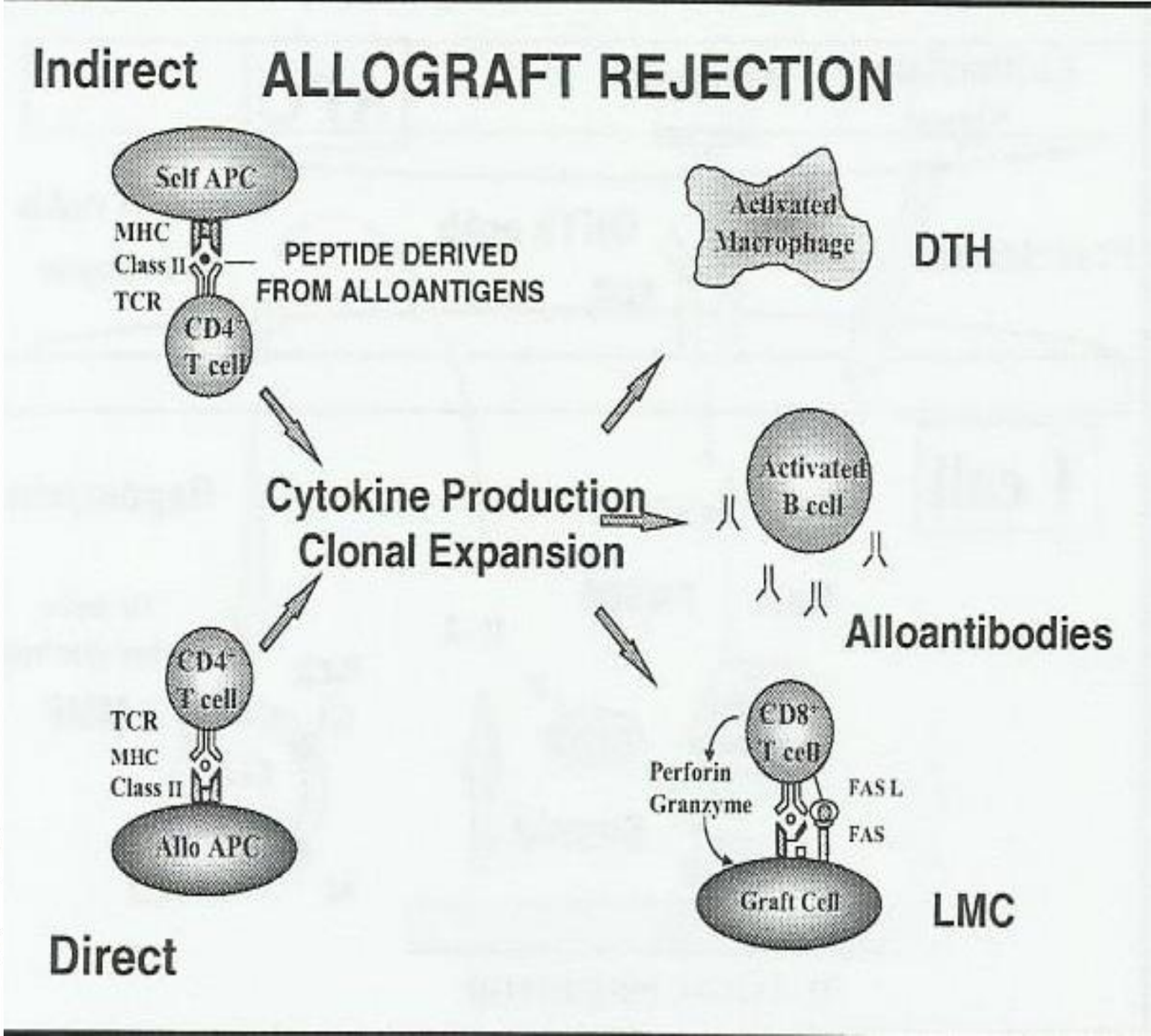
IL-2
IFN-g

Lakkis et al.

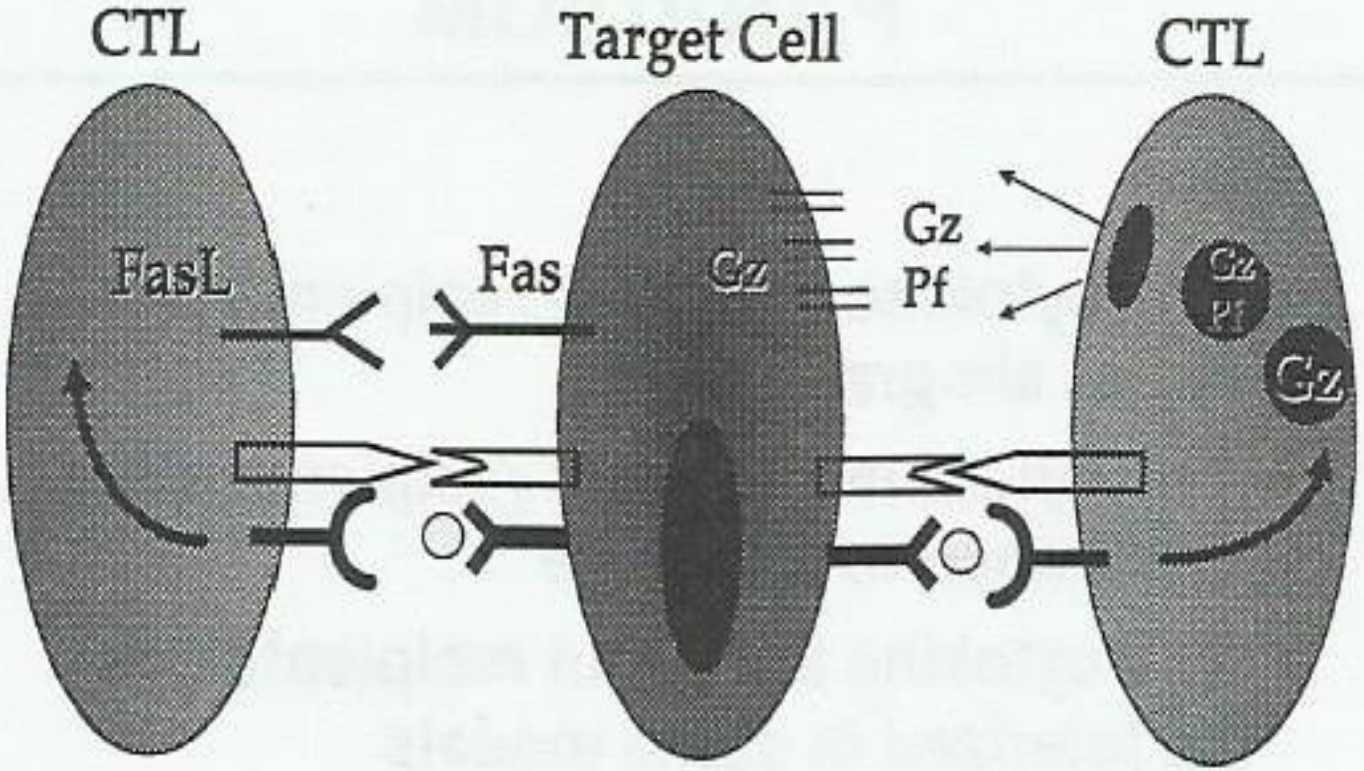

Adherence
Integrin
Rolling
Activated Integrin
Selectin
Cell-surface Proteoglycans
Tissue Inflammation
Endothelium
Chemokines
Extravasation

STEP THREE

EFFECTOR MECHANISMS


Indirect
ALLOGRAFT REJECTION
Self APC
MHC
Class II
TCR
PEPTIDE DERIVED
FROM ALLOANTIGENS
CD4+
T cell
Activated
Macrophage
DTH
Cytokine Production
Clonal Expansion
Activated
B cell
Alloantibodies
CD4+
T cell
TCR
MHC
Class II
Allo APC
CD8+
T cell
Perforin
Granzyme
FAS L
FAS
Graft Cell
LMC
Direct

CTL-Mediated Lymphocytotoxicity

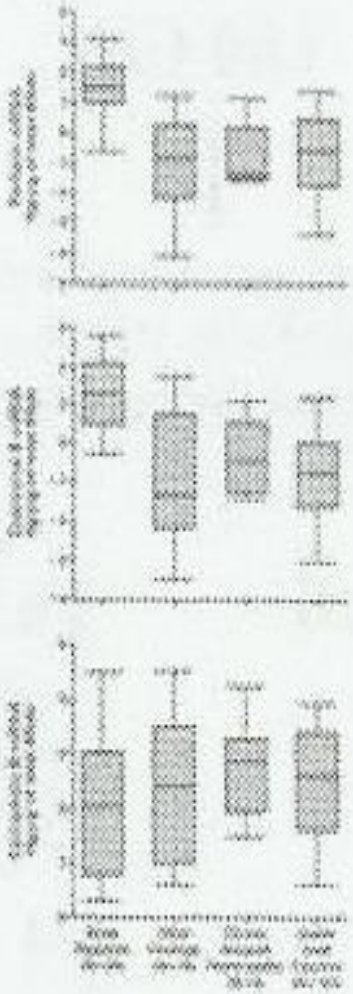


Non-Secretory
Receptor Mediated

Apoptosis

Secretory
Perforin + Granzyme
Mediated

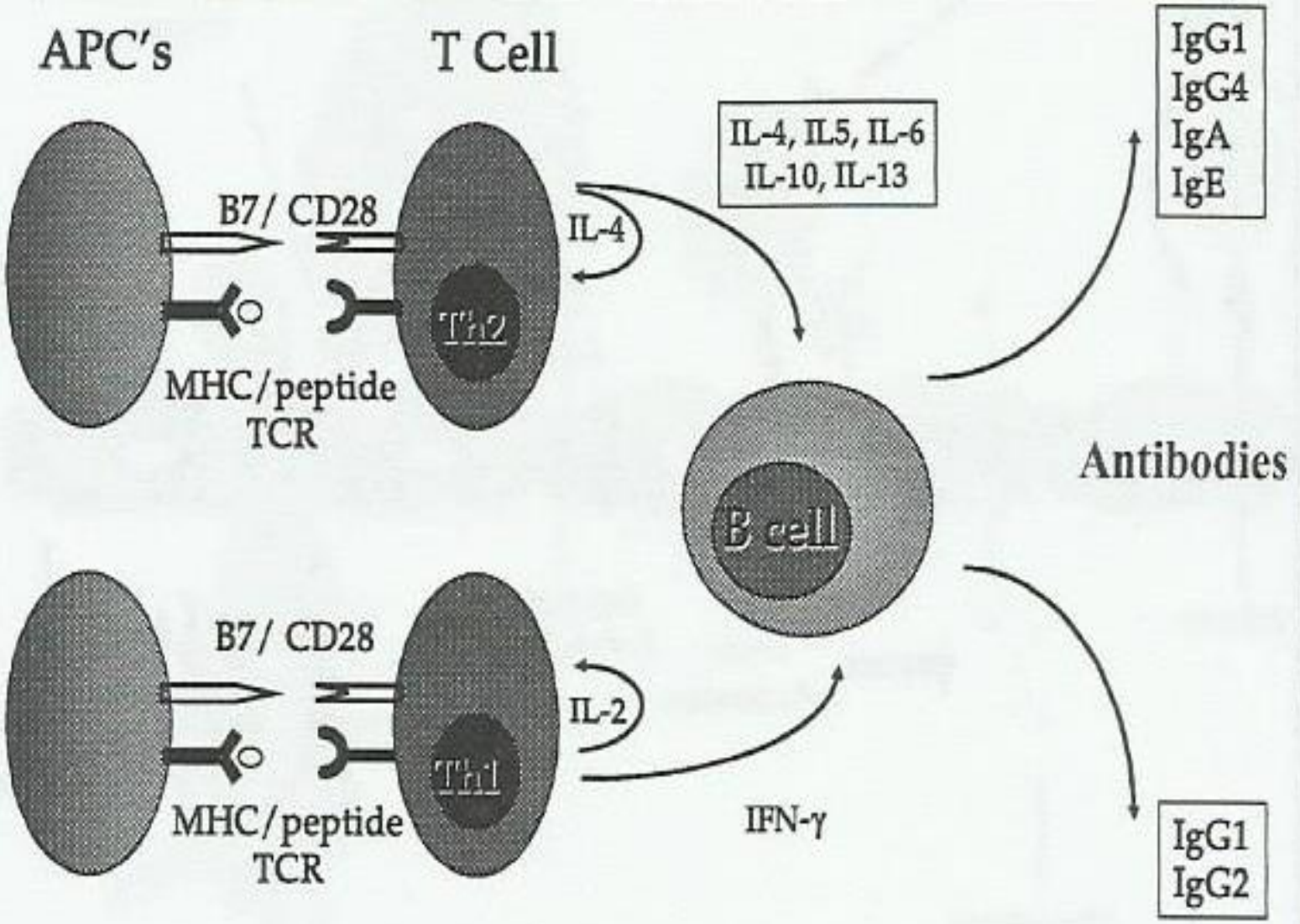
Levels of Cytotoxic Molecules mRNA in Urinary Cells



Li, B. et al. N Engl J Med 2001;344:947-954

THE NEW ENGLAND JOURNAL of MEDICINE

Humoral Responses



ALLOREACTIVE ANTIBODIES

- Antibodies recognize polymorphic (exposed) regions of MHC molecules
- May develop upon exposure to alloantigens through pregnancy, blood transfusion, previous transplant or by cross reactivity
- Cross matches and PRA are used to detect alloantibodies that are present pre-transplant (prevent hyperacute rejection)
- Alloantibodies can also develop post transplant (contribute to acute and chronic rejection)

Humoral Alloreactivity

Pre Tx

Preformed Ab´s

Post Tx

De novo Ab´s

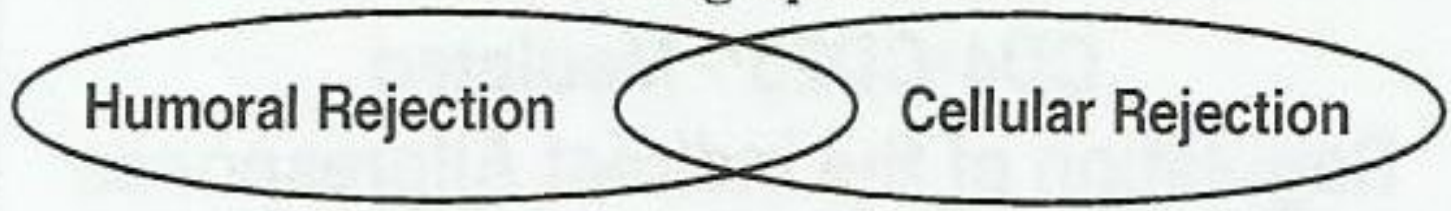
historic

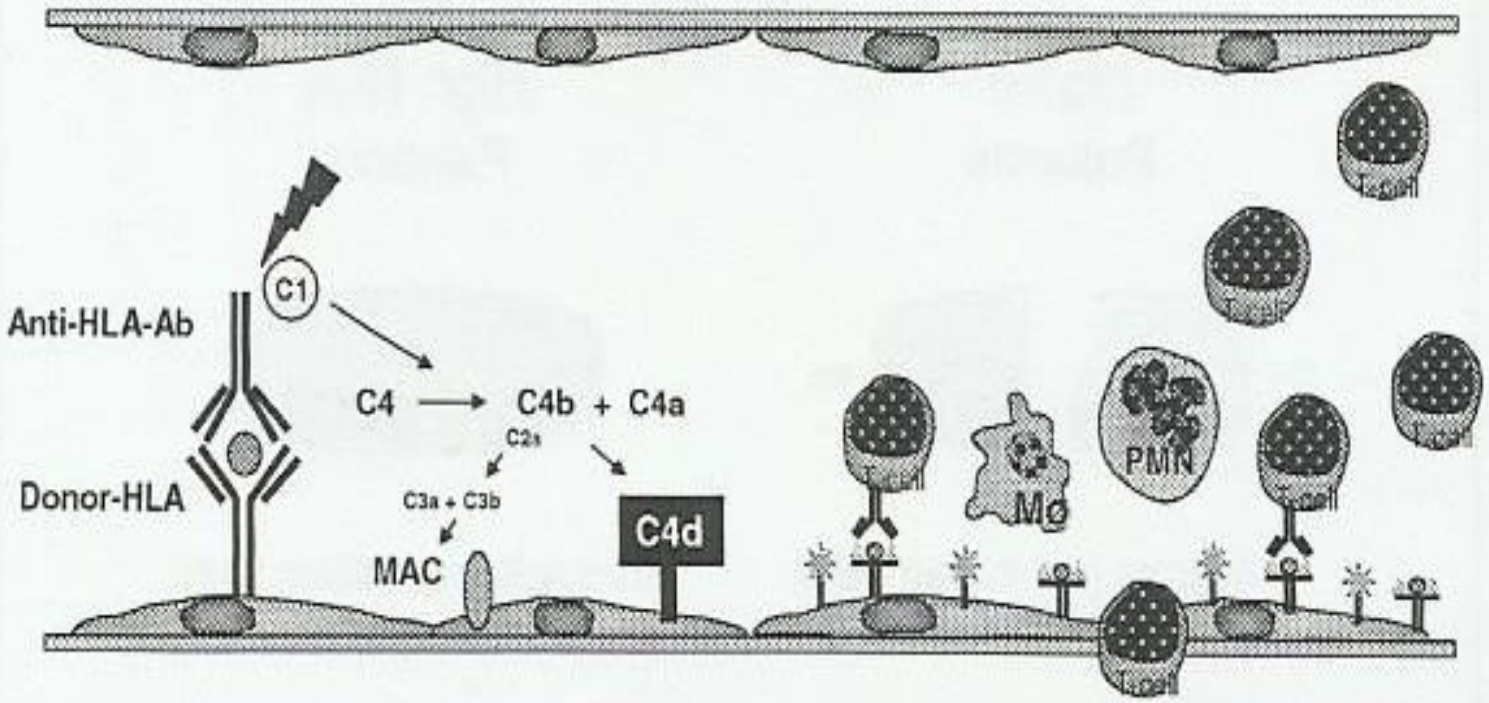
current

de novo

time

A Shifting Spectrum





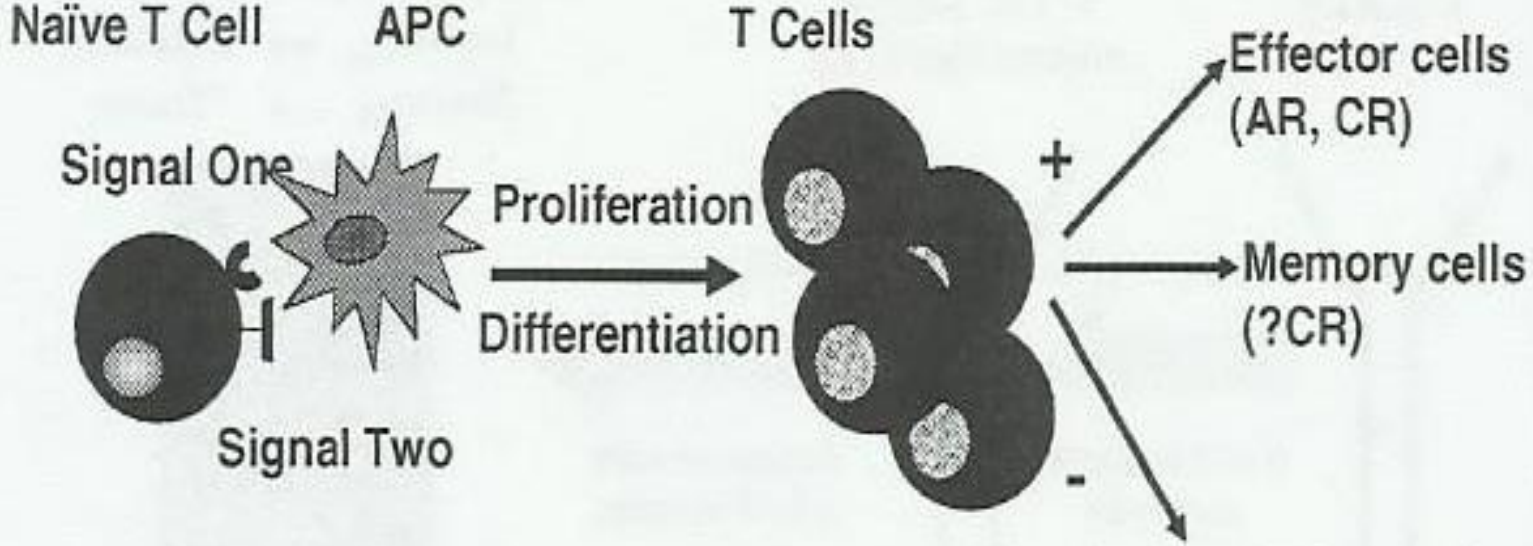
DTH Response

- Mediated by CD4+ T cells and monocytes/ macrophages
- Endothelial cell role: chemokines, adhesion, migration, promotion of direct and indirect allorecognition
- Starts as an antigen-specific immune response but ends with tissue injury and repair
- Role of apoptosis ?

STEP FOUR

RESOLUTION OF THE RESPONSE WITH RESIDUAL MEMORY

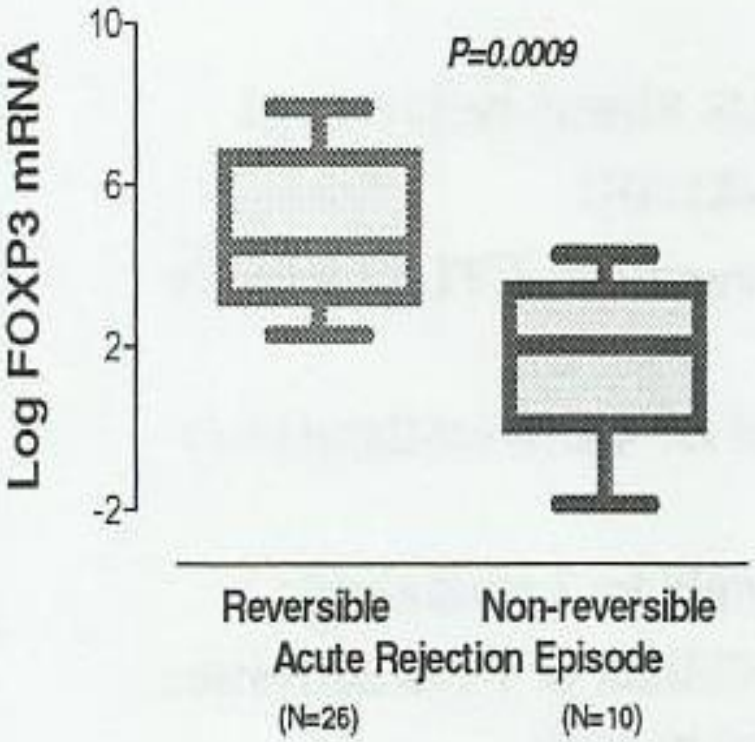
Fate of T Cells Determines Immune Response

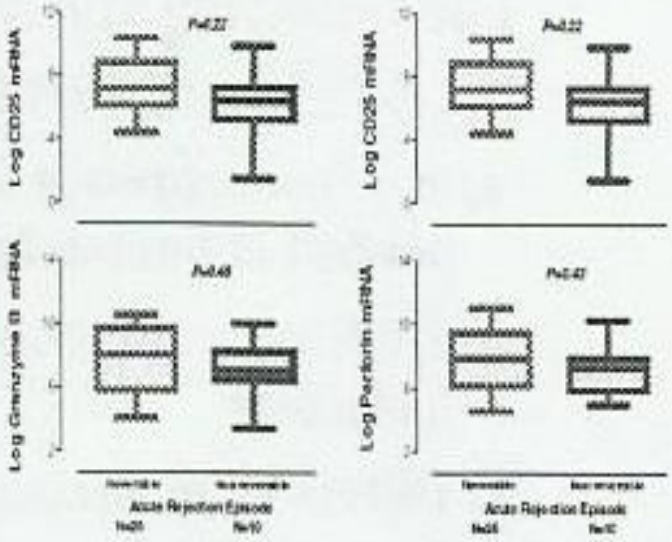


Najafian, Khoury and Sayegh, 2001

Figure 1

FOXP3 mRNA Levels are Higher in Patients with Successfully Reversed Acute Rejection Episode

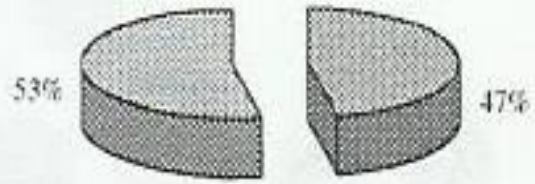




Urine specimens were collected from 36 patients with acute rejection. Twenty six of the 36 acute rejections qualified as successful reversal and the remaining 10 did not. Urinary cell Foxp3 mRNA was significantly higher in the group with successful reversal than in the group with out reversal (mean±SE: 4.7±0.5 vs. 1.5±0.7, P=0.0009). The levels of CD25 (7.3±0.4 vs. 6.0±0.9, P=0.23), CD3ε (8.5±0.5 vs. 7.4±0.8, P=0.35), and perforin (7.8±0.5 vs. 7.3±0.7, P=0.43) were not informative of acute rejection outcome .

$CD4^+CD25^+$- Mediated Regulation of the Indirect Alloresponse

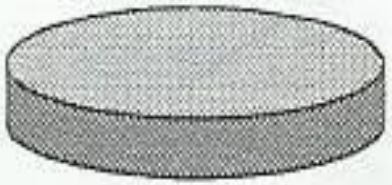
Stable Patients



< 60 spots per million cells

Regulation

High Risk Patients



60 spots per million cells
$P = 0.018$

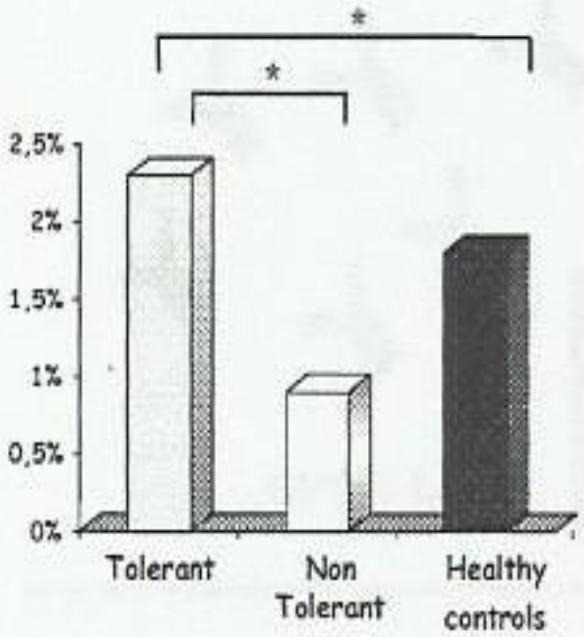
No Regulation

Salama et al
JASN, 2003

... and what about tolerant humans?

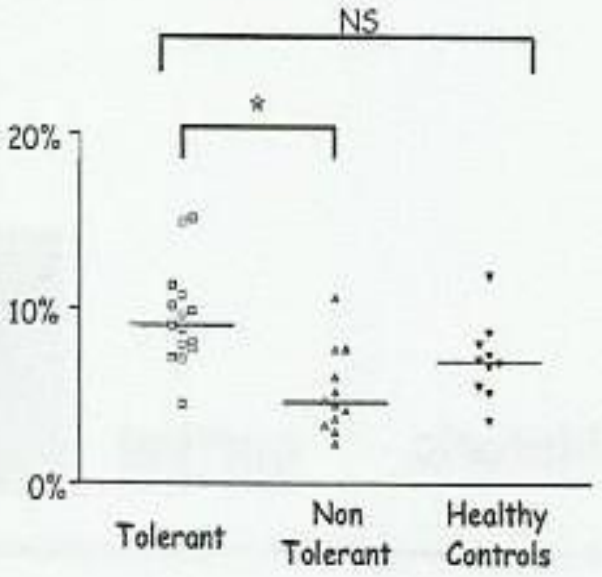
Potential role of CD4+CD25+ regulatory T cells in tolerance to liver allografts in humans

Pediatric living donor liver Tx



Y. Li et al. Am J Transplant 2004

Adult cadaveric liver Tx

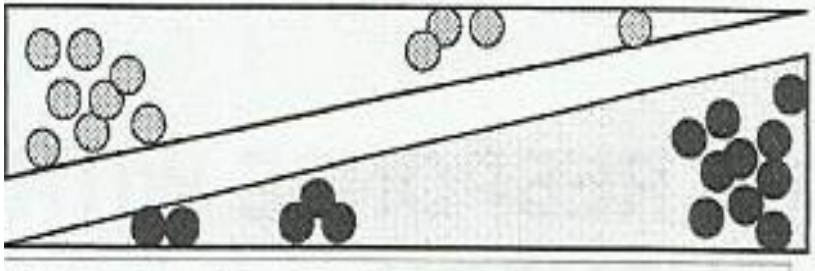


M.Martínez-Llordella, submitted

* p <0,05

Altering The Balance Of Alloreactivity

Regulatory Cells and Suppressor Factors



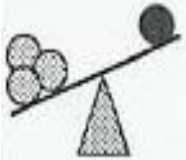
Effector T cell clone size

How to tolerize a T cell?

-Inactivate (anergy)

-Delete (apoptosis)

-Regulate (cells/factors)



Tolerance

Rejection

Strom and Turka

From Abatacept to Belatacept (LEA29Y)
Rational design of a drug

provides more potent immunosuppression required for transplantation

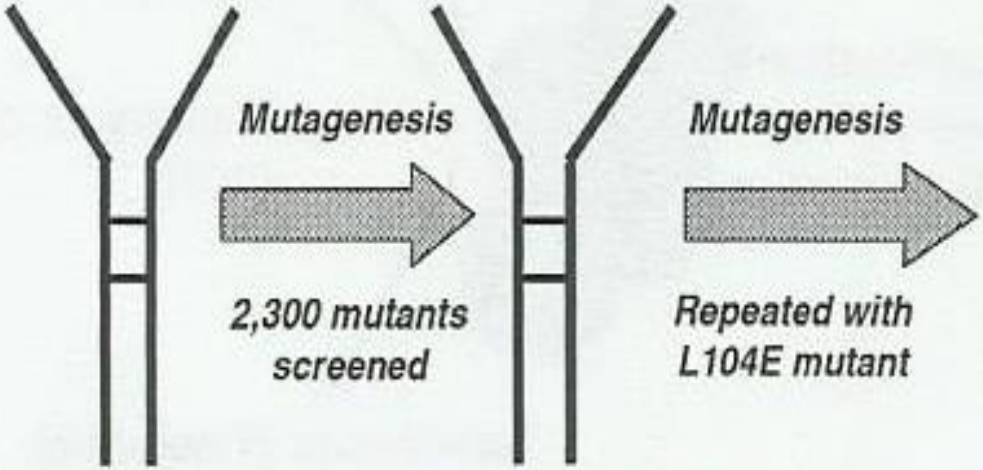
CTLA4Ig

L104E (CDR3) mutant identified

Belatacept (LEA29Y)

Leucine$_{104}$ → GlutamatE

Alanine$_{29}$ → TYrosine



Results: 2–fold slower dissociation from CD80 + 4–fold slower dissociation from CD86 = ~10-fold more potent inhibition of T-cell activation *In vitro*

Larsen CP et al. *Am J Trans 2005;5:443–453*