Immunobiology of Immunosuppressant Agents
(2003)

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Vanderbilt University School of Medicine

GEORGE D. SNELL 1903

P. A. GORER 1907 - 1961

SIR PETER MEDAWAR 1915

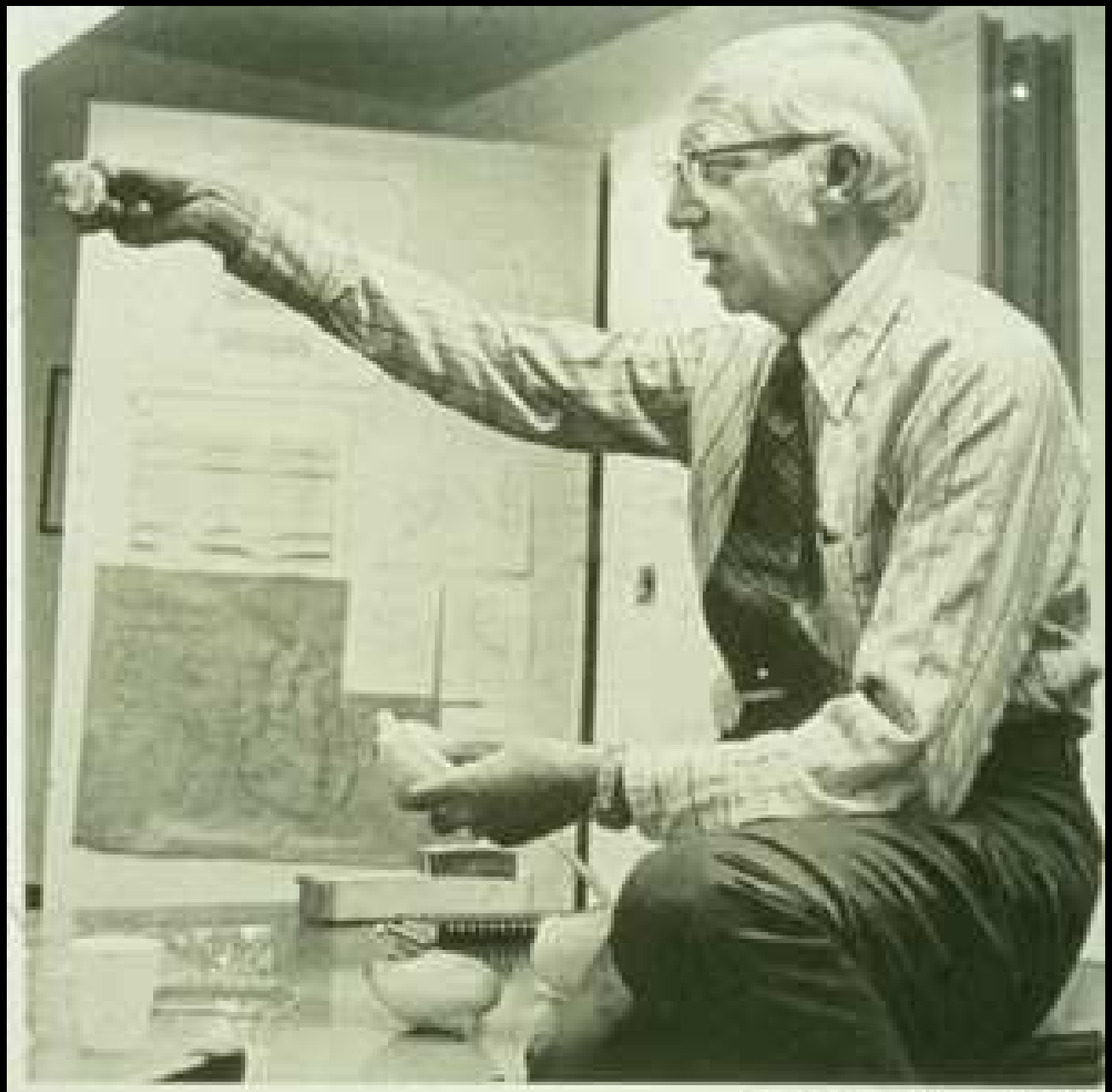


Photo by Ron Simpson, Sag. Y

WILLEM KOLFF

Fig. 1.7 Dr J.E. Murray, Dr J.P. Merrill and Dr J.H. Harrison, who successfully carried out renal transplantation between this set of identical twins on 23 December 1954.

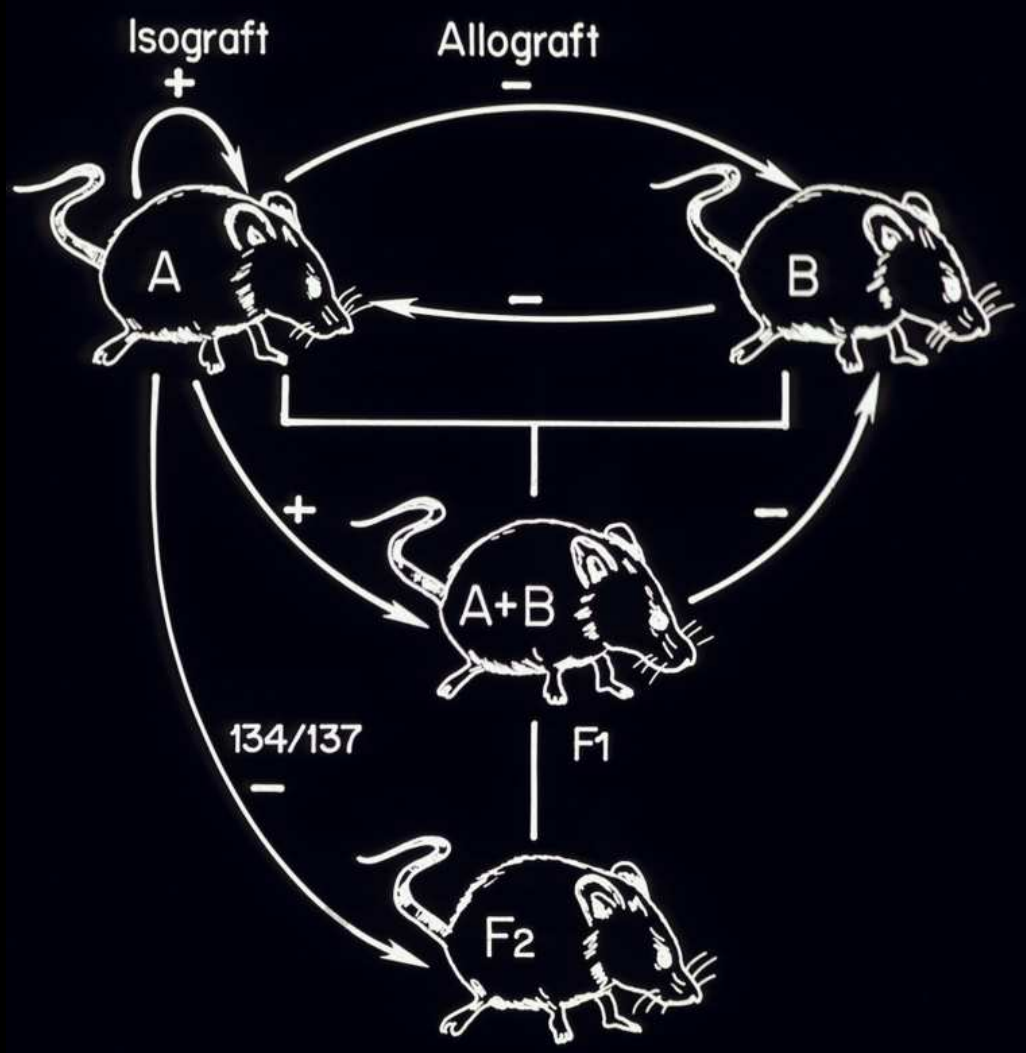
RENAL TRANSPLANT IN THE 20TH CENTURY

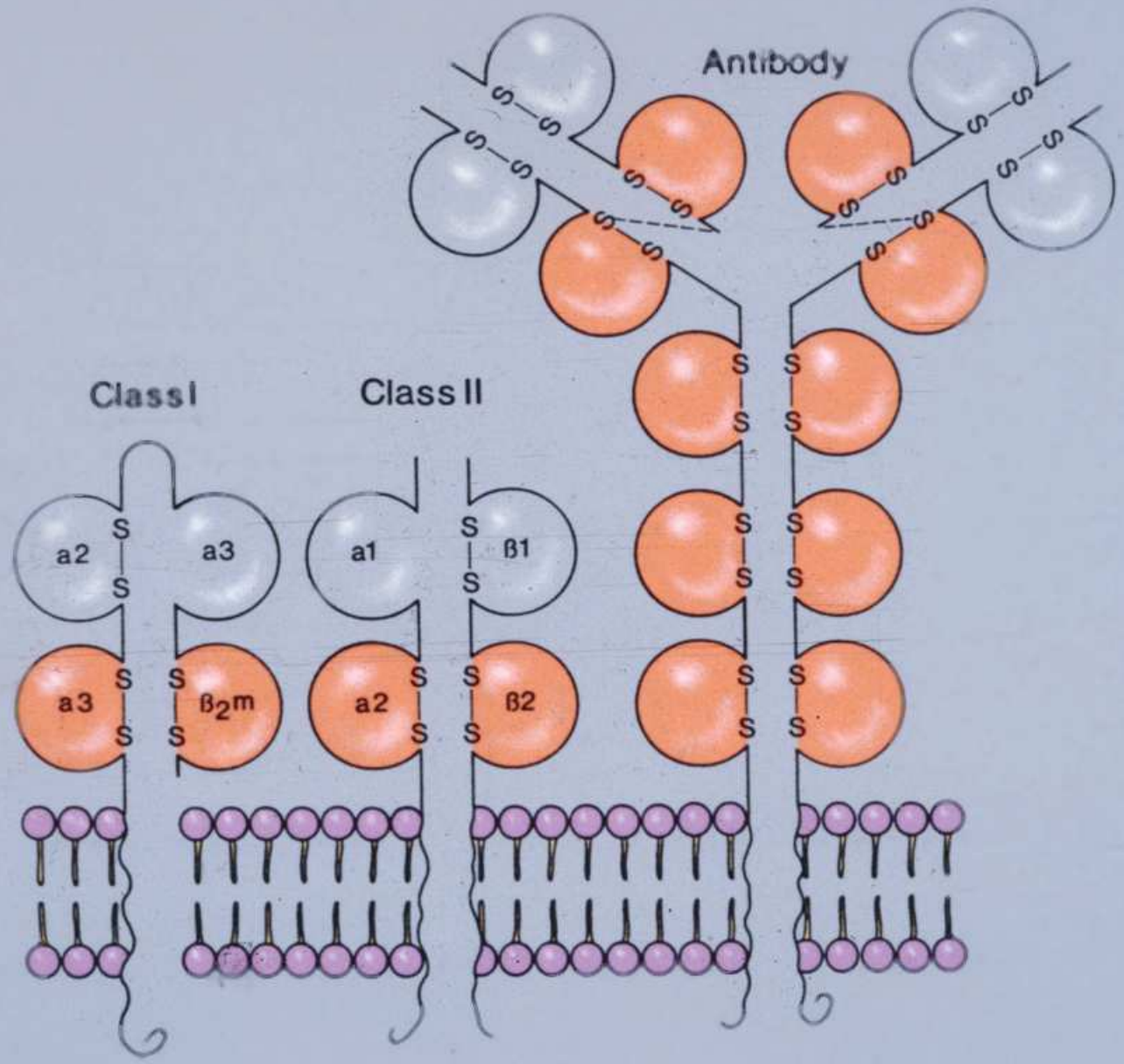
A HISTORY OF IMMUNOSUPPRESSIVE DRUGS

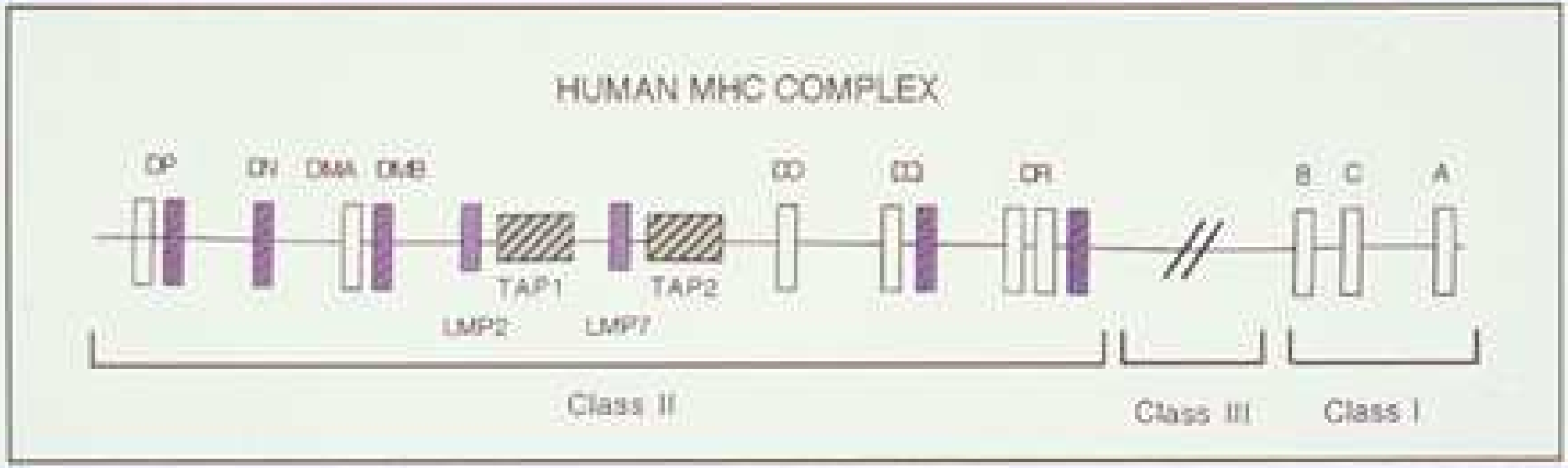
ERA 1	1953-63	THE EXPERIMENTAL PERIOD
ERA 2	1963-83	THE AZATHIOPRINE ERA-INCREASING PATIENT POPULATION FOR TRANSPLANT, WITH INCREASING PT SURVIVAL AT EXPENSE OF GRAFT SURVIVAL
ERA 3	1983-93	THE CYCLOSPORINE A ERA-MARKED IMPROVEMENT IN EARLY GRAFT SURVIVAL, ALL ORGANS ARE NOT CLINICALLY TRANSPLANTABLE, CHRONIC REJECTION REMAINS A PROBLEM
ERA 4	1993-?	THE DESIGNER DRUG ERA-KNOWLEDGE OF TRANSPLANT IMMUNOLOGY ALLOWS GRAFTING OF NEW AGENTS

LAWS OF TRANSPLANTATION

Prehn & Main, 1958

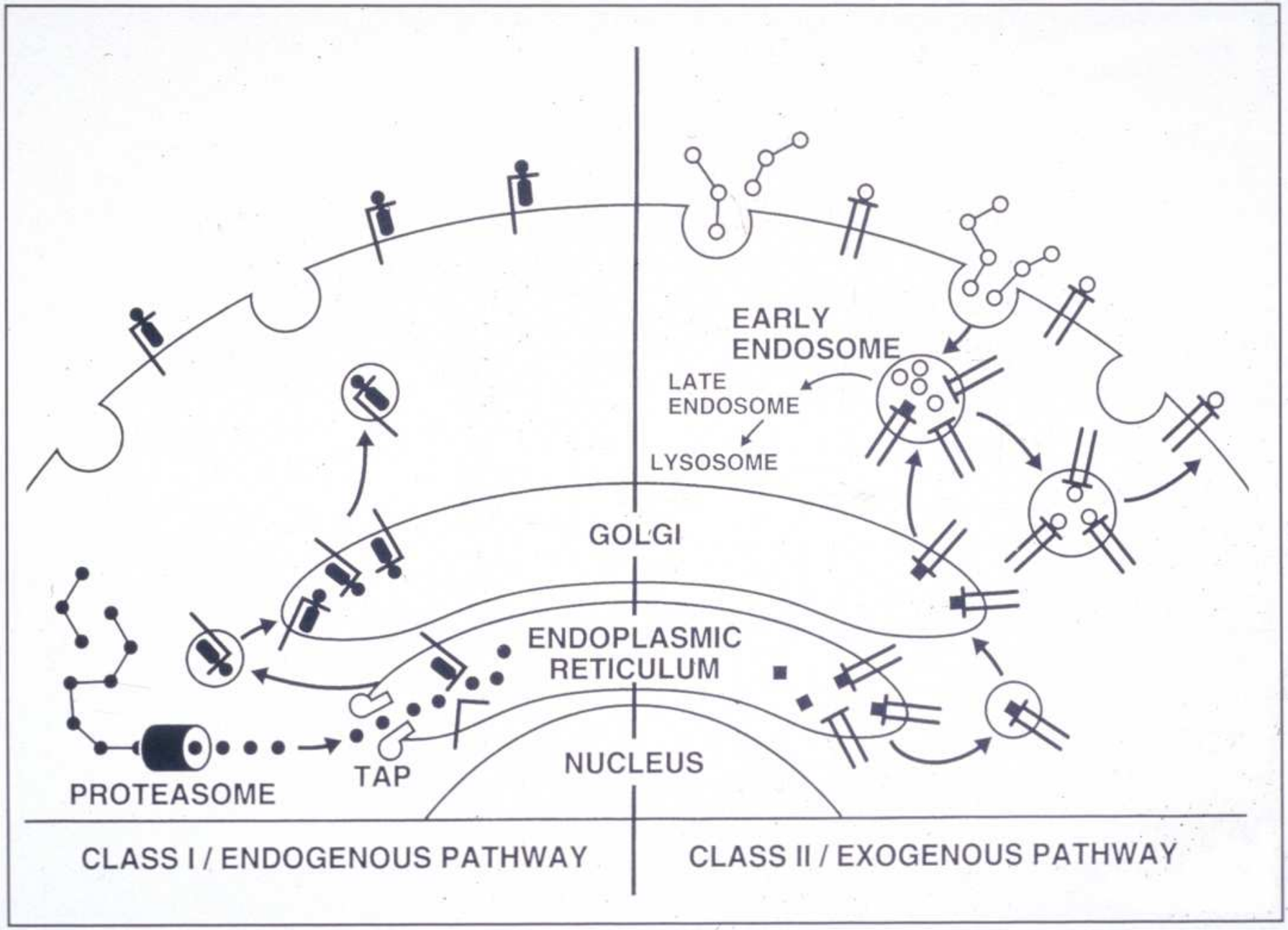


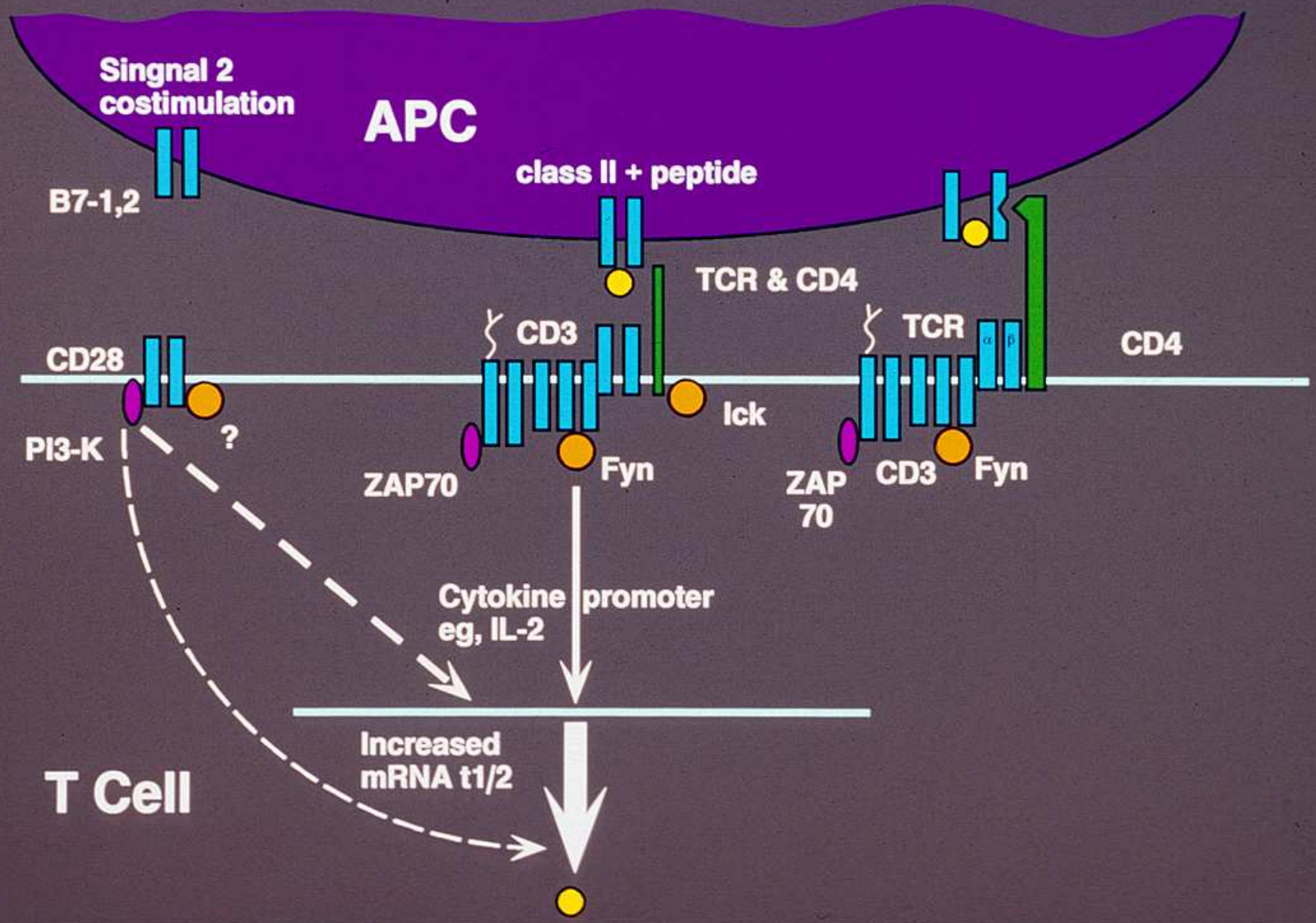

Antibody
Class I
Class II
a2
a3
a1
β1
a3
β2m
a2
β2
S

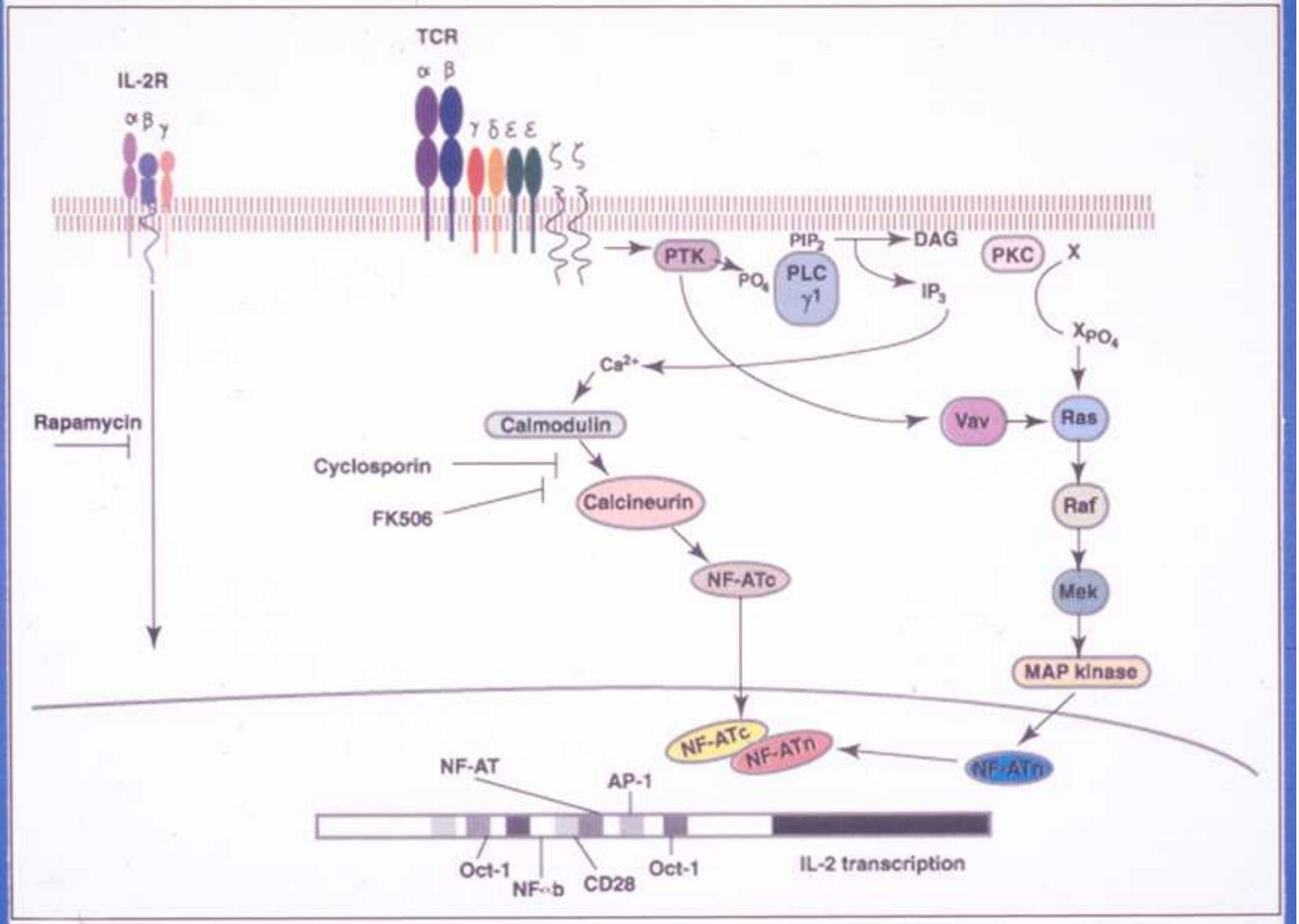

HUMAN MHC COMPLEX
DP
DN
DMA
DMB
TAP1
TAP2
LMP2
LMP7
DR
B
C
A
Class II
Class III
Class I

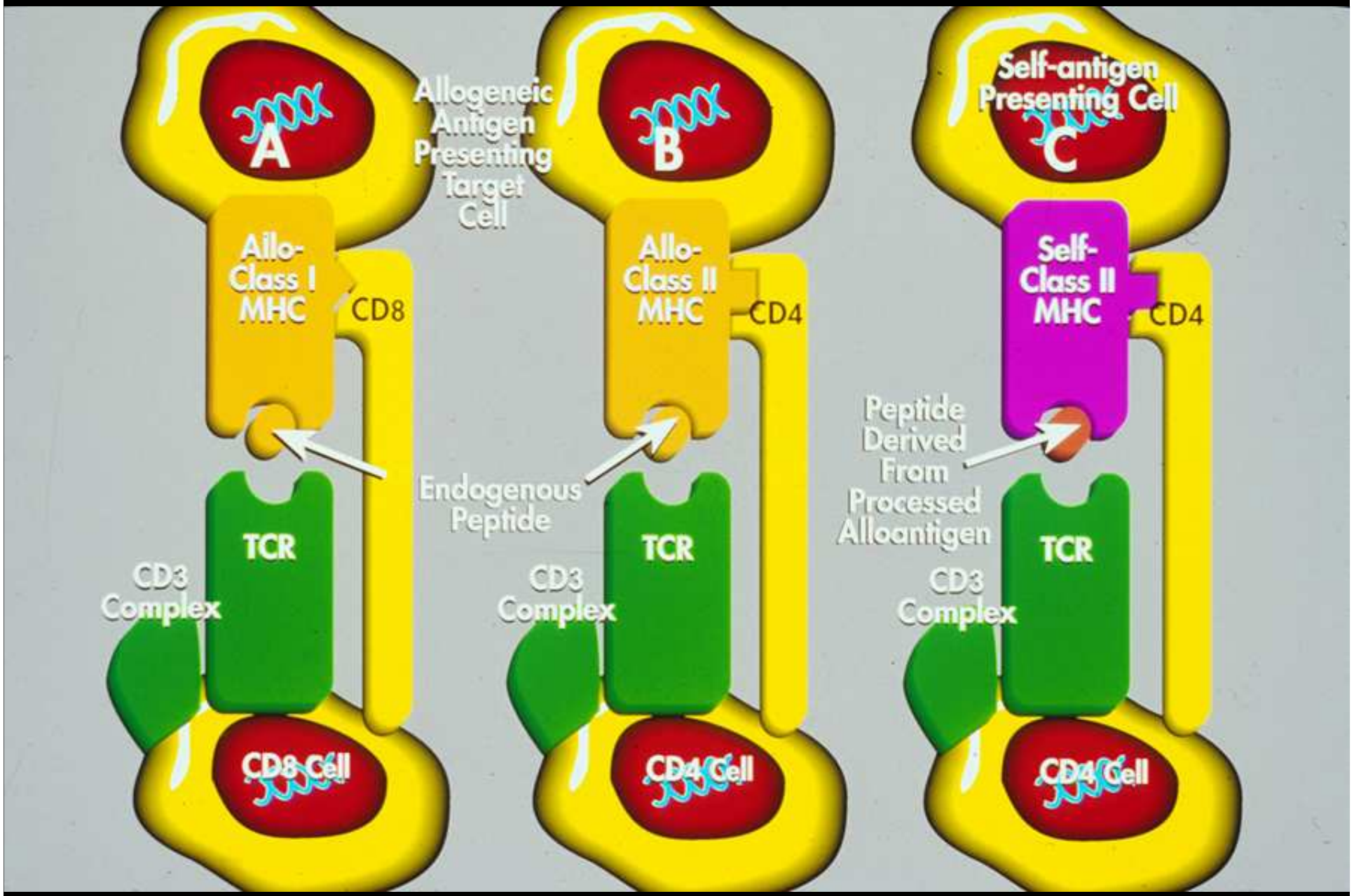

A


B

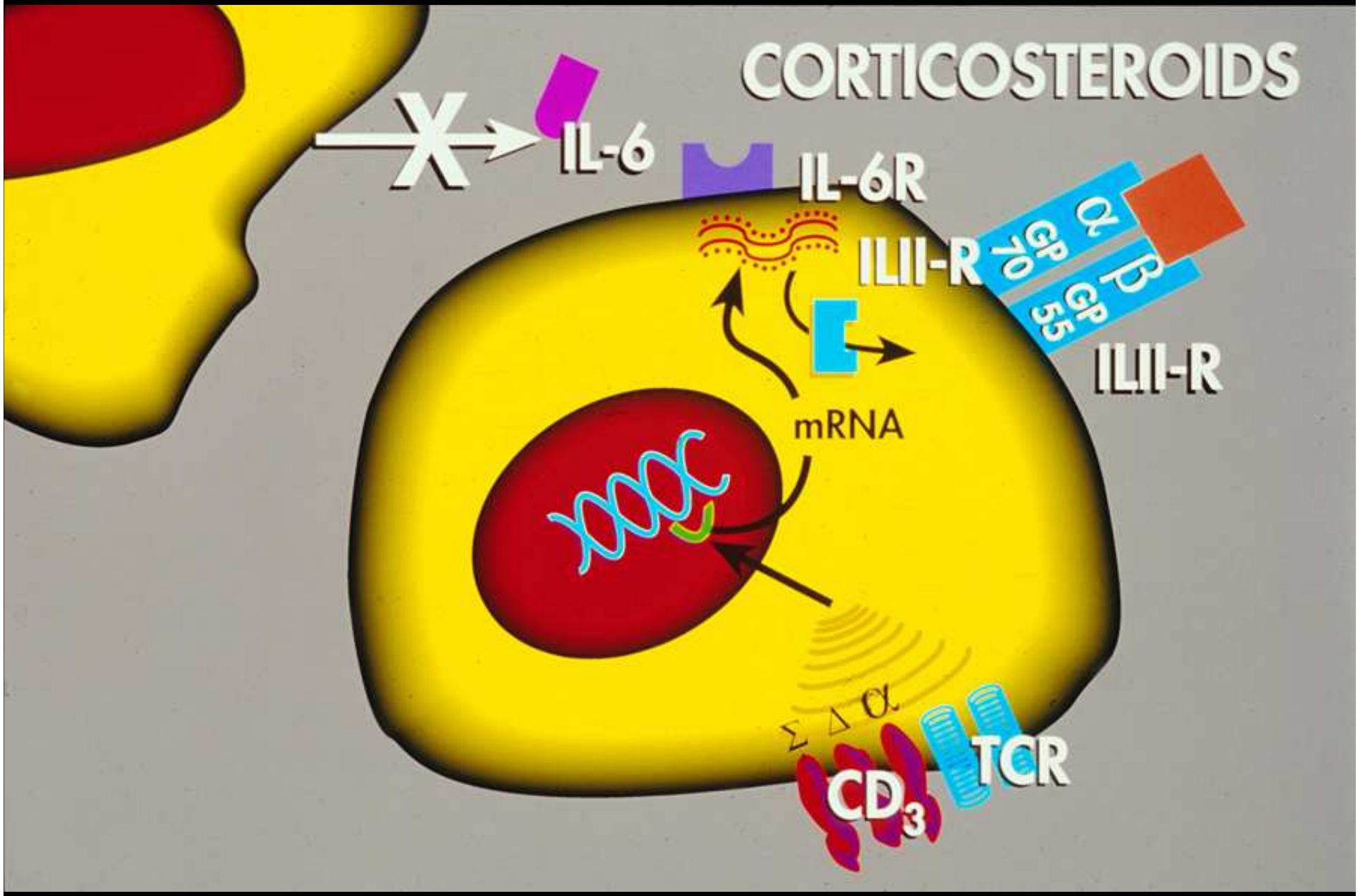

EARLY
ENDOSOME
LATE
ENDOSOME
LYSOSOME
GOLGI
ENDOPLASMIC
RETICULUM
NUCLEUS
TAP
PROTEASOME
CLASS I / ENDOGENOUS PATHWAY
CLASS II / EXOGENOUS PATHWAY

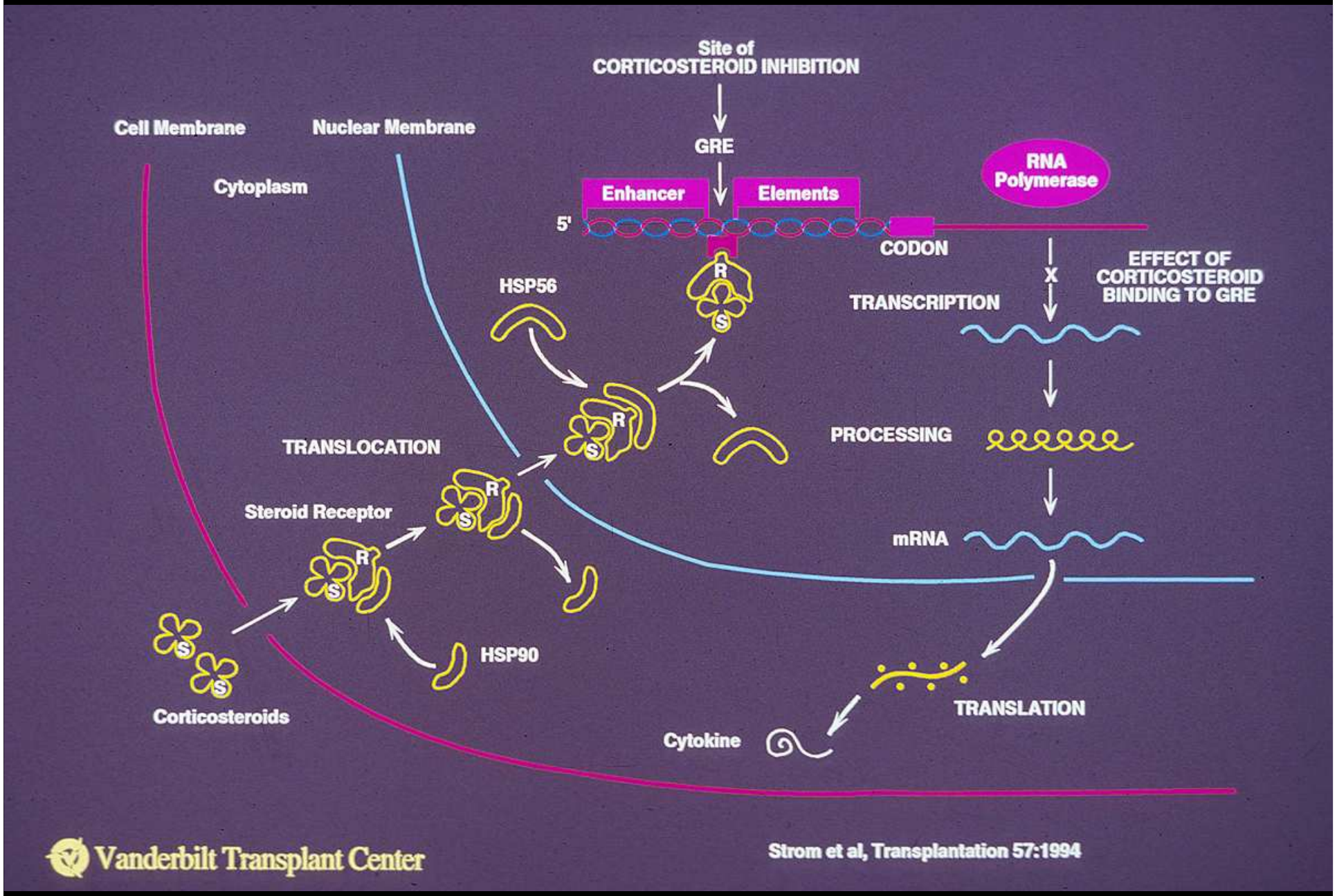

Singnal 2
costimulation
APC
B7-1,2
class II + peptide
TCR & CD4
CD3
TCR
CD4
CD28
Lck
PI3-K
?
ZAP70
Fyn
ZAP
70
CD3
Fyn
Cytokine promoter
eg, IL-2
Increased
mRNA t1/2
T Cell


TCR
α β
IL-2R
α β γ
γ δ ε ε
ζ ζ
PTK
PO4
PIP2
PLC γ1
DAG
IP3
PKC
X
XPO4
Ca2+
Calmodulin
Vav
Ras
Raf
Mek
MAP kinase
Rapamycin
Cyclosporin
FK506
Calcineurin
NF-ATc
NF-ATc
NF-ATn
NF-ATn
NF-AT
AP-1
Oct-1
NF-κb
CD28
Oct-1
IL-2 transcription

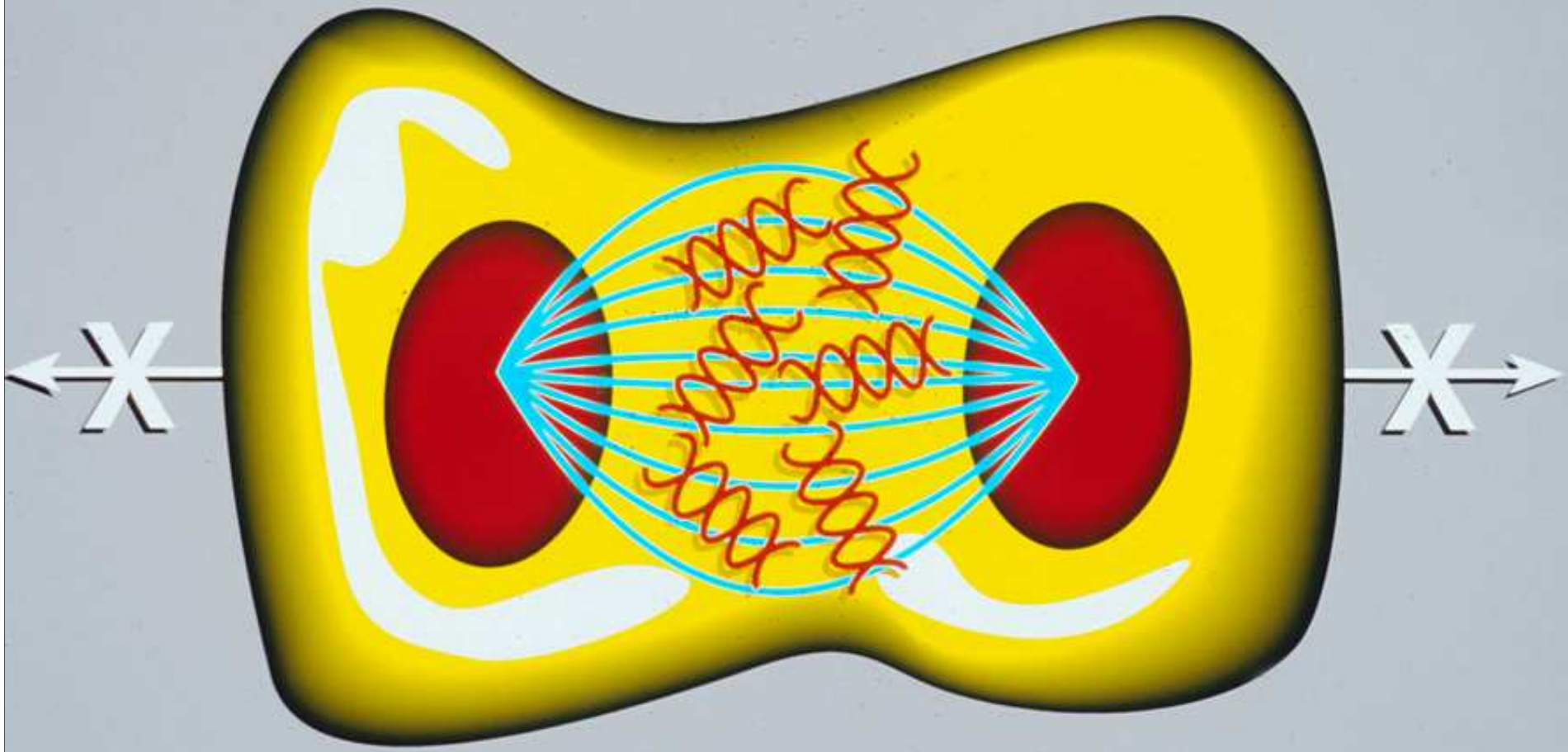

A
Allogeneic Antigen Presenting Target Cell
B
Self-antigen Presenting Cell
C
Allo-Class I MHC
CD8
Allo-Class II MHC
CD4
Self-Class II MHC
CD4
Endogenous Peptide
Peptide Derived From Processed Alloantigen
TCR
CD3 Complex
TCR
CD3 Complex
TCR
CD3 Complex
CD8 Cell
CD4 Cell
CD4 Cell

IMMUNOSUPPRESSANTS

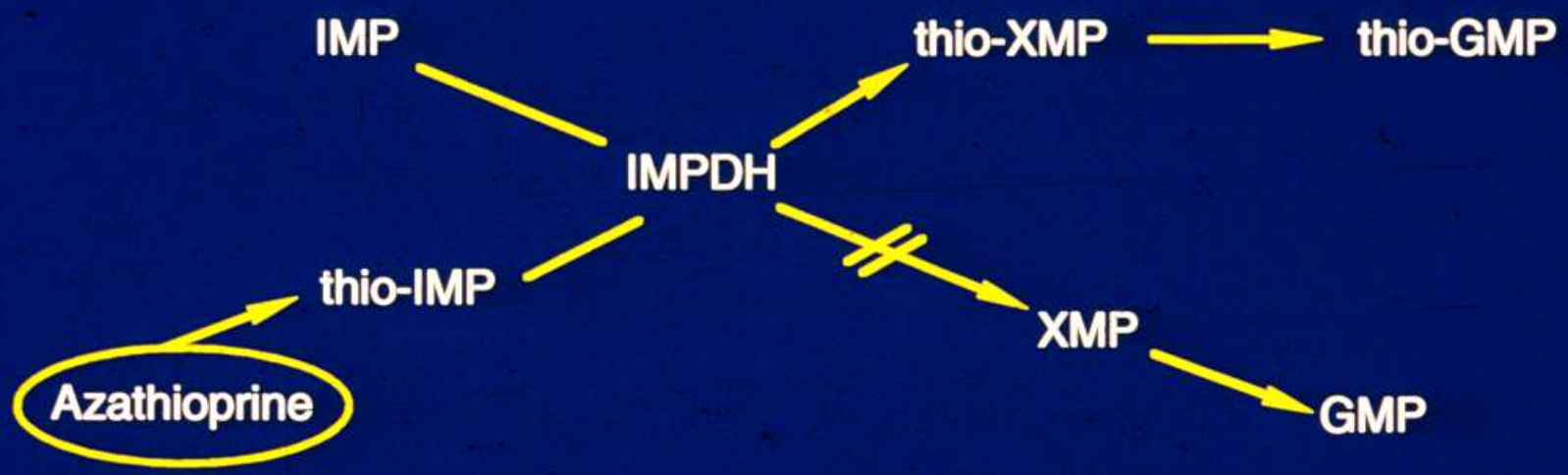

CORTICOSTEROIDS
IL-6
IL-6R
ILII-R
α
GP 70
β
GP 55
ILII-R
mRNA
Σ Δ α
CD3
TCR


Site of
CORTICOSTEROID INHIBITION
GRE
Cell Membrane
Nuclear Membrane
Cytoplasm
Enhancer
Elements
RNA
Polymerase
5'
CODON
X
EFFECT OF
CORTICOSTEROID
BINDING TO GRE
HSP56
TRANSCRIPTION
R
S
TRANSLOCATION
PROCESSING
Steroid Receptor
mRNA
HSP90
Corticosteroids
TRANSLATION
Cytokine
Vanderbilt Transplant Center
Strom et al, Transplantation 57:1994

Antimetabolites - Azathioprine, Mycophenolate Mofetil, Brequinar



Nonselective Action of Azathioprine



Results:

- 5'-phosphoribosylamine ↓
- XMP ↓ PRPP ↑ AMP ↑
- thio-dGTP incorporated into DNA ↑
  - DNA strand breaks
  - delayed cytotoxicity

Selective, Noncompetitive Inhibition of IMPDH by Mycophenolic Acid

MPA inhibition of inosine monophosphate dehydrogenase (IMPDH)

↓

Depletion of dGTP; excess AMP, ADP, dATP

↓

Glycoprotein Synthesis

Inhibition of ribonucleotide reductase
PRPP synthetase

↓

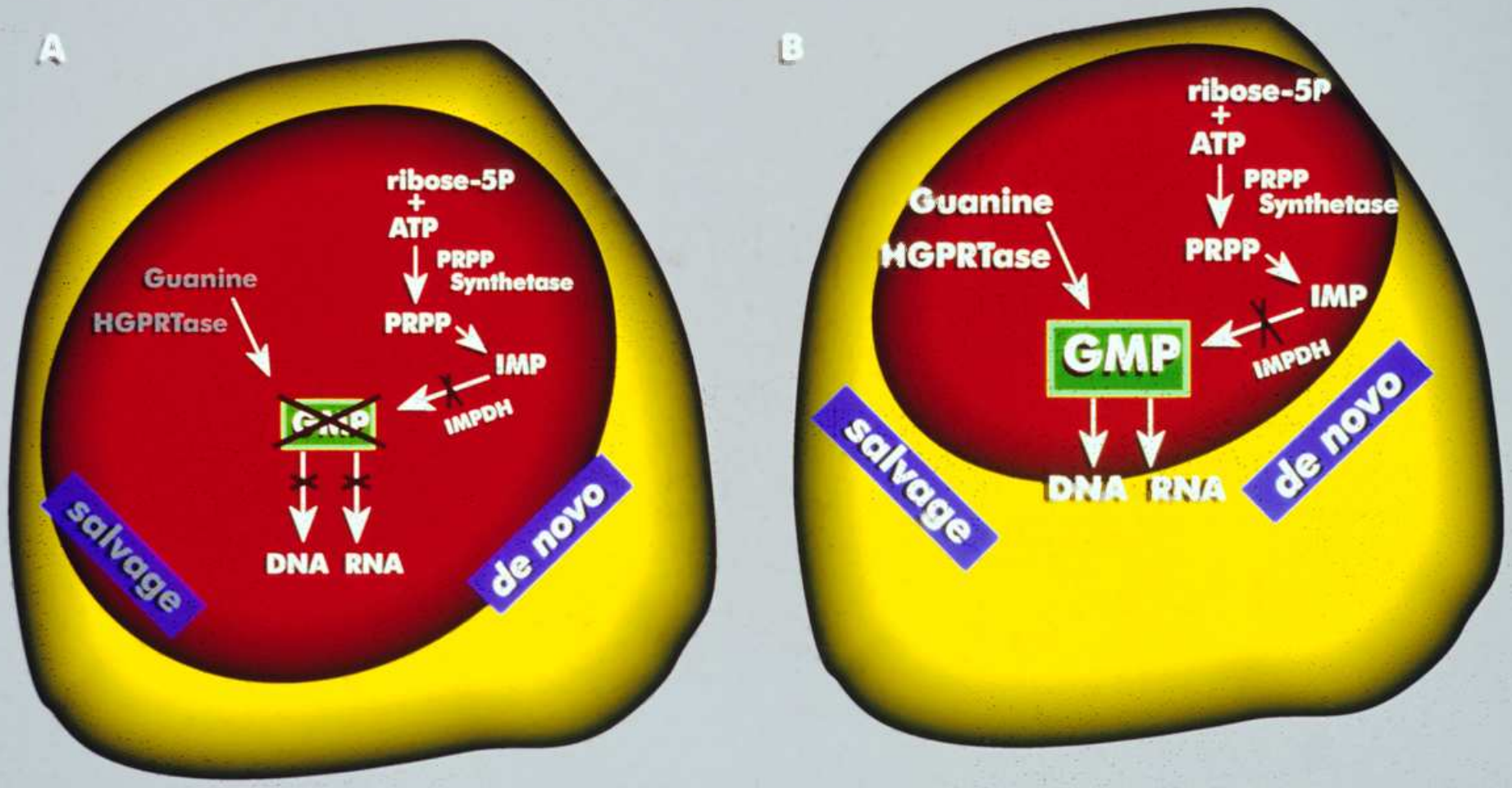
Depletion of deoxyribonucleotide triphosphates

DNA Polymerase

↓

DNA

Mycophenolate Mofetil



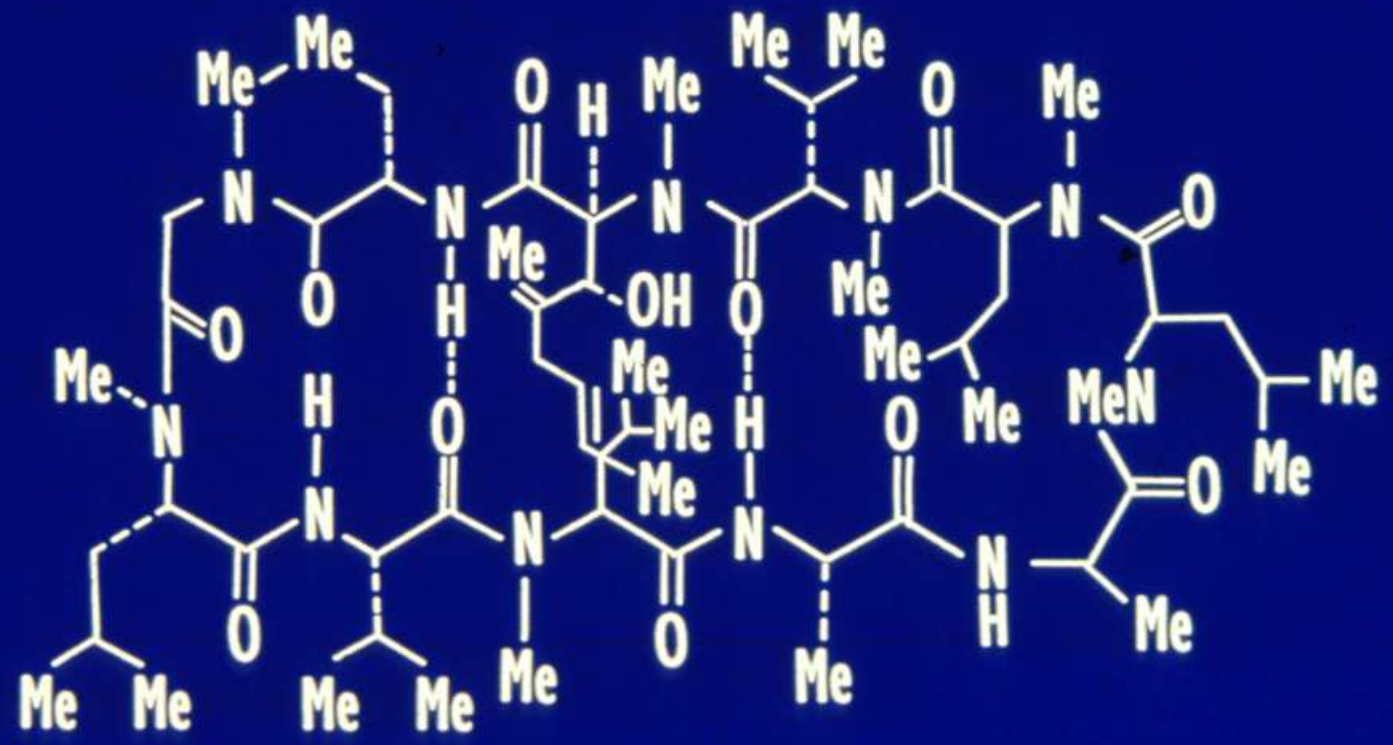
Lymphocyte

Parenchymal Cell

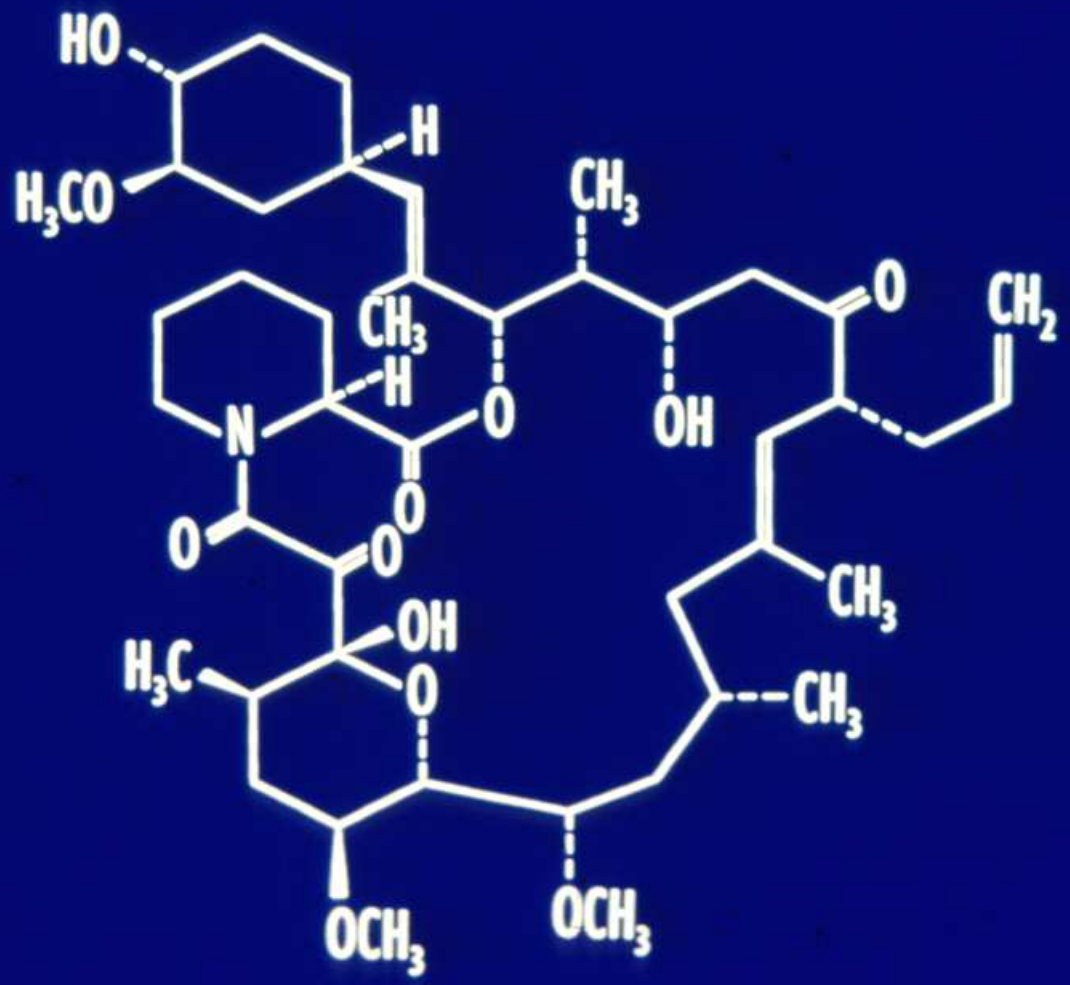
Immunophyllin Binding Agents

1. Cyclosporines (Sandimmune and Neoral)
2. Tacrolimus (FK506, Prograf)
3. Sirolimus (Rapamycin)

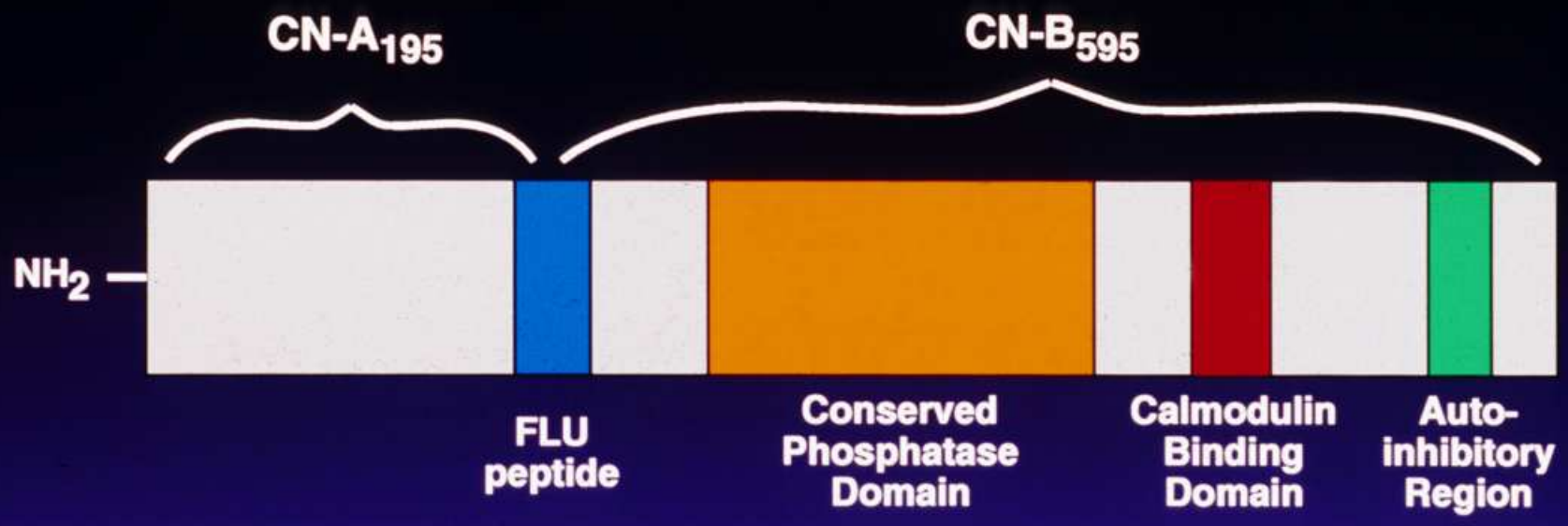
Cyclic undecapeptide cyclosporin A (CSA)



Tacrolimus (FK506)



Structure of Calcineurin



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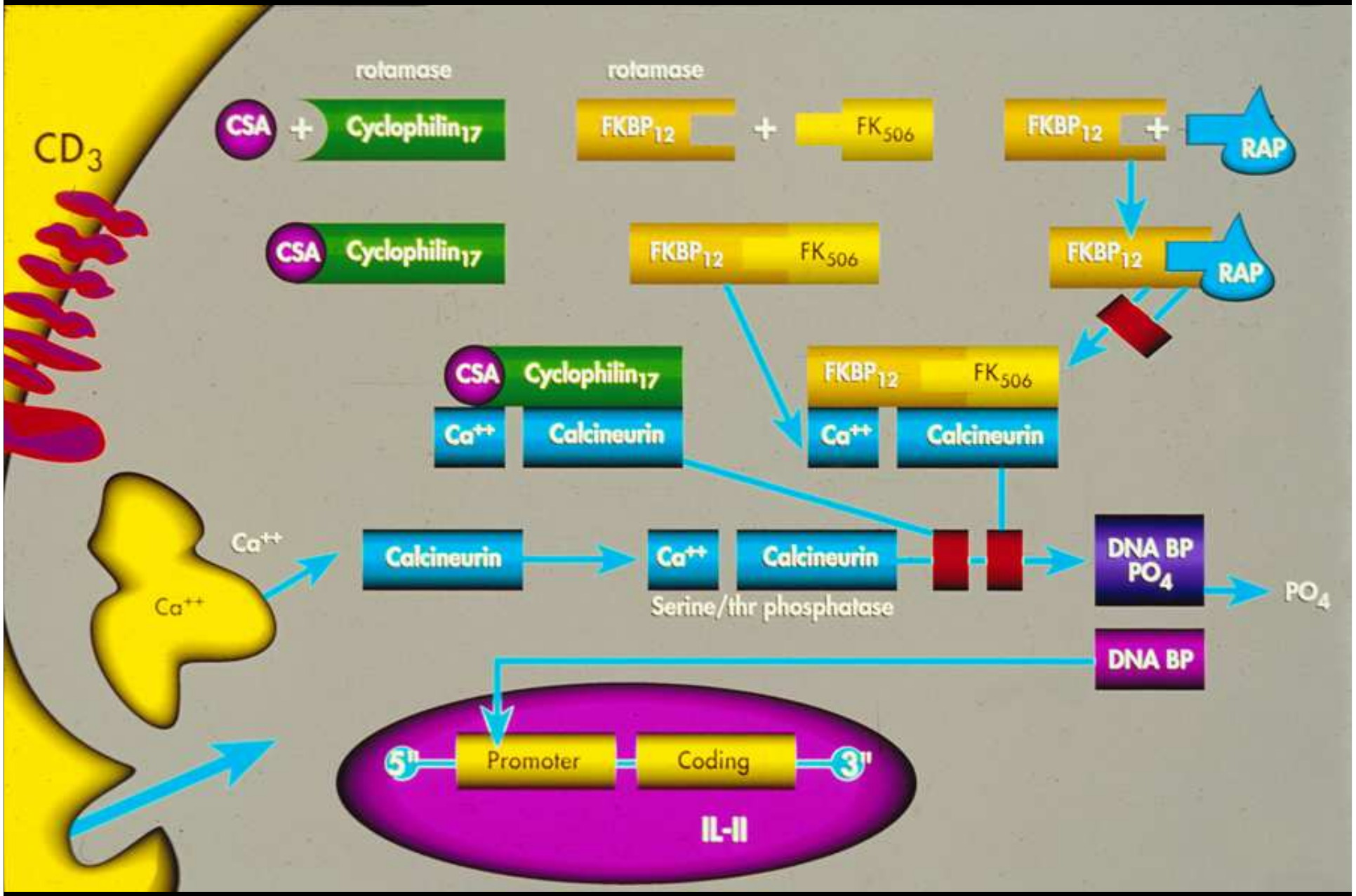
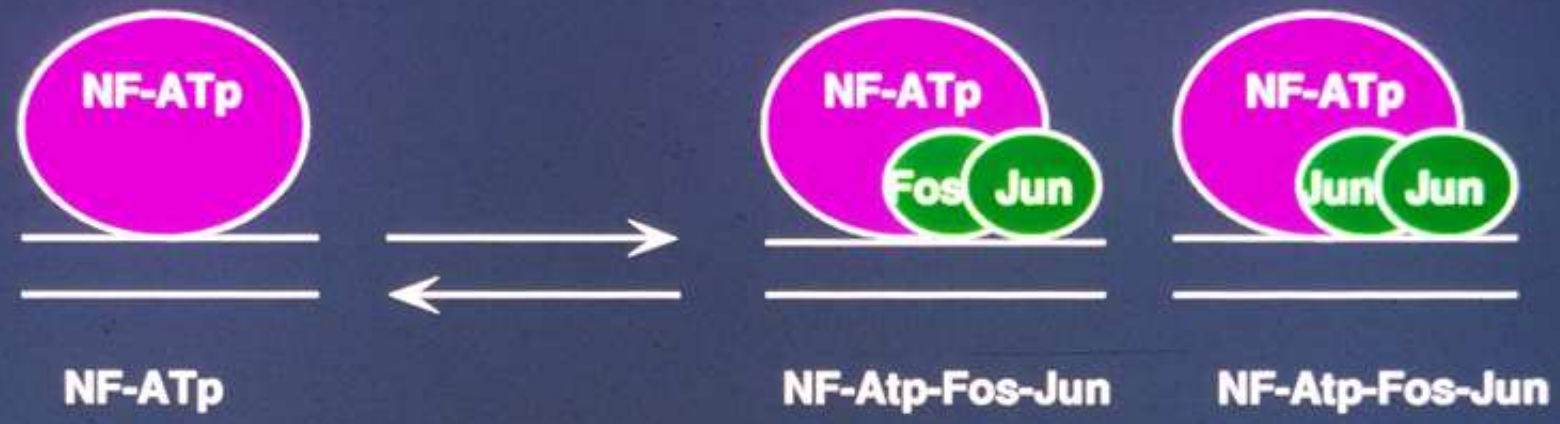
After Clipstone, Fiorentino, & Crabtree
J.Bio. Chem. 269:94


rotamase
rotamase
CD3
CSA
Cyclophilin17
FKBP12
FK506
RAP
Ca++
Calcineurin
Serine/thr phosphatase
DNA BP
PO4
5'
Promoter
Coding
3'
IL-II

(a)



Rapid
dissociation rate
(<2 min)

Slow
dissociation rate
(>20 min)

(b)

Distal murine NF-AT site

GCCCAAAGAGGAAAATTTGTTTCATACAG

Anjana Rao, Immunology Today 1994:15

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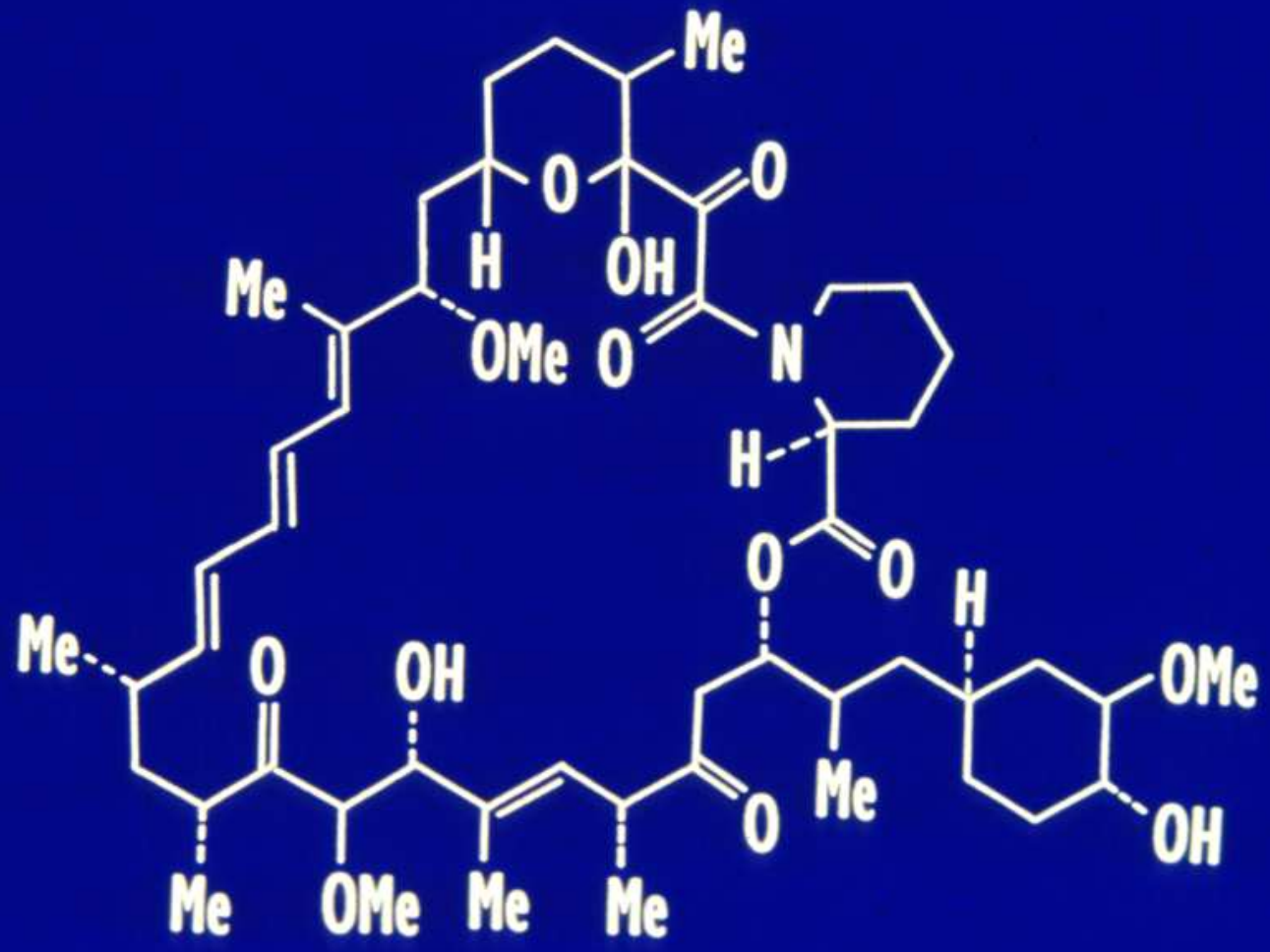
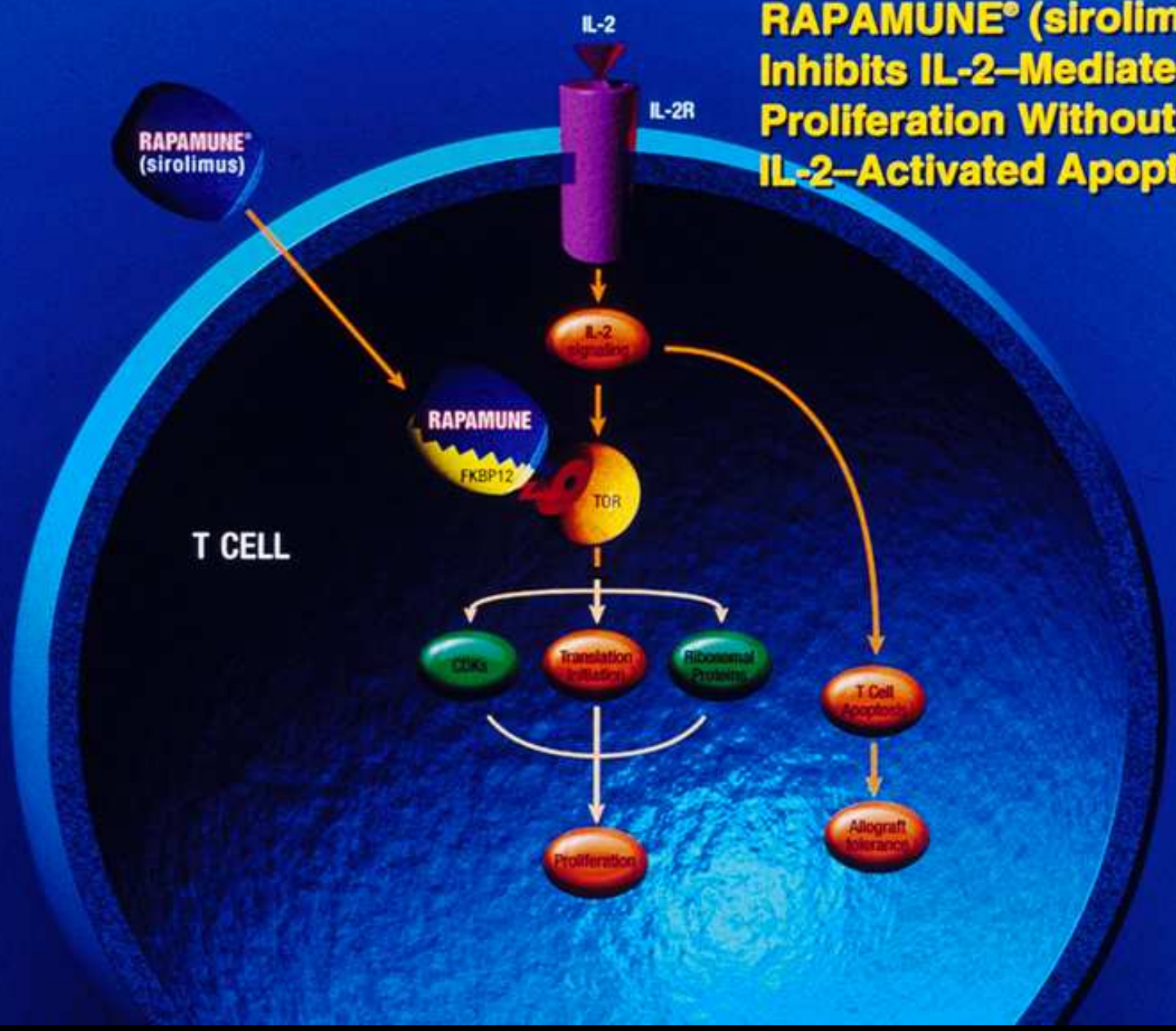
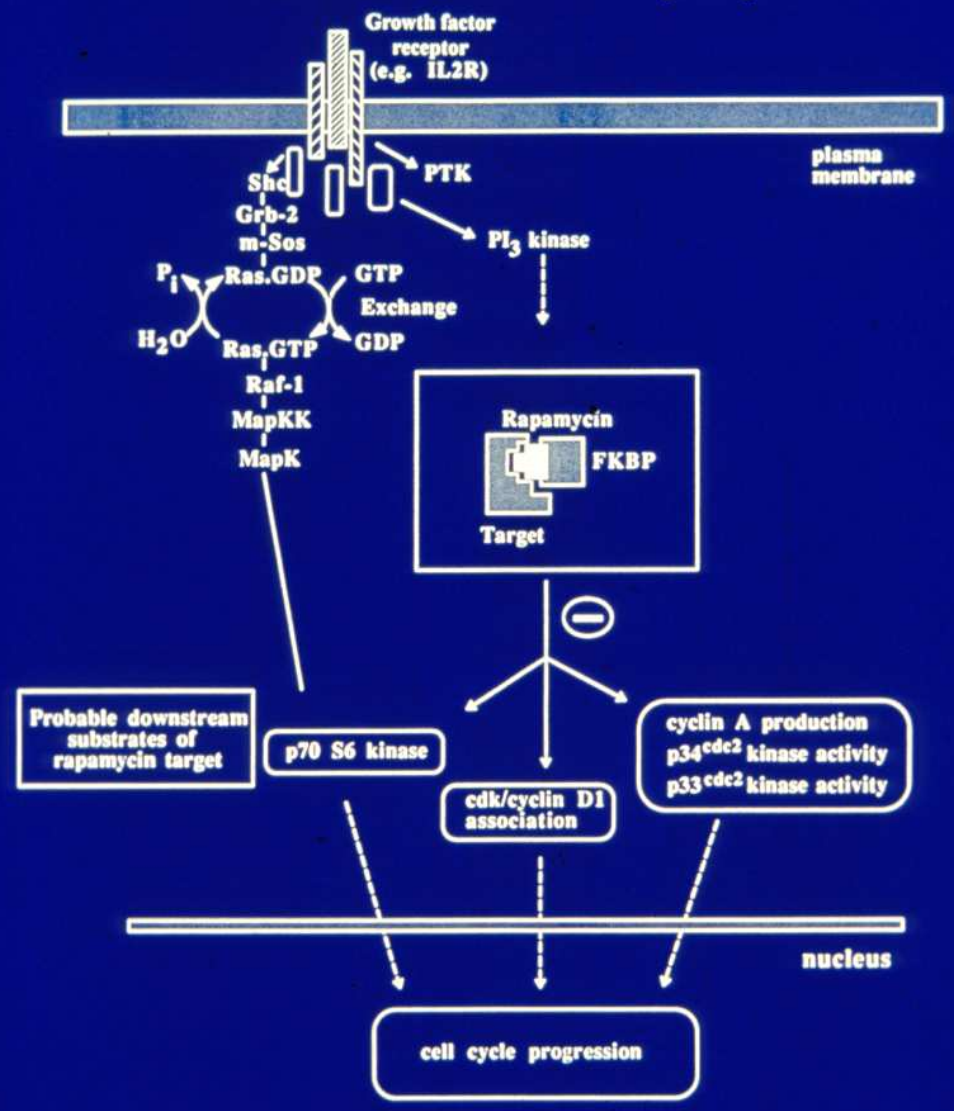
Rapamycin



RAPAMUNE® (sirolimus) Inhibits IL-2–Mediated Cell Proliferation Without Altering IL-2–Activated Apoptosis



Mechanism of Action of Rapamycin

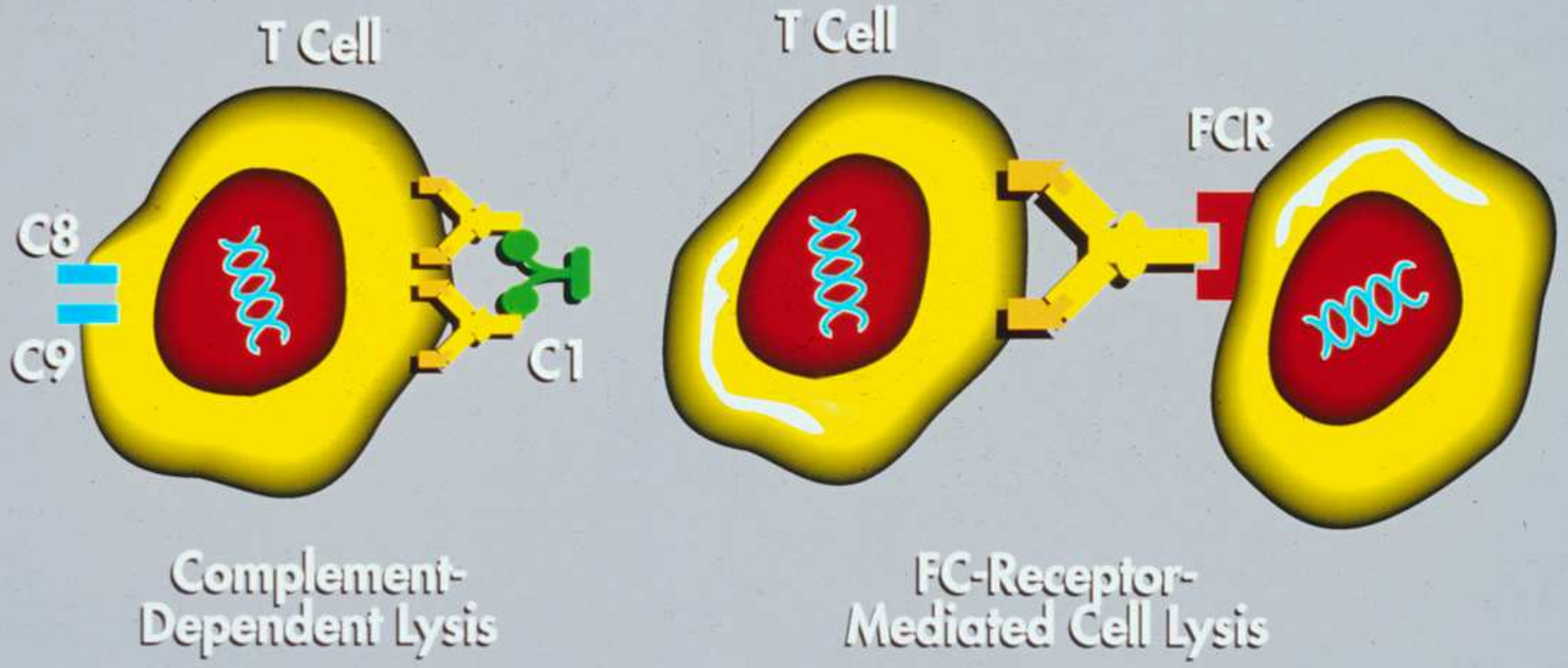


RAPAMUNE® (sirolimus) Has a Distinct Mechanism of Action

	Cyclosporine	RAPAMUNE
Binding protein	Cyclophilin	FKBP12
Effector protein	Calcineurin	mTOR
IL-2 message	Inhibited	–
IL-2 response	–	Inhibited
Cell-cycle effect	G_0-G_1	G_1-S

Galat A, et al. *Prog Biophys Mol Bio*. 1995;63:67-118. Wiederrecht G, et al. *Prog Cell Cycle Res*. 1995;1:53-71.
Liu J, et al. *Cell*. 1991;66:807-815. Flanagan WM, et al. *Nature*. 1991;352:803-807.
McCaffrey PG, et al. *J Biol Chem*. 1993;268:3747-3752.

Polyclonal Anti–T-Cell Antibodies



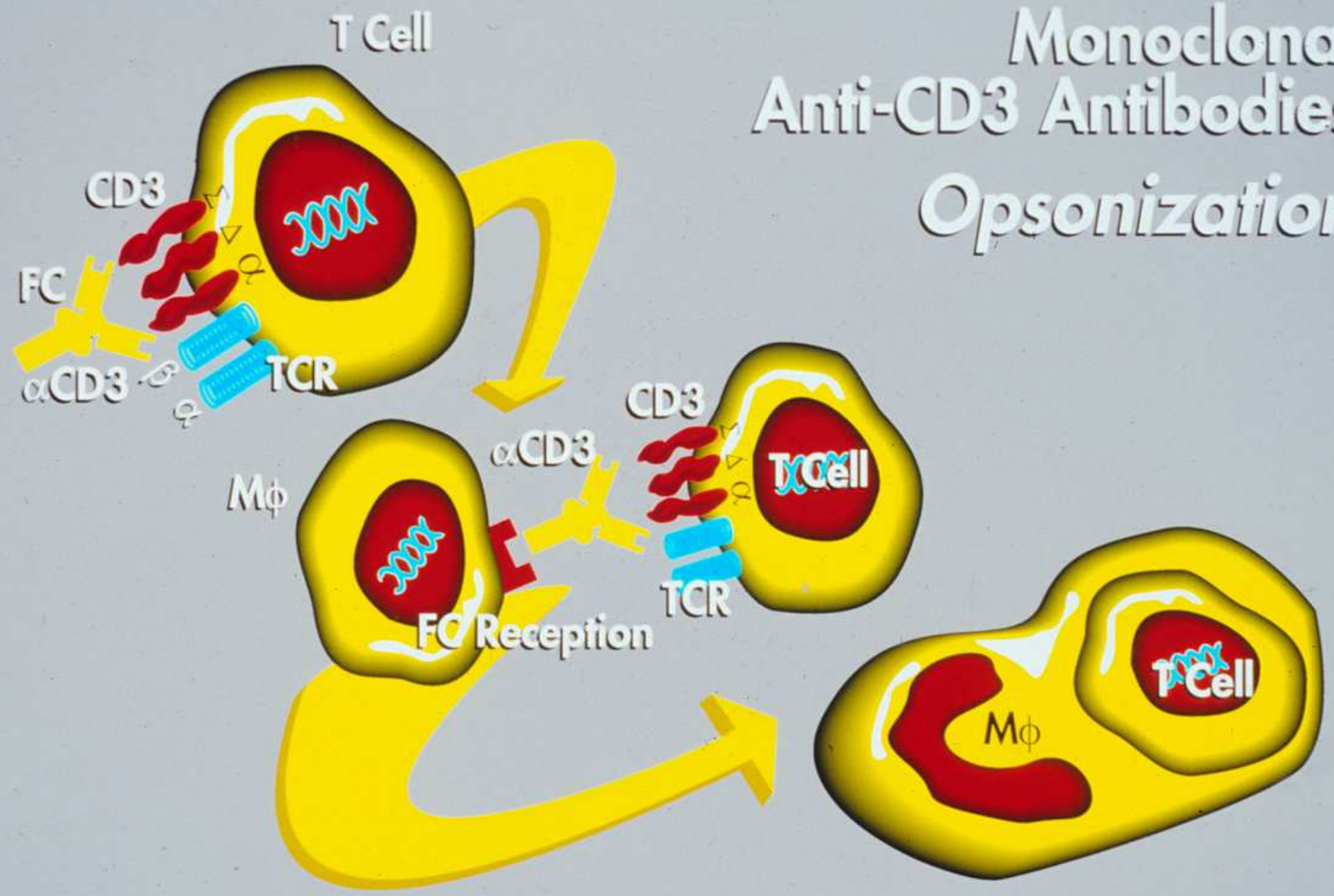
SPECIFICITIES IN ANTIBODY PREPARATIONS

ANTIBODY	ANTIGEN TARGET
ATGAM	CD2, CD3, CD4, CD5, CD7 CD8, CD11a, CD18, CD45, TCR
OKT3	CD3
OKT4	CD4
ENLIMOMAB	ICAM-1
T10 B9	TCR
ANTI LFA-1	LFA-1
ANTI-TAC	IL2 Receptor

Source: Bourdage JS, Hamlin DM. Comparative polyclonal antithymocyte globulin and antilymphocyte/antilymphoblast globulin anti-cd antigen analysis by flow cytometry. *Transplantation*. April 27, 1995; 59(8).

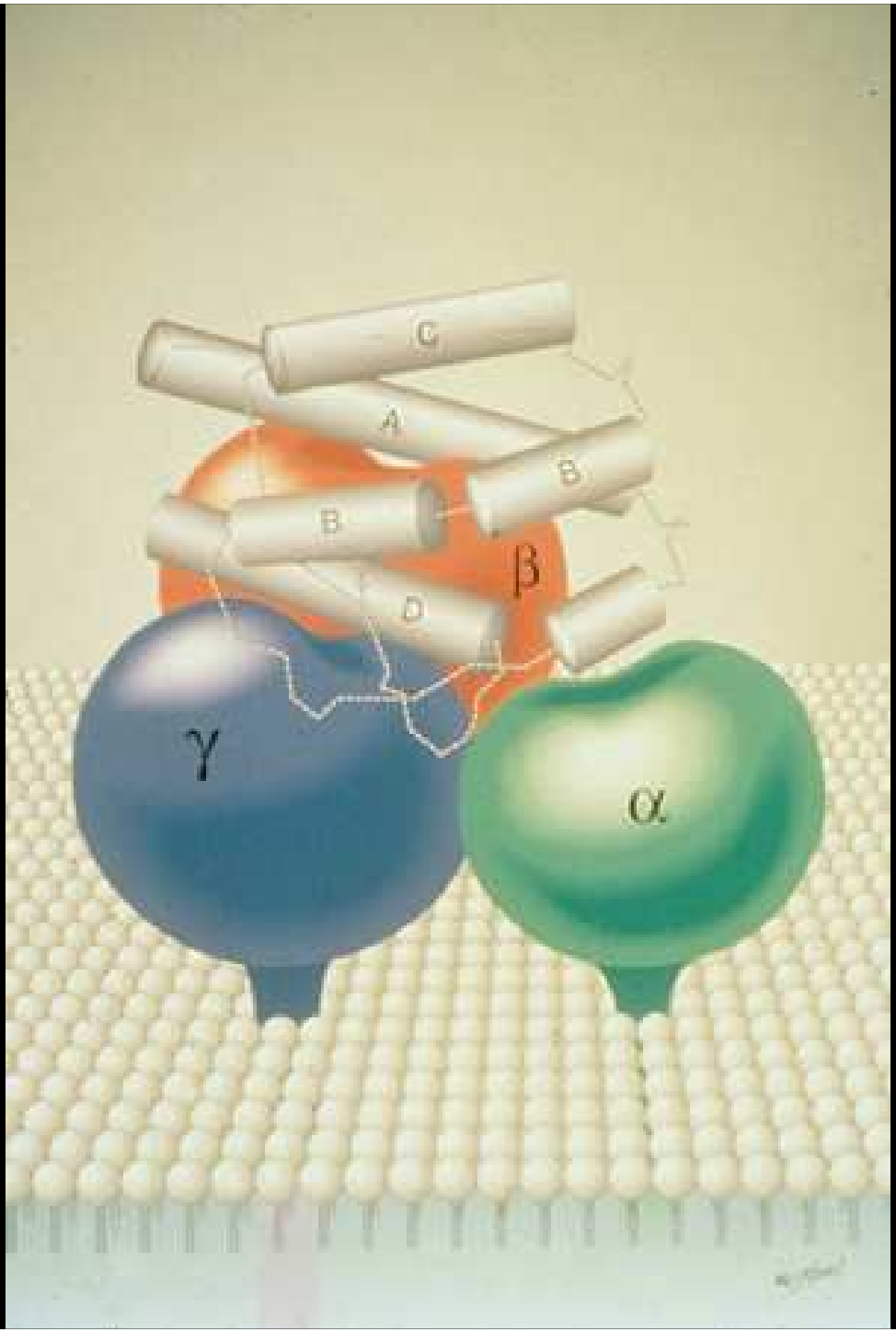
Monoclonal Anti-CD3 Antibodies

Opsonization

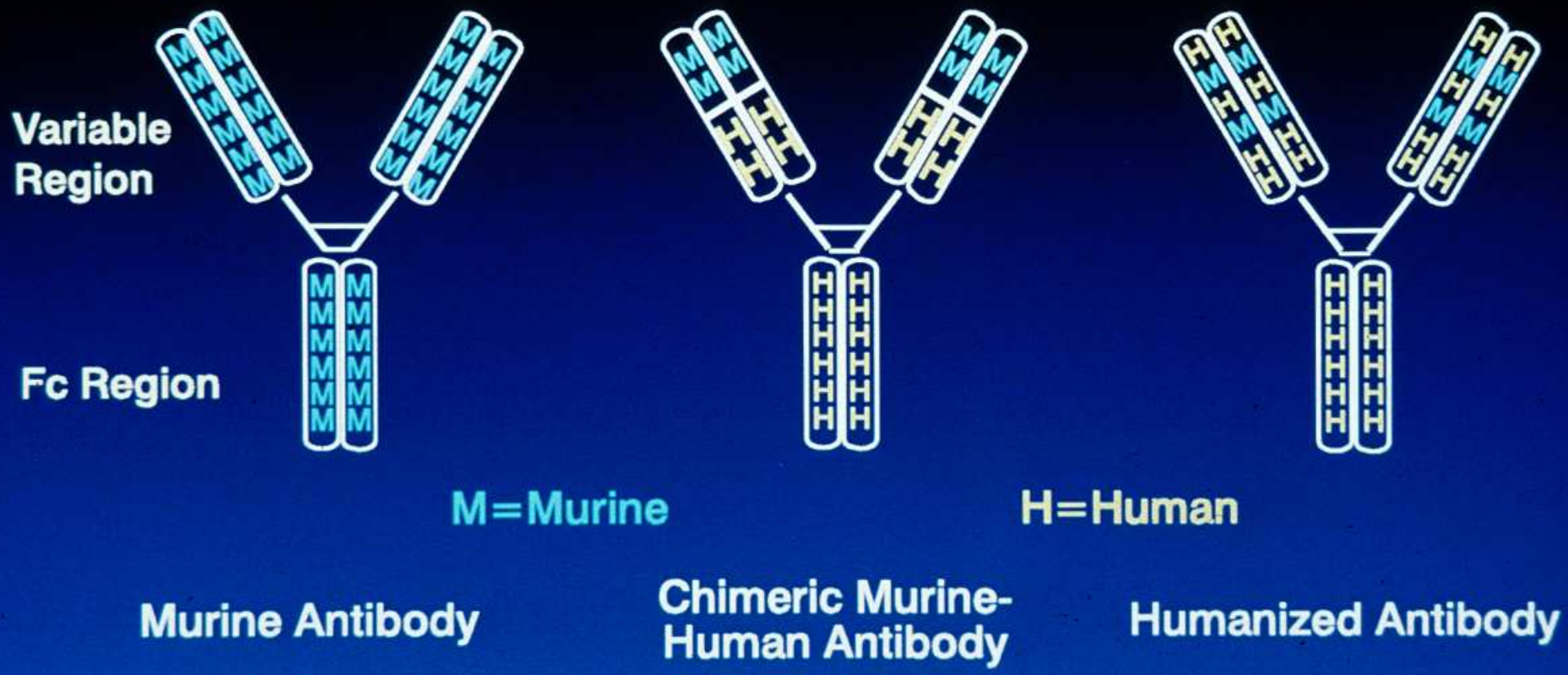


IL-2 RECEPTOR ANTIBODIES

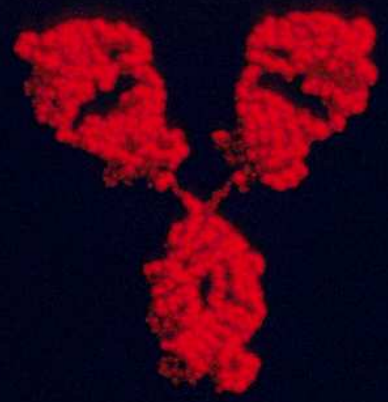
1. TARGET: NONCONSTITUTIVE IL-2R, MOSTLY β CHAIN
2. IN ANIMALS HIGHLY CYTOTOXIC AND PROLONGS GRAFTS
3. IN MAN MODULATES RECEPTOR
4. FRENCH STUDY: AS INDUCTION AGENT
   a. FEWER SIDE EFFECTS THAN P ON T CELL AB
   b. GRAFT SURVIVAL THE SAME
   c. TREND FOR MORE REJECTIONS


C
A
B
B
β
D
γ
α

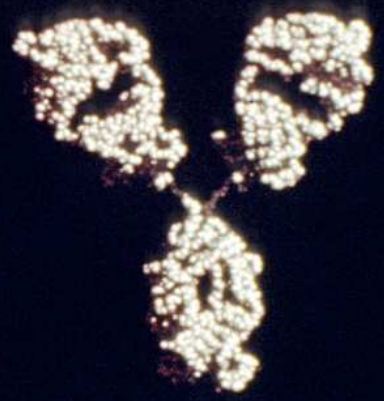
Murine and Humanized Monoclonal Antibodies



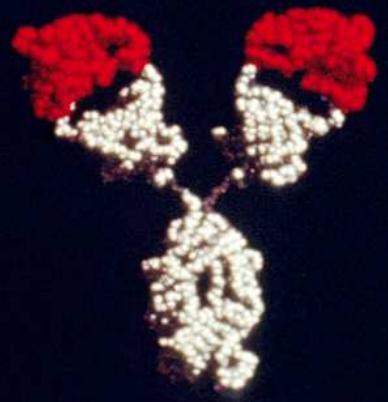
RECOMBINANT MONOCLONAL ANTIBODIES



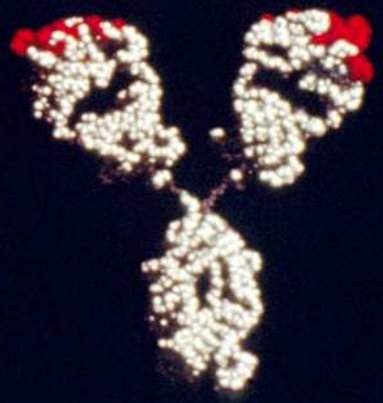
Mouse



Human



Chimeric



Humanized

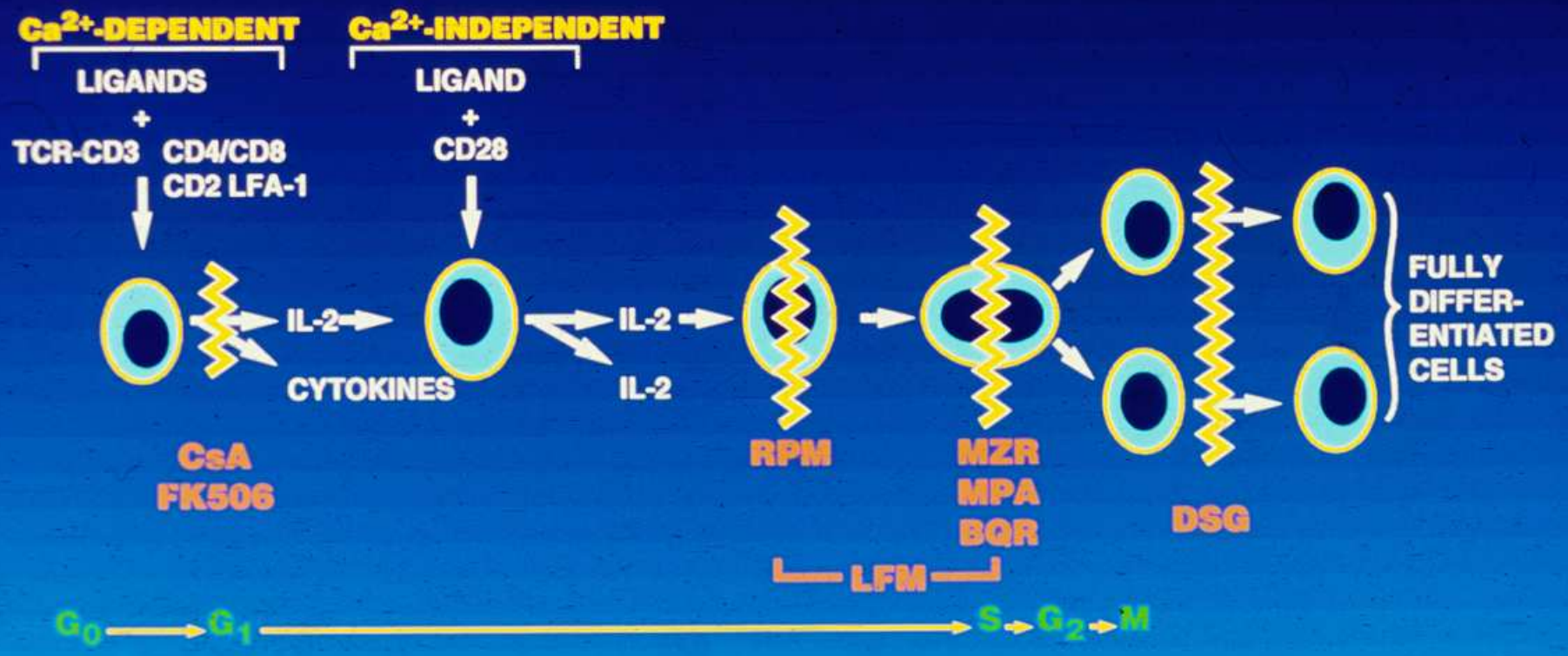
FTY 720
Potential Advantages

- Unique Action – Alteration of Lymphocyte Traffic
- Synergy With Calcineurin Inhibitors and Sirolimus Permitting Dose Reductions
- Side Effects Are Not Additive to Other I.S. Drugs
- Once Daily Dosing
- Minimal Drug-Drug Interactions
- Low Intra-subject Pharmacologic Variability

FTY 720

- Immunosuppressive *In Vitro*
- Depletes T and B cells from peripheral blood
- Increases Lymphocyte homing to mesenteric nodes and Peyer's patches

POSSIBLE SITES OF ACTION IN T CELLS OF NEW XENOBIOTIC IMMUNOSUPPRESSANTS



R.Morris '94