

LABORATÓRIO DE BIOLOGIA CELULAR E MOLECULAR
Instituto de Ciências Biomédicas
Universidade de São Paulo

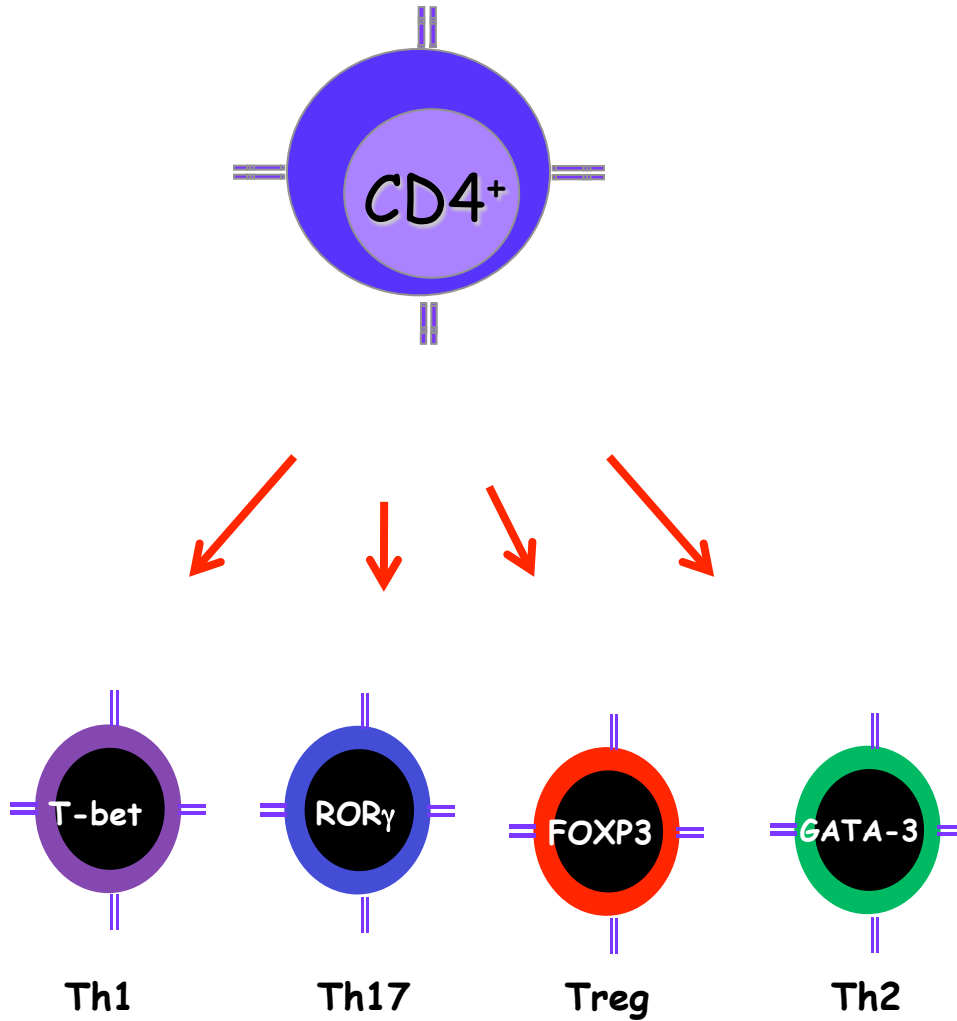


Ativação e diferenciação de linfócitos T

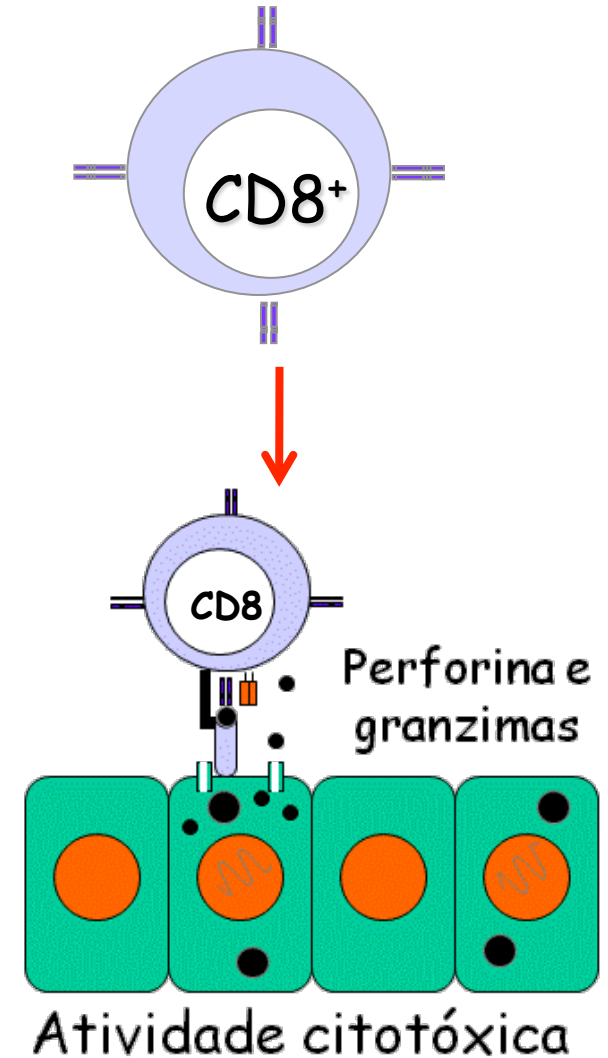
Prof. Dr. Gustavo P. Amarante-Mendes
Nutrição 2020



Linfócito T CD4 Células auxiliares



Linfócito T CD8 Células citotóxicas



Ativação de Linfócitos T (Conseqüências)

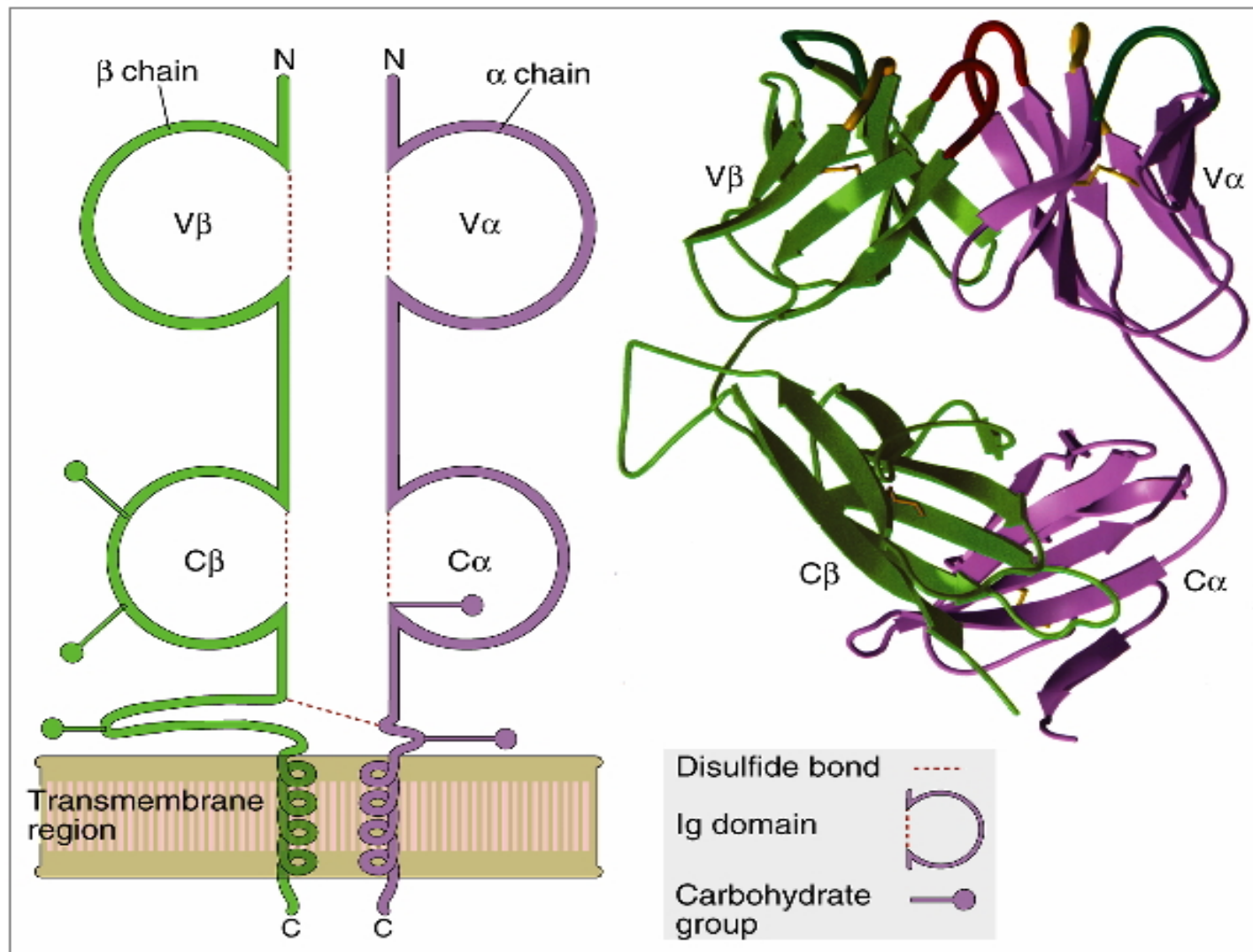
- Estimulação do complexo TcR resulta na ativação de uma cascata de eventos bioquímicos que levam à **proliferação** e **diferenciação** celular
- Geração de células **Efetoras** e de **Memória**
- Produção de **citocinas** (CD4) e atividade **citotóxica** (CD8)

Estrutura do TCR

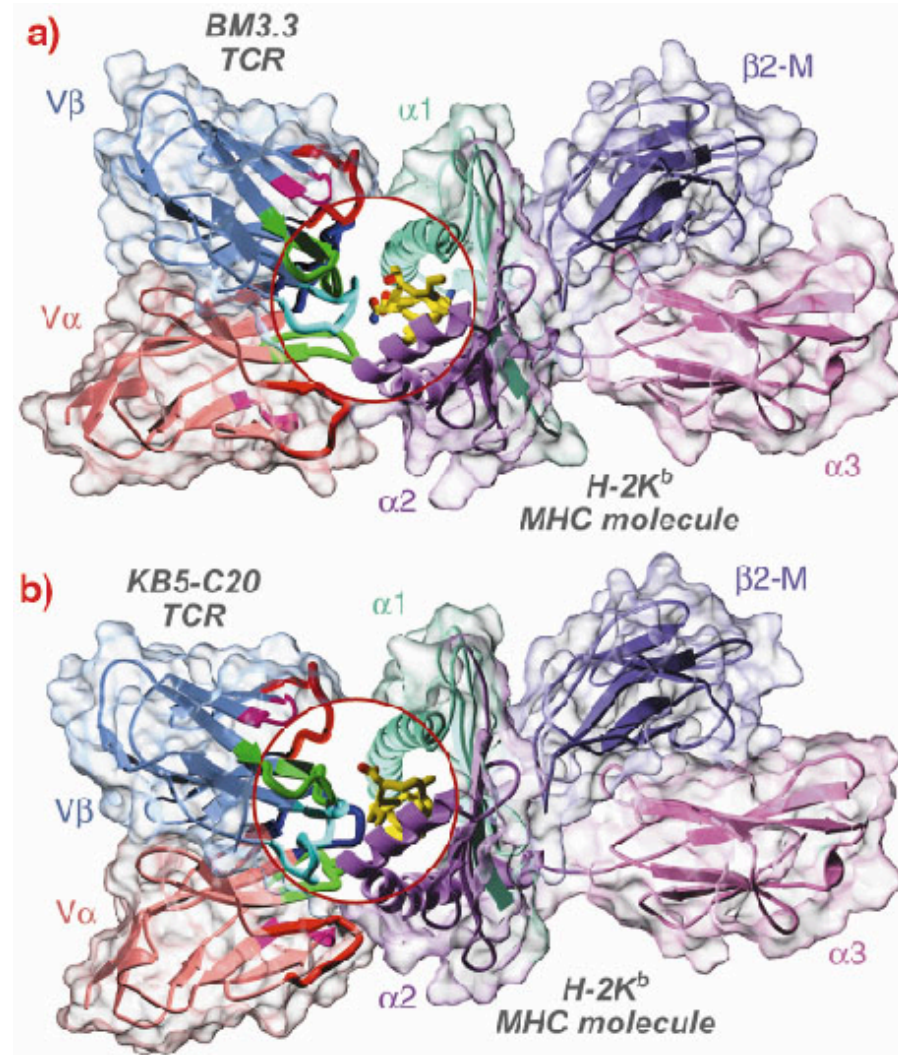
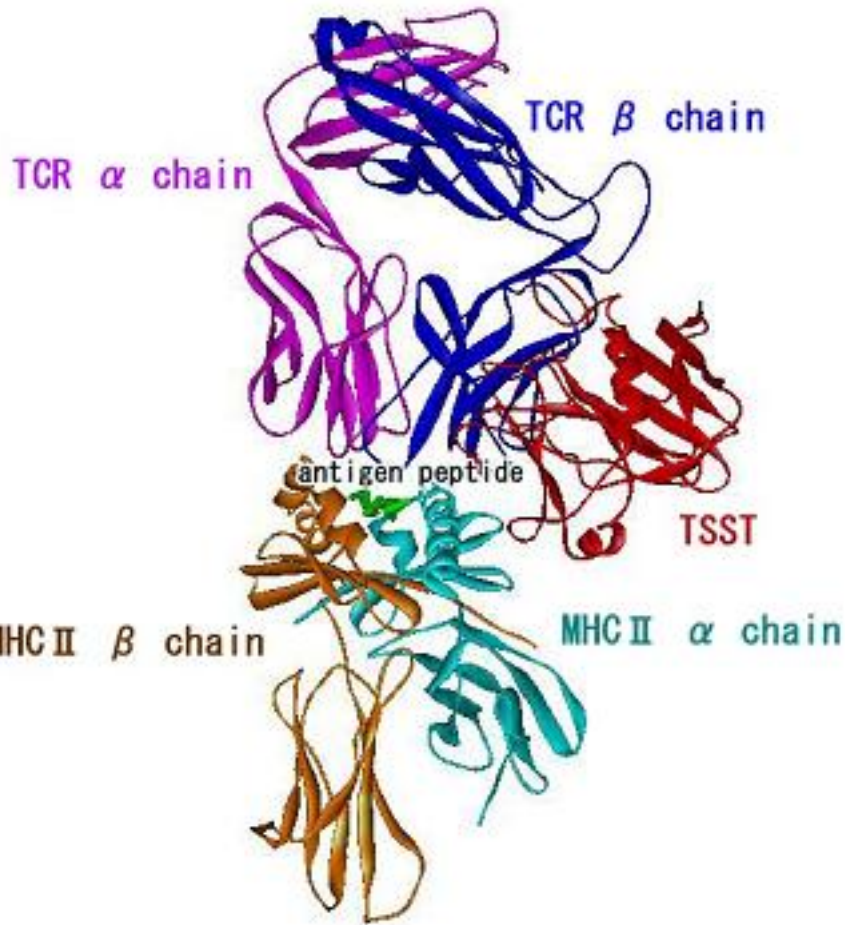
(Conceitos Básicos)

- TCR é formado por duas cadeias ligadas não-covalentemente
- Reconhecimento antigênico ocorre através da interação com **peptídeo + MHC**
- Transdução do sinal é dado por moléculas acessórias (CD3 e cadeias ζ)
- CD3 e cadeias ζ contém **ITAMS!**

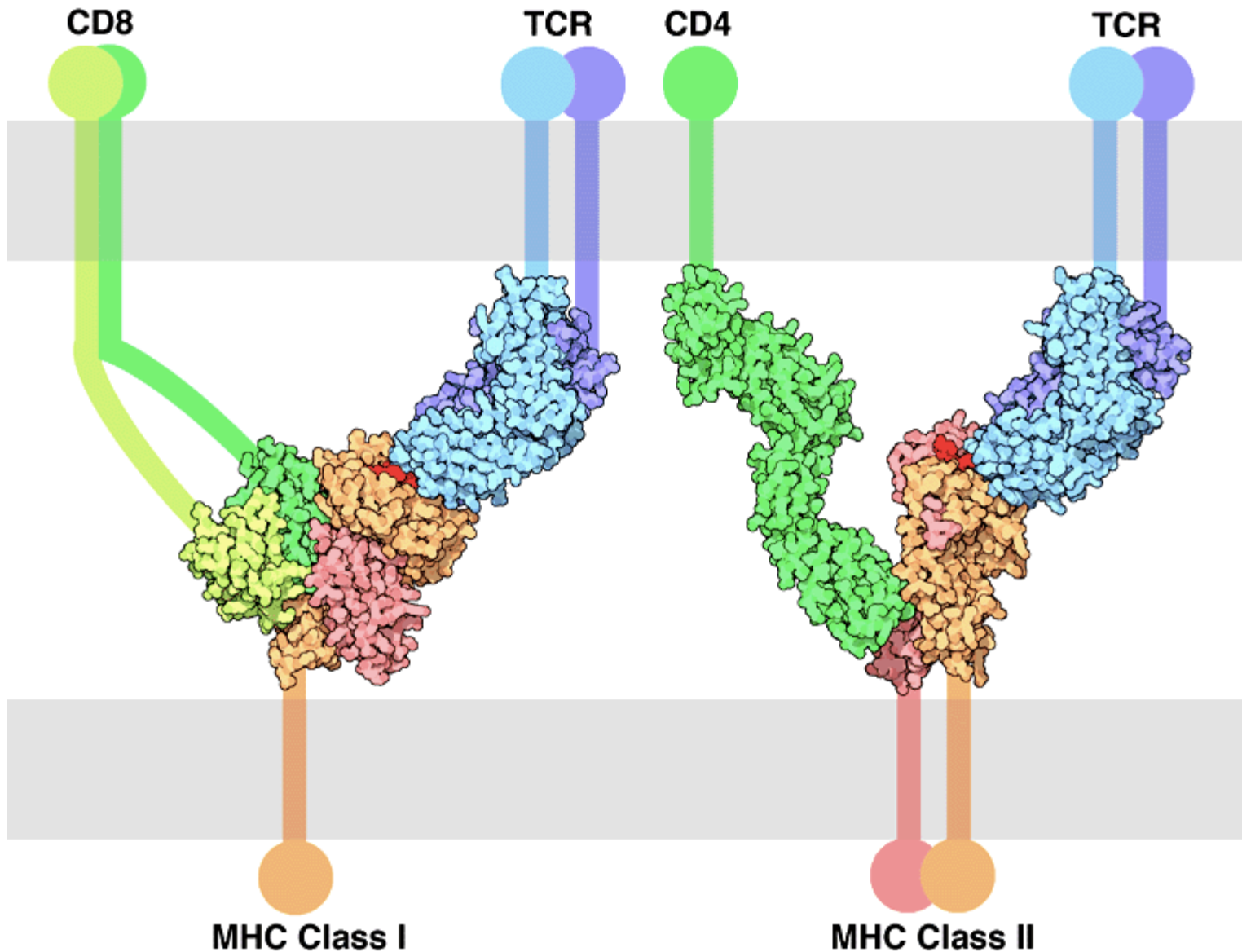
Estrutura do TCR



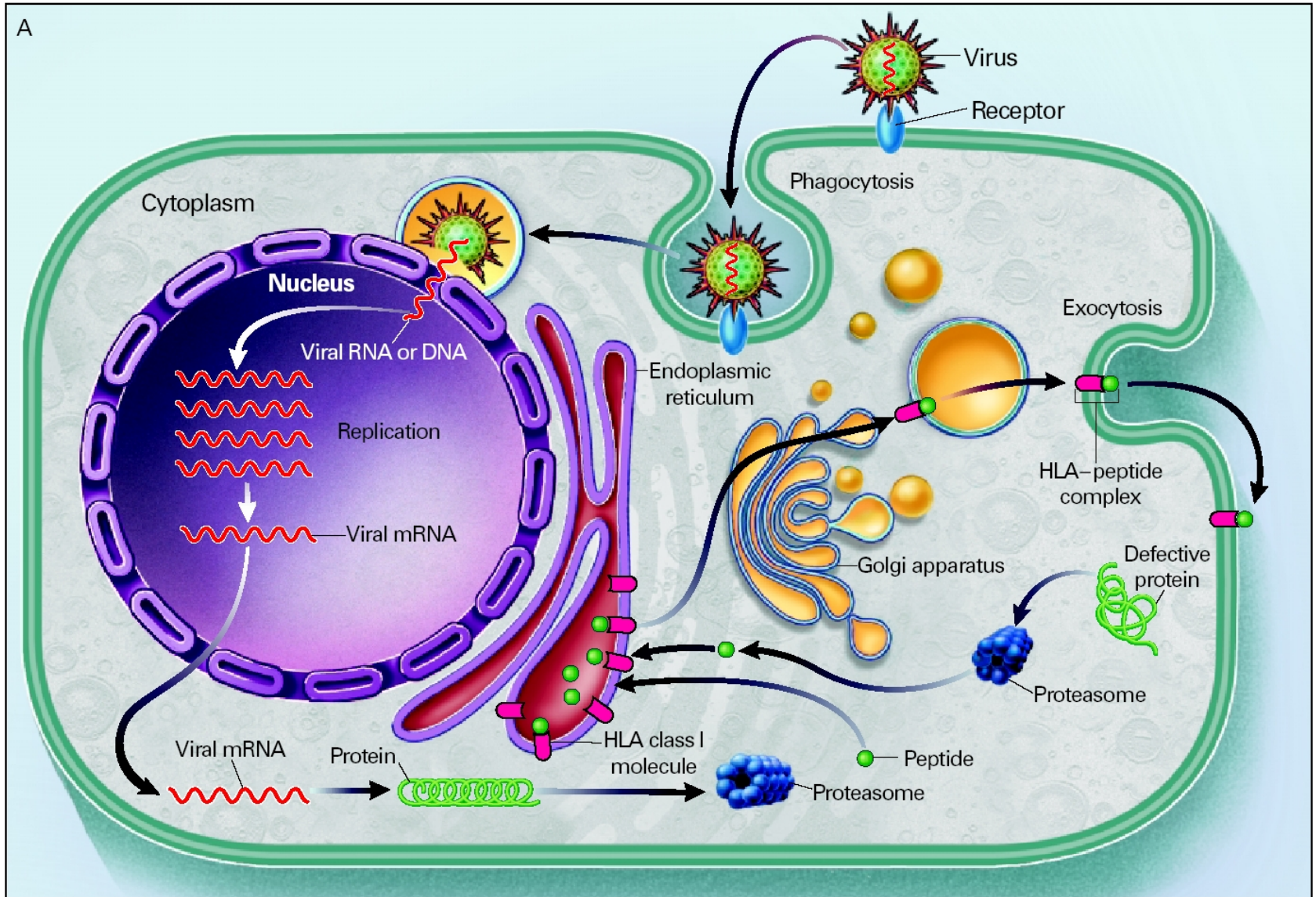
Interação TCR-MHC+peptídeo



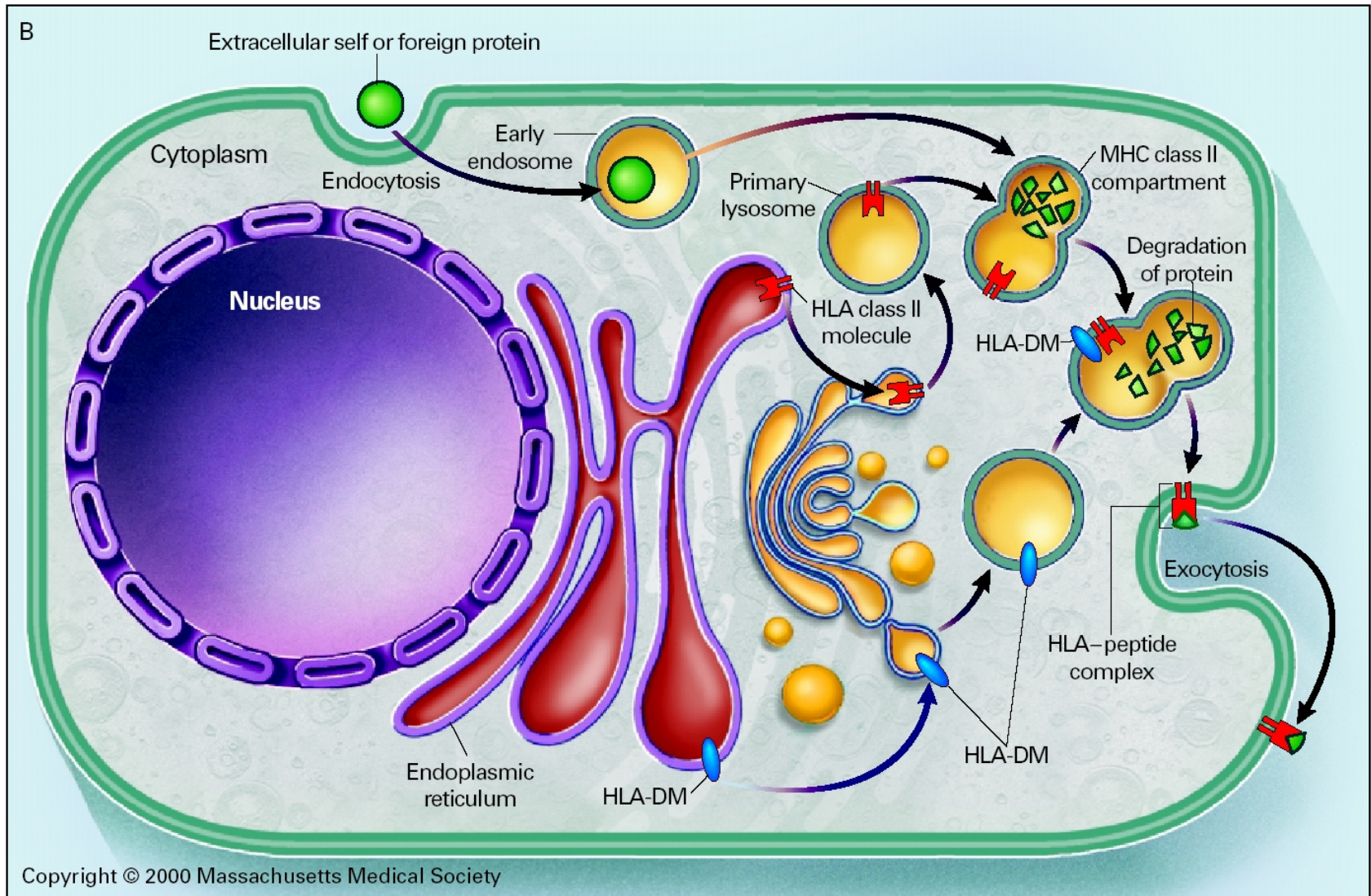
Receptor e Co-receptores



Apresentação MHC classe I



Apresentação MHC classe II

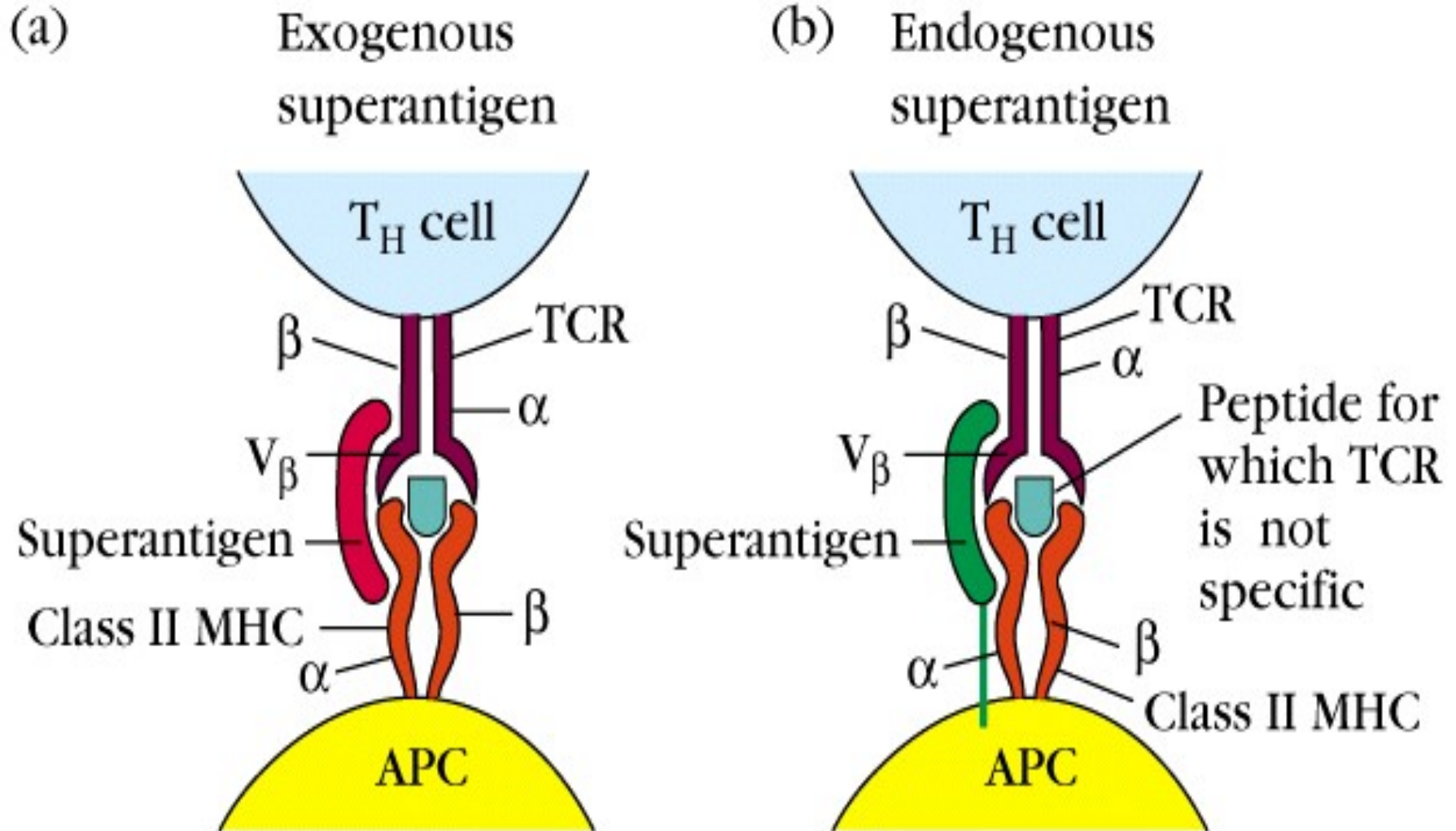


Klein J, Sato A. The HLA System. First of Two Parts. N Engl J Med 2000; 343:702-9.

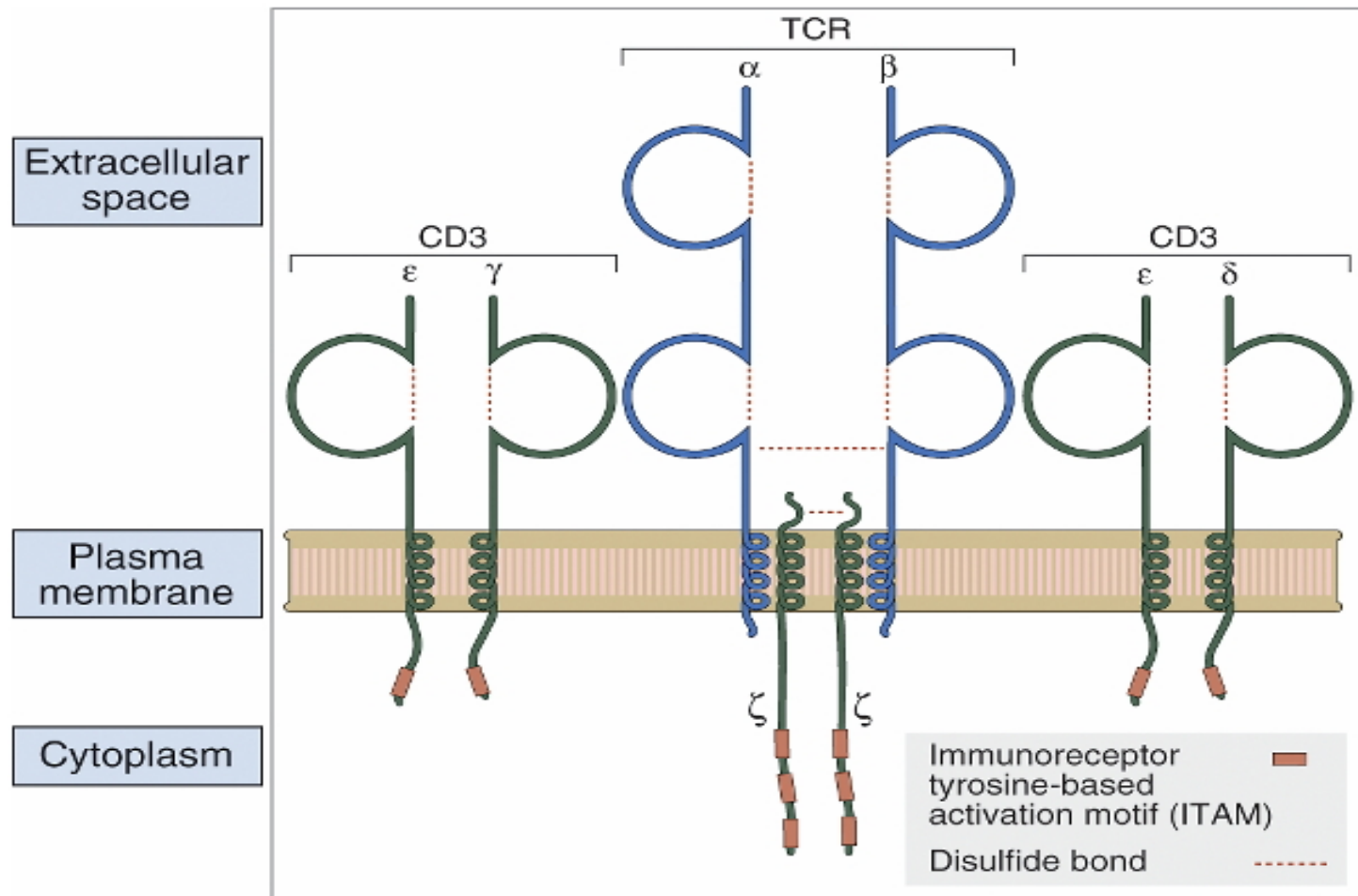


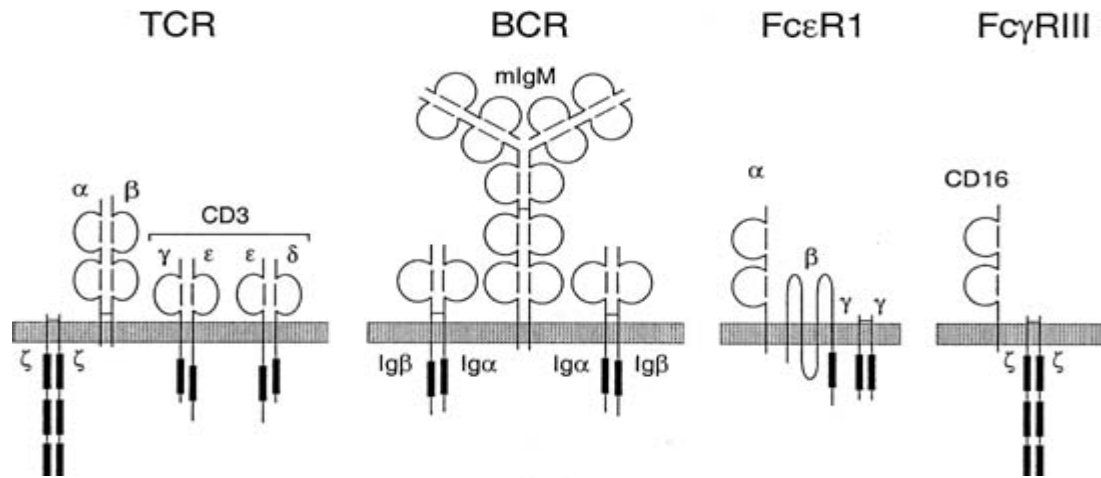
The New England
Journal of Medicine

Superantigenos



Componentes do complexo TCR/CD3





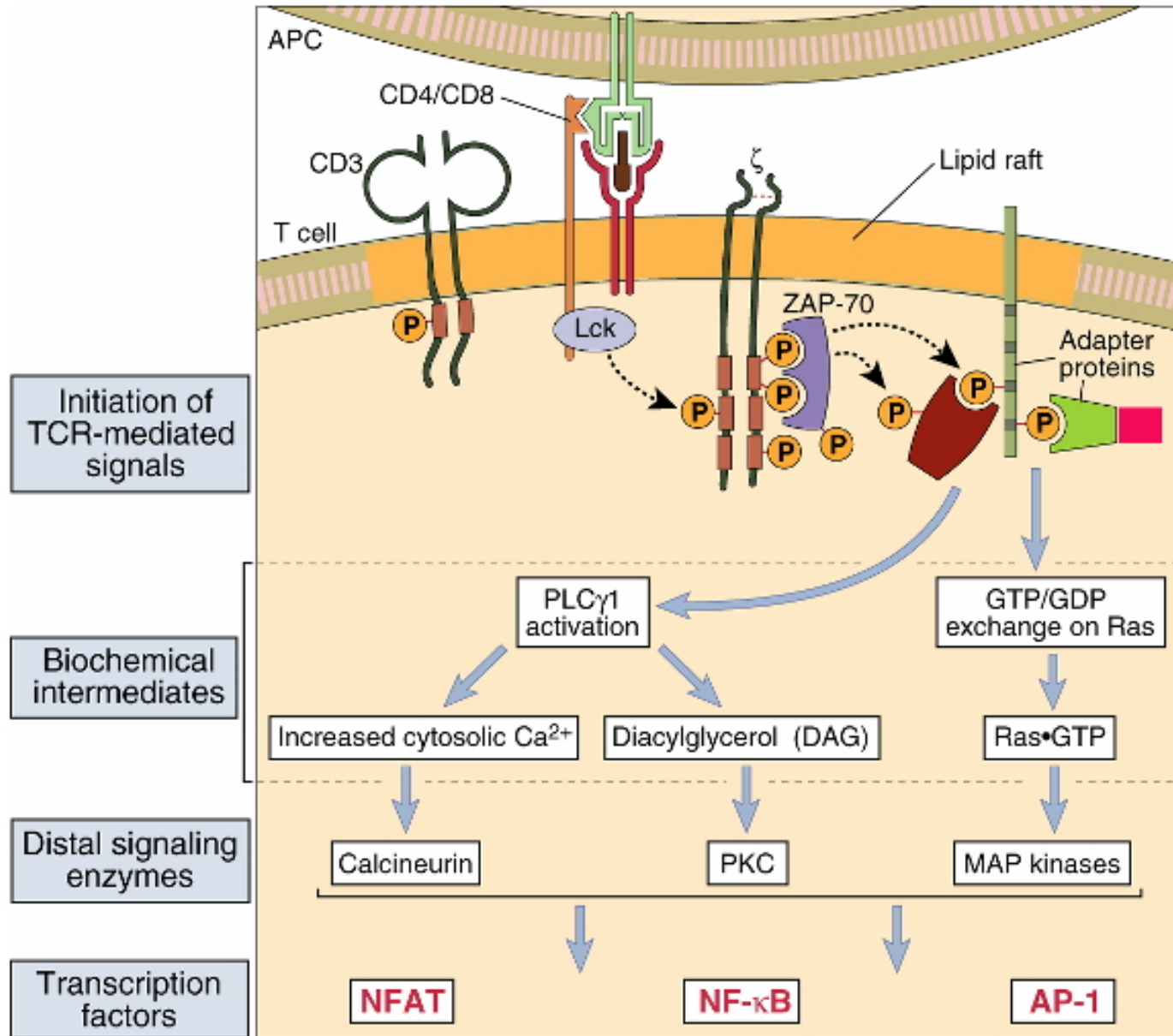
ITAMs

(Immunoreceptor Tyrosine-Based Activation Motif)

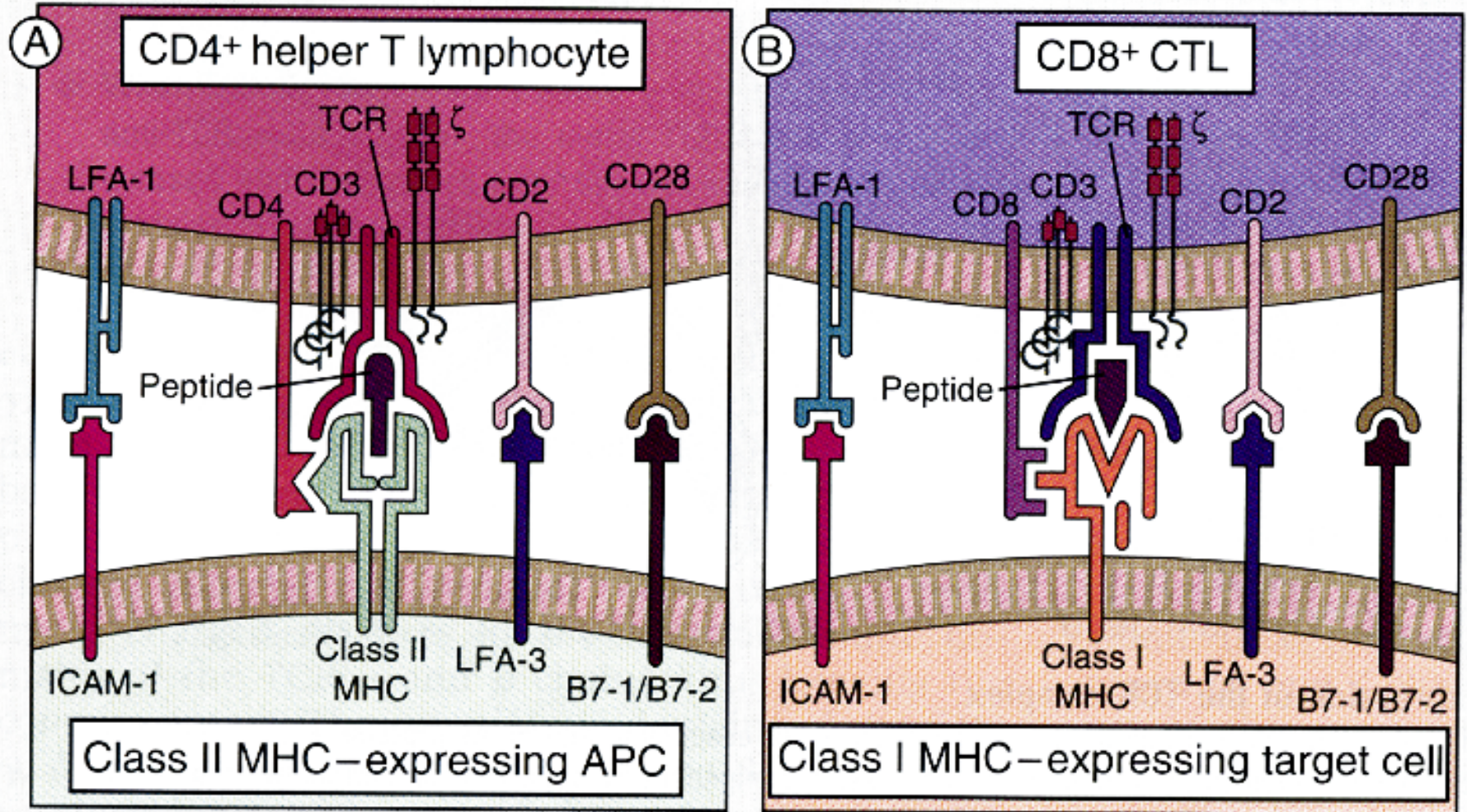
h ζ 1	N Q L Y N E L N L G R R E E - Y D V L
h ζ 2	E G L Y N E L Q K D K M A E A Y S E I
h ζ 3	D G L Y Q G L S T A T K D T - Y D A L
hCD3 γ	D Q L Y Q P L K D R E D D Q - Y S H L
hCD3 ϵ	N P D Y E P I R K G Q R D L - Y S G L
hCD3 δ	D Q V Y Q P L R D R D D A Q - Y S H L
rIgE FcR γ	D A V Y T G L N T R N Q E T - Y E T L
rIgE FcR β	D R L Y E E L - H V Y S P I - Y S A L
mIg α	E N L Y E G L N L D D C S M - Y E D I
mIg β	D H T Y E G L N I D Q T A T - Y E D I
BLV gp30	D S D Y Q A L L P S A P E I - Y S H L
EBV LMP-2	H S D Y Q P L G T Q D Q S L - Y L G L
SIV Nef	G D L Y E R L L R A R G E T - Y G R L
HHV8 K1	L Q D Y Y S L H D L C T E D - Y T Q P

Consensus D/E- - Y - - L - - - - - Y - - L

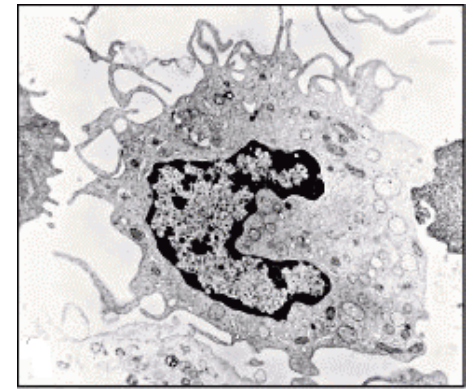
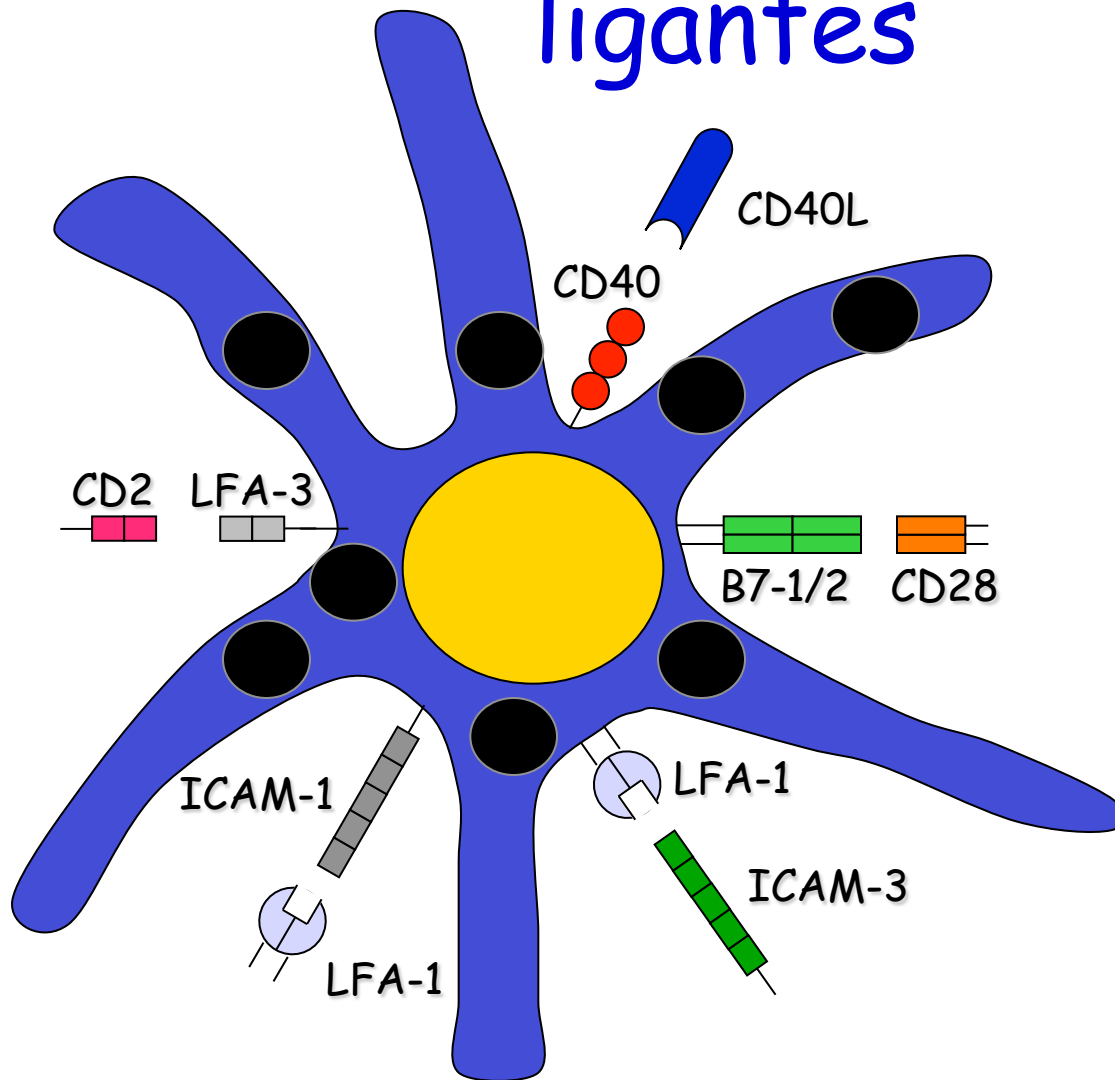
Sinalização intracelular



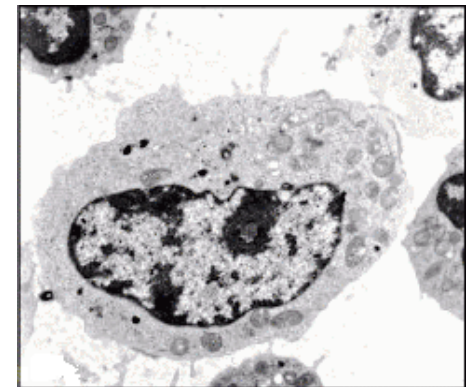
Interações Celulares



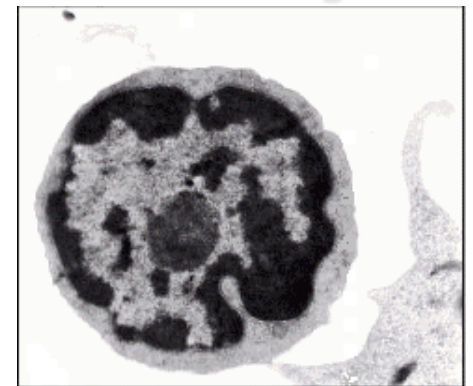
APCs, moléculas co-estimuladoras e seus ligantes



Célula dendrítica

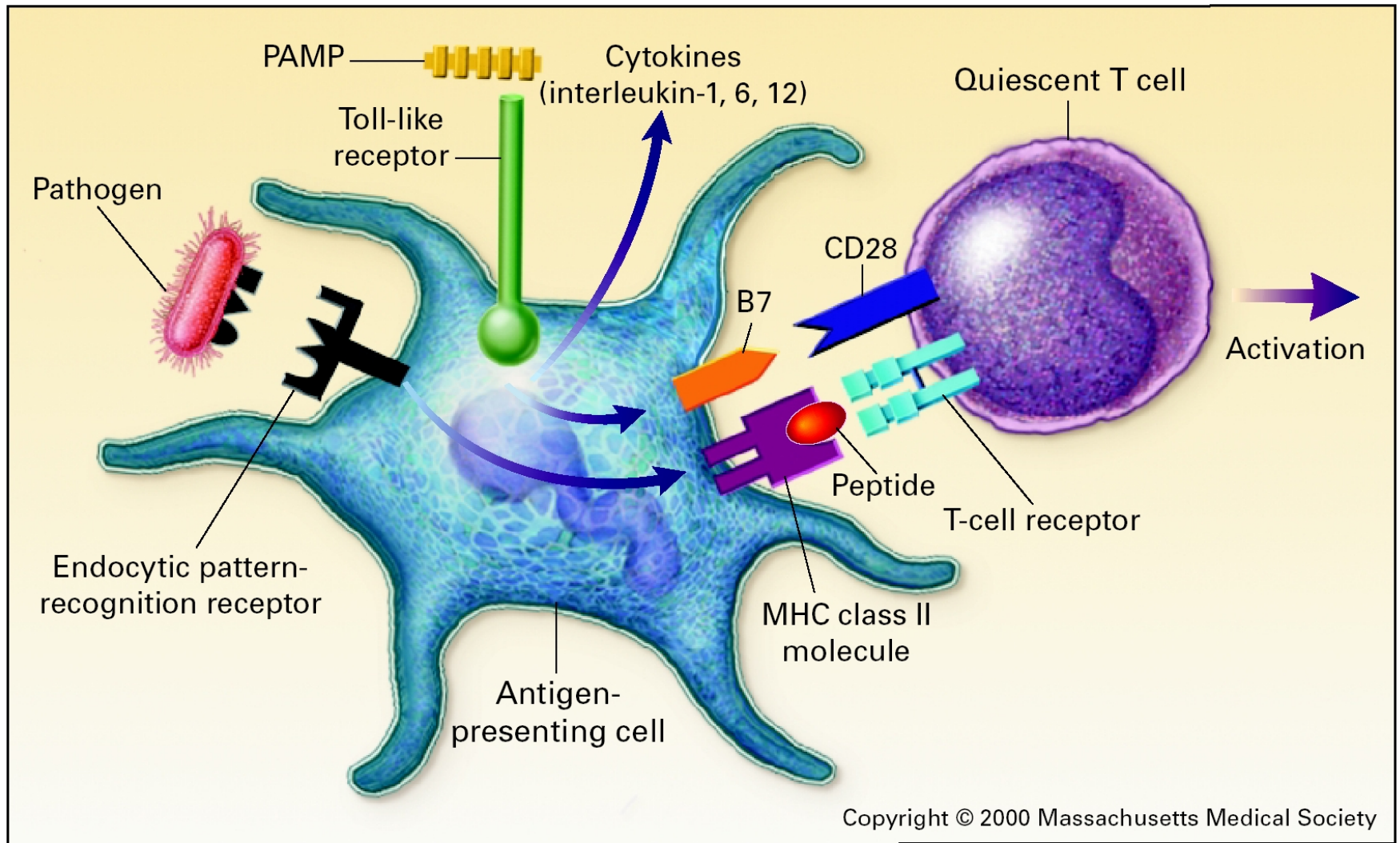


Macrófago



Linfócito B

PAMPs activate APCs

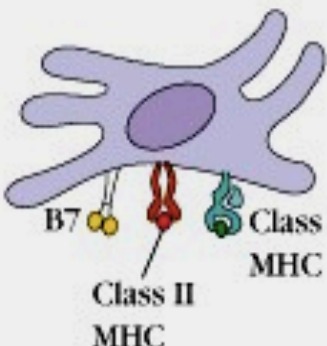

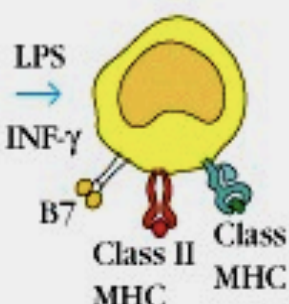
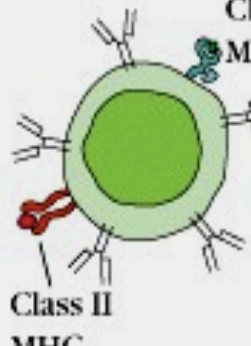
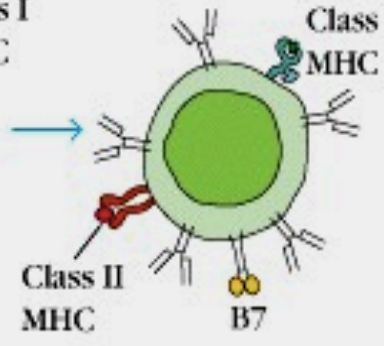


Medzhitov R, Janeway C Jr. Innate Immunity. N Engl J Med 2000; 343:338-44.



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Journal of Medicine

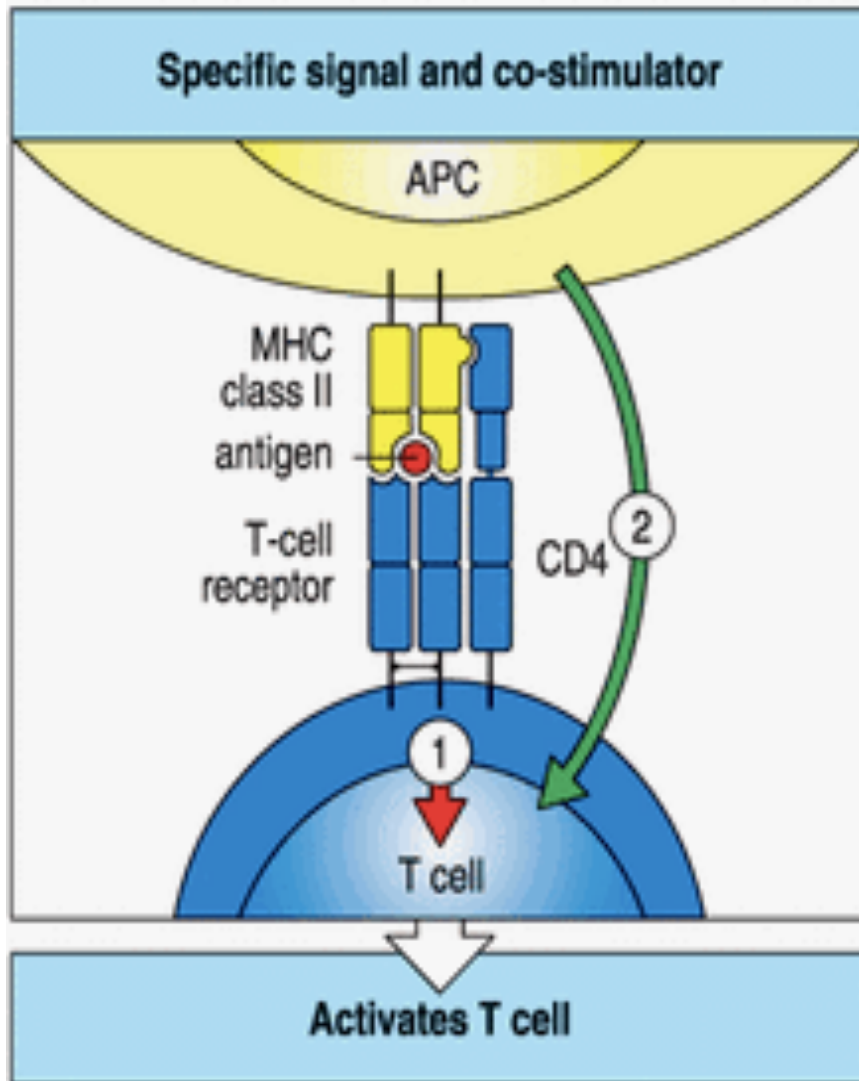
Atividade Co-estimuladora

	Dendritic cell	Macrophage		B Lymphocyte	
					
Antigen uptake	Endocytosis phagocytosis (by Langerhans cells)	Phagocytosis	Phagocytosis	Receptor-mediated endocytosis	Receptor-mediated endocytosis
Class II MHC expression	Constitutive (+++)	Inducible (-)	Inducible (++)	Constitutive (++)	Constitutive (+++)
Co-stimulatory activity	Constitutive B7 (+++)	Inducible B7 (-)	Inducible B7 (++)	Inducible B7 (-)	Inducible B7 (++)
T-cell activation	Naive T cells Effector T cells Memory T cells	(-)	Effector T cells Memory T cells	Effector T cells Memory T cells	Naive T cells Effector T cells Memory T cells

Major events in T cell activation

Event	Example
Cell-cell interaction	T cell-APC CTL-target cell
Receptor-ligand binding	TCR-antigen/MHC
Transmembrane signal transduction	Activation of Lck
Generation of second messengers	1,4,5-IP ₃ and DG
Second-messenger effects	Ca ²⁺ mobilization Protein kinase C activation
Biochemical pathways	Phosphatidylinositol pathway Ras pathway
Cellular events	MTOC reorganization Secretion of cytolytic granules
Early gene activation	c-Myc, c-Fos
Intermediate gene activation	Lymphokines, lymphokine receptors, nutrient receptors
Late gene activation	Genes involved in cell proliferation 4F2, VLA-2

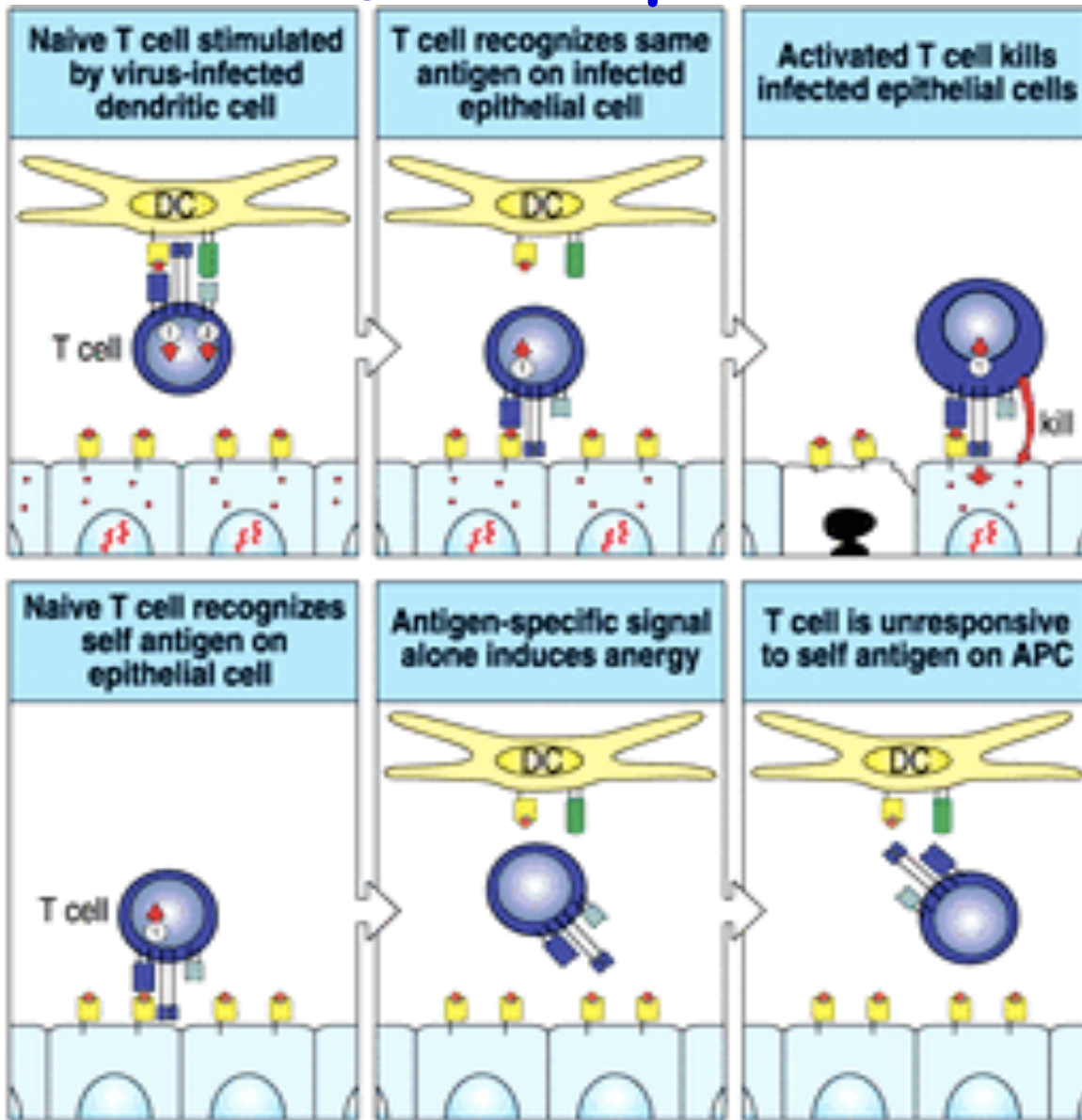
Ativação depende de dois sinais



(1) Sinalização do TcR/CD3 necessita do reconhecimento do MHC+peptídeo e colaboração de co-receptores CD4 ou CD8

(2) Interação entre moléculas co-estimuladoras (CD28/CD80,CD86)

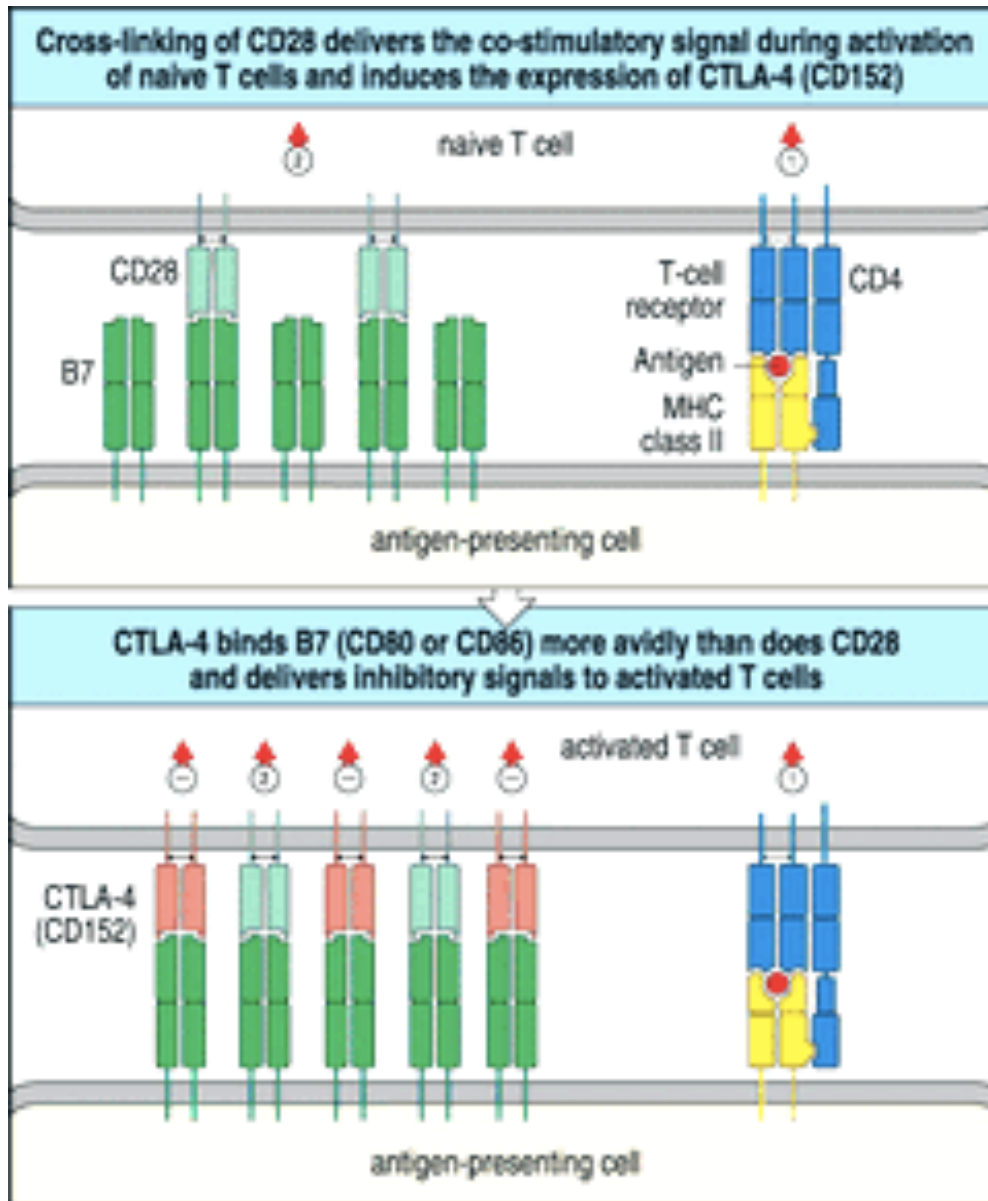
Ativação depende de dois sinais



Sinais 1 e 2 precisam estar presentes na mesma célula

Na falta do sinal 2, o resultado é ANERGIA

Moléculas Co-estimuladoras



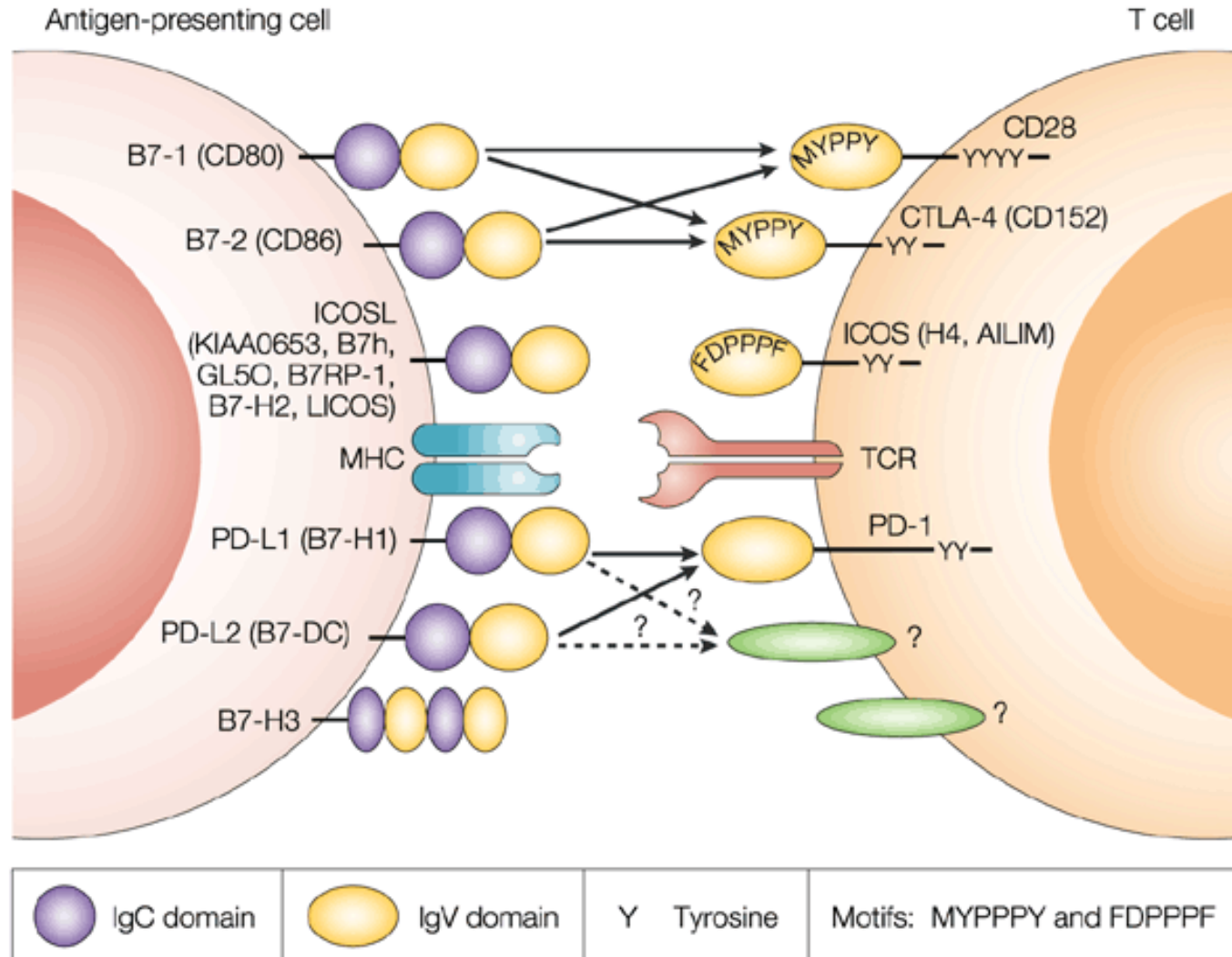
Sinal 2 - CD28 na célula T virgem e CD80 (B7.1) ou CD86 (B7.2) nas APCs

SINAL POSITIVO

CD154 (CTLA-4) expresso nas células T ativadas também interage com CD80 ou CD86

SINAL NEGATIVO

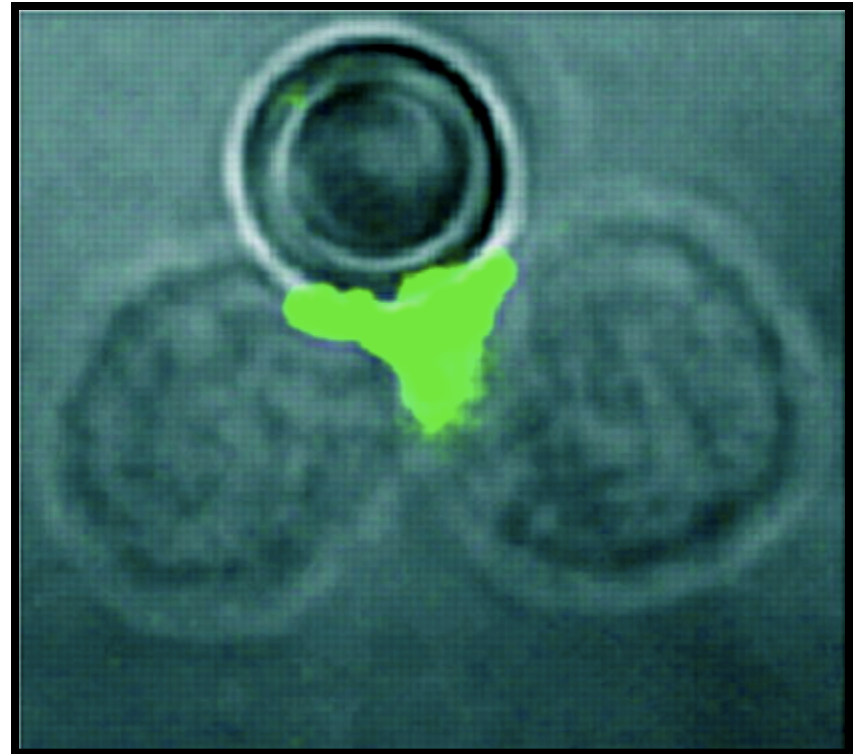
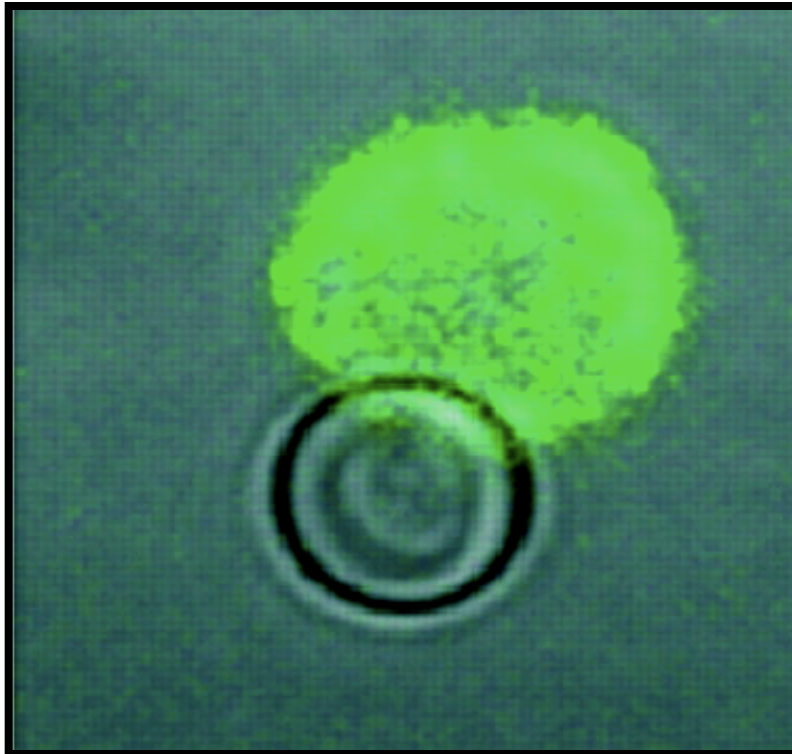
Moléculas Co-estimuladoras

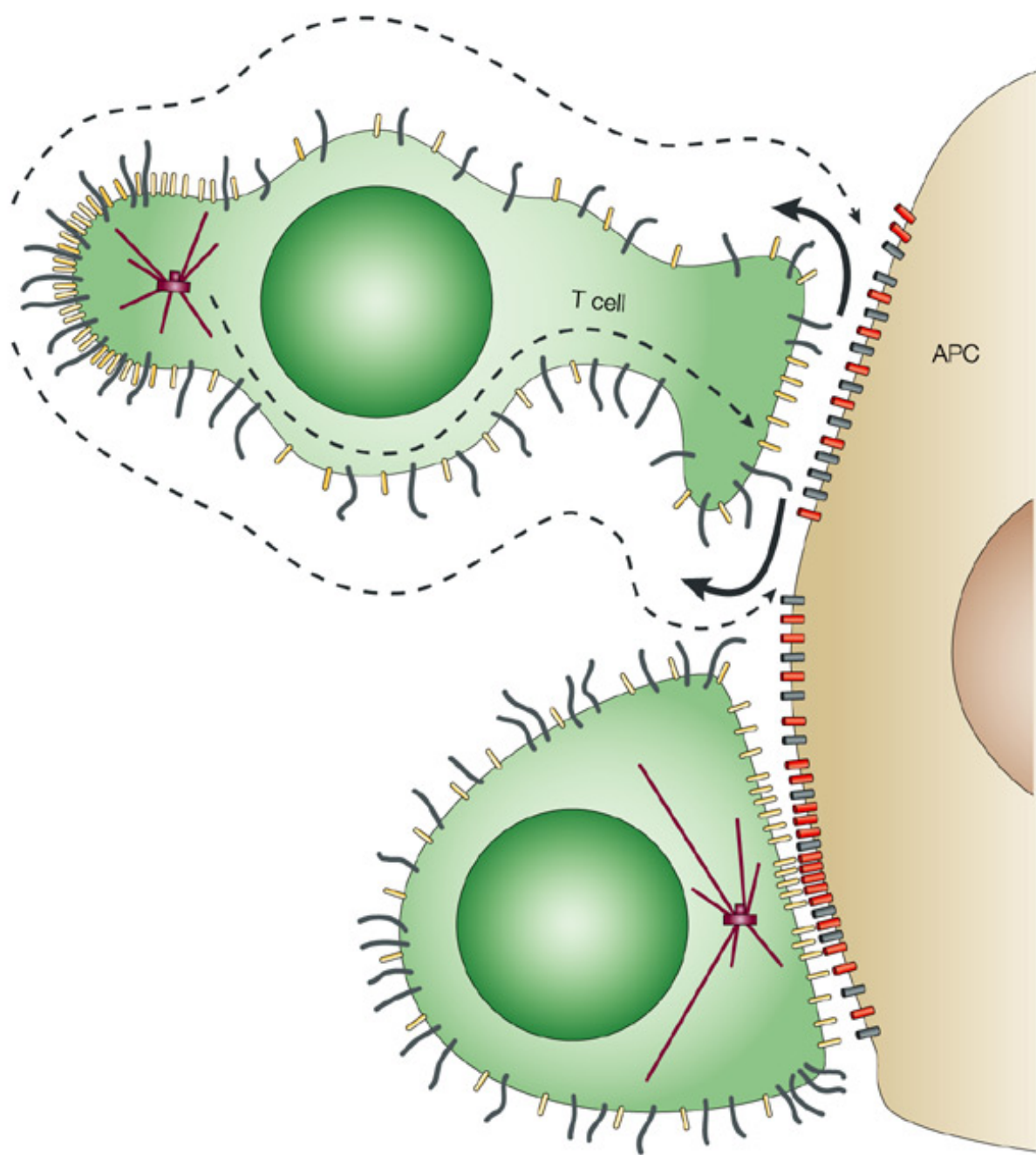


Co-estimulação promove a polarização dos "lipid rafts"

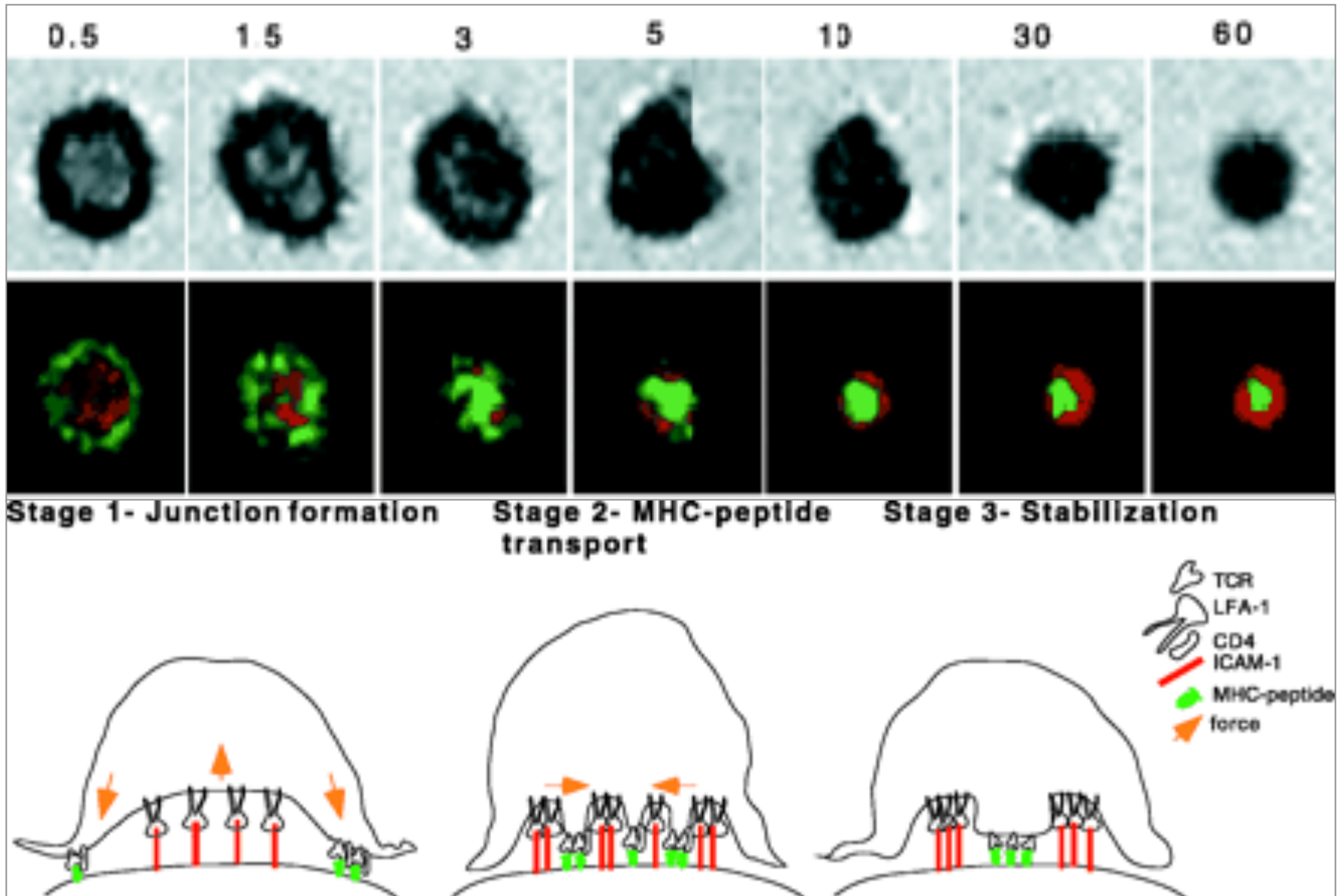
MHC+peptídeo

MHC+peptídeo+B7

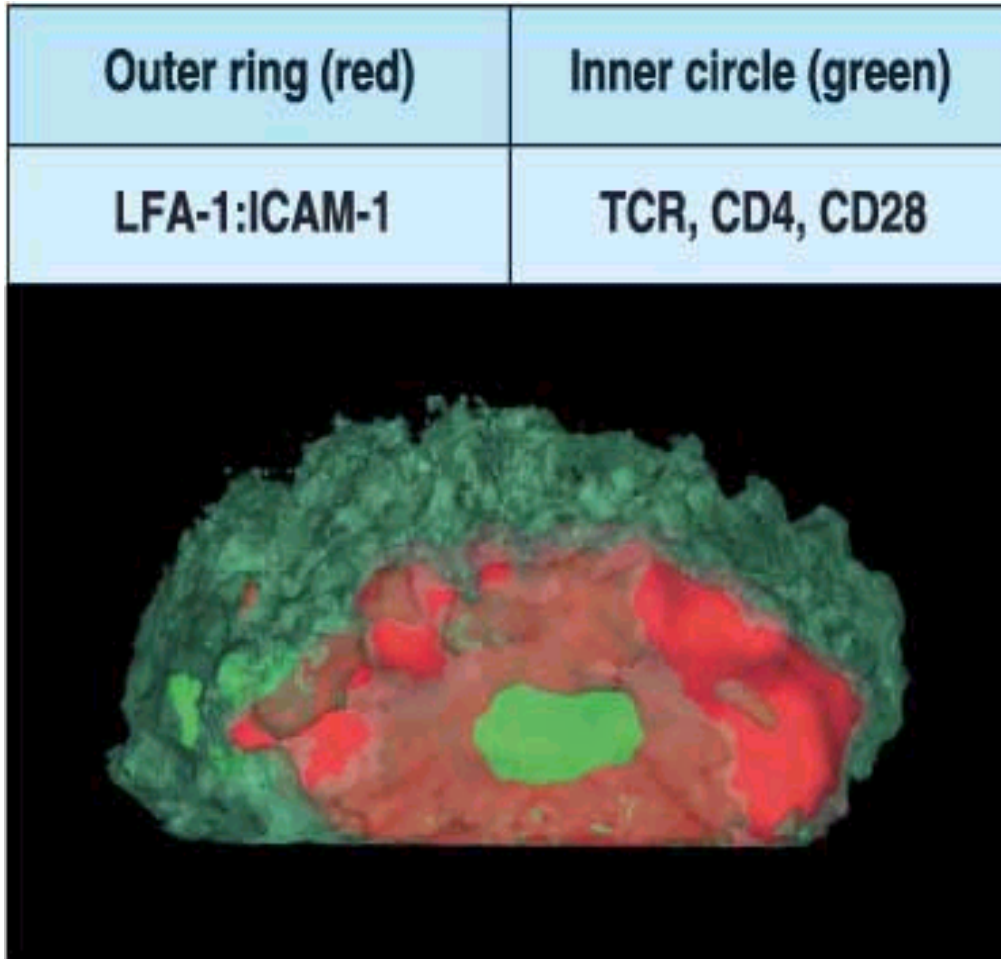




Formação da Sinapse Imunológica



Sinapse Imunológica

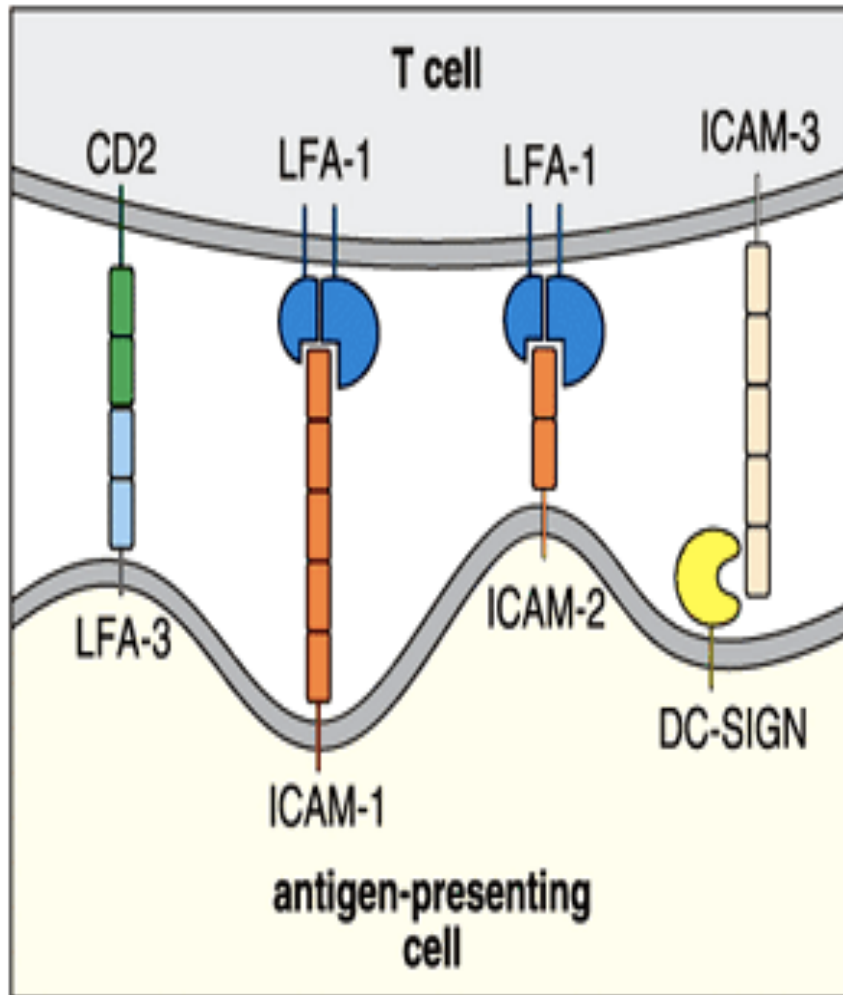


Interação entre as moléculas de adesão mantém as células T e APCs fortemente ligadas.

Agregação de TcRs e moléculas co-estimuladoras amplificam o sinal de ativação.

Fig 8.30 © 2001 Garland Science

Moléculas de Adesão



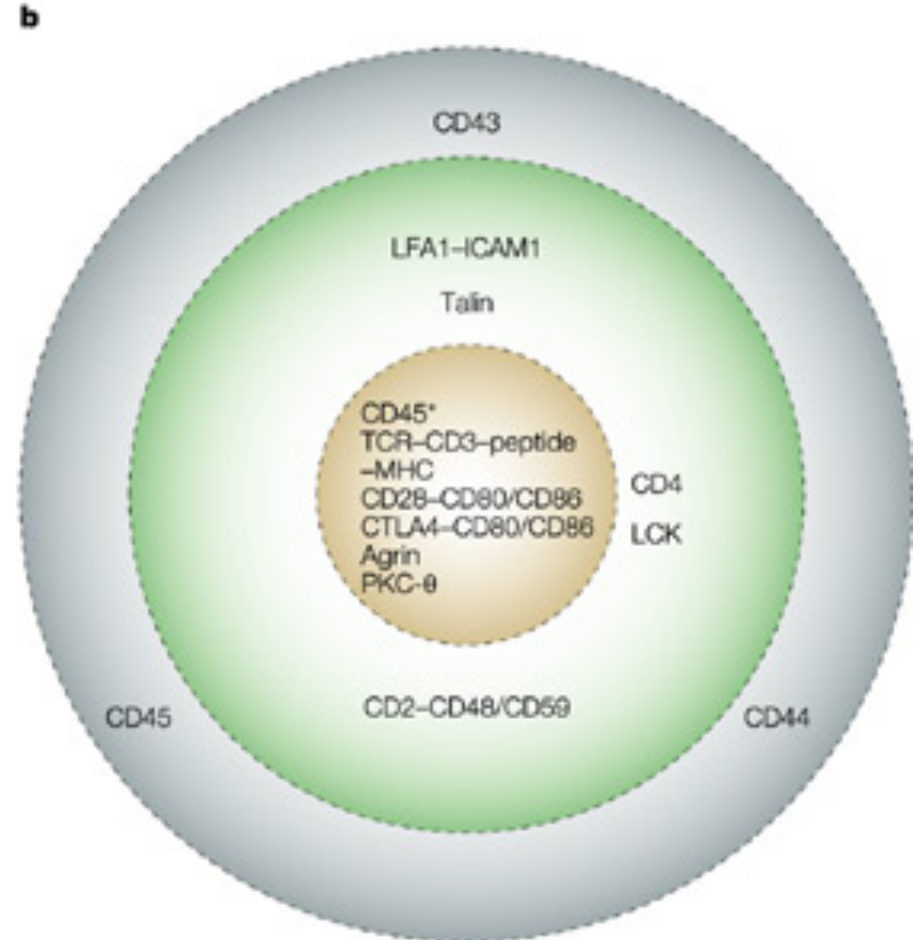
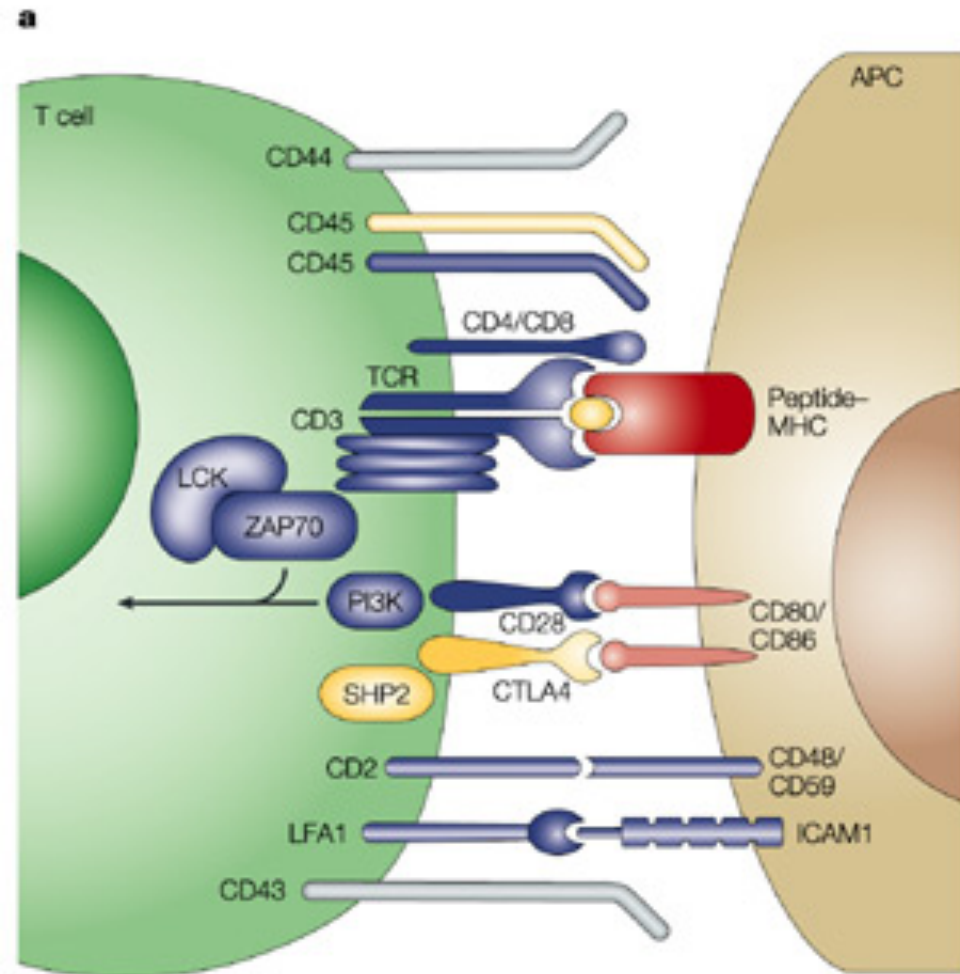
LFA - leucocyte
functional antigen

ICAM - intercellular
adhesion molecule

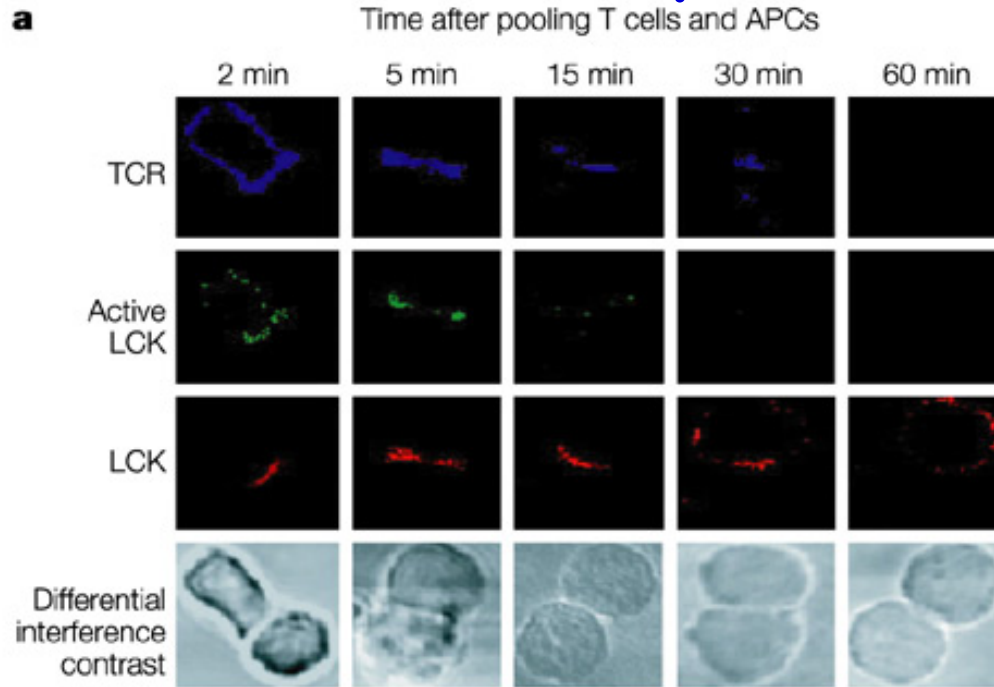
DC-SIGN - specific
intercellular adhesion
molecule grabbing non-
integrin

Fig 8.8 © 2001 Garland Science

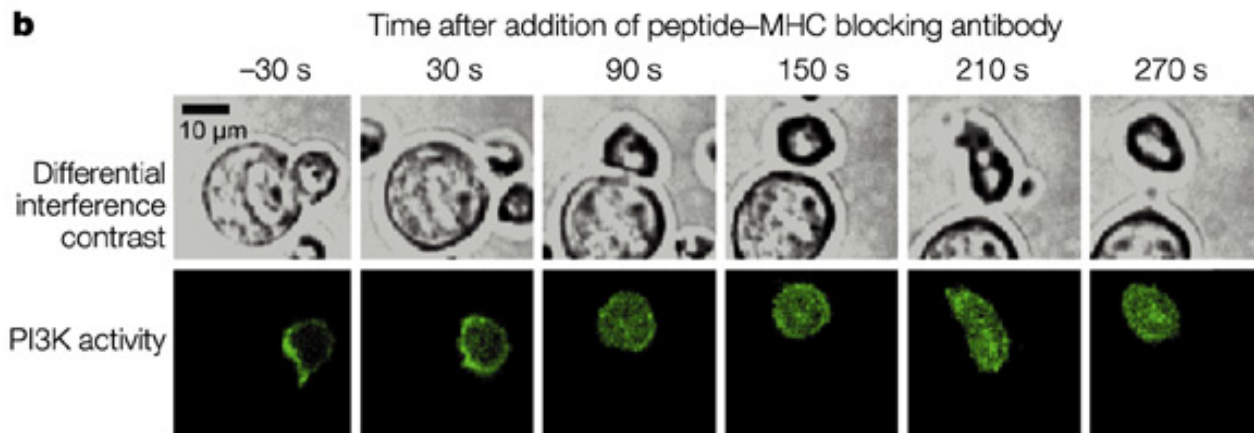
Sinapse Imunológica



Sinapse Imunológica

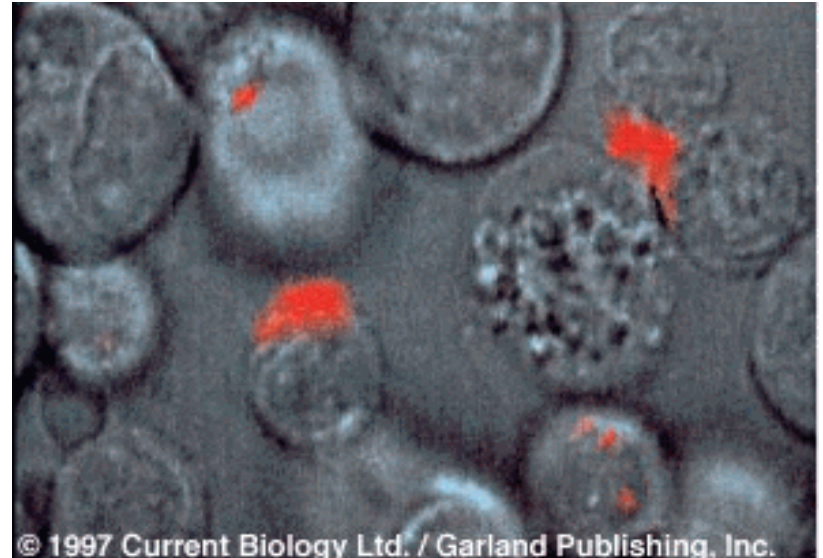
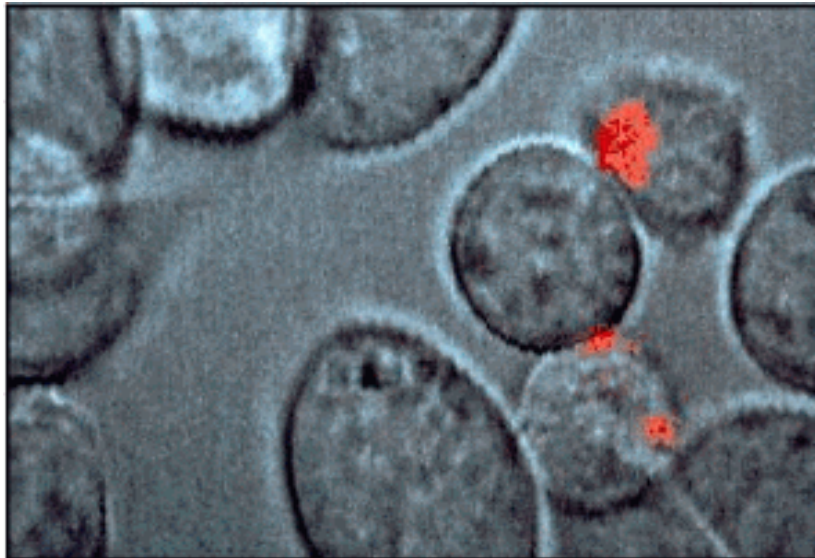
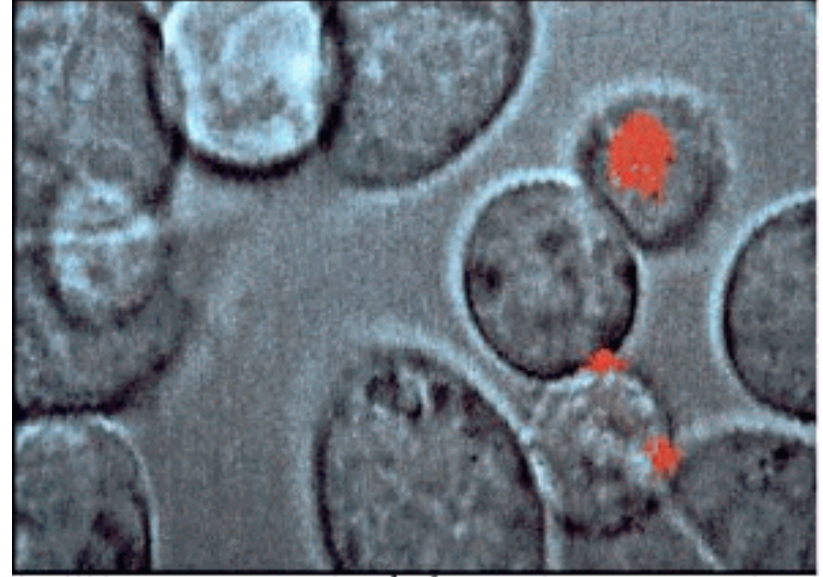
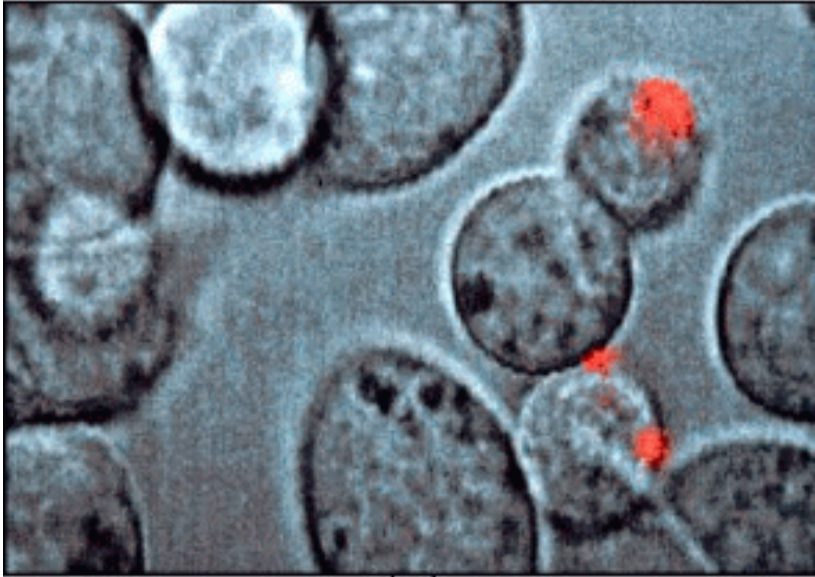


Ativação precoce de
tirosina-quinases



Sinal contínuo do
TcR é necessário
para manter a
sinapse

Polarização dos grânulos citoplasmáticos.



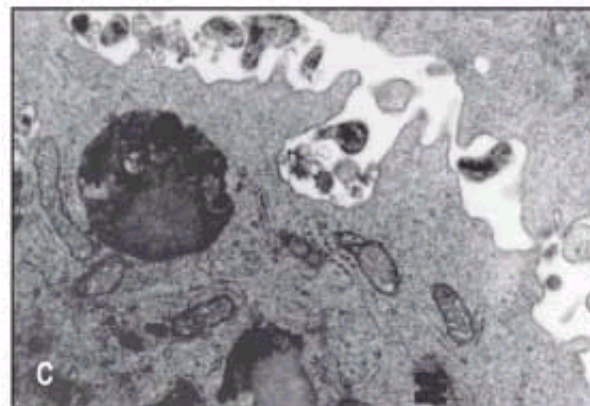
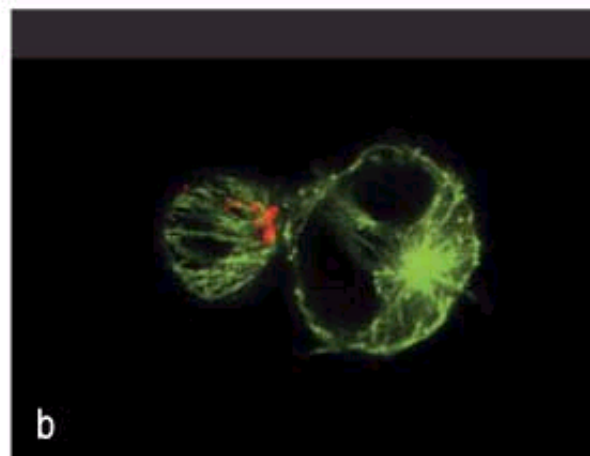
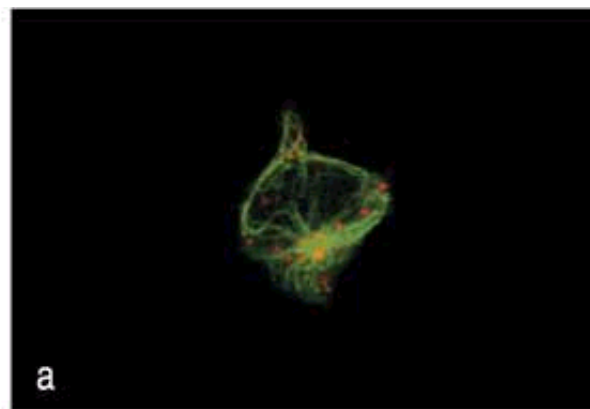
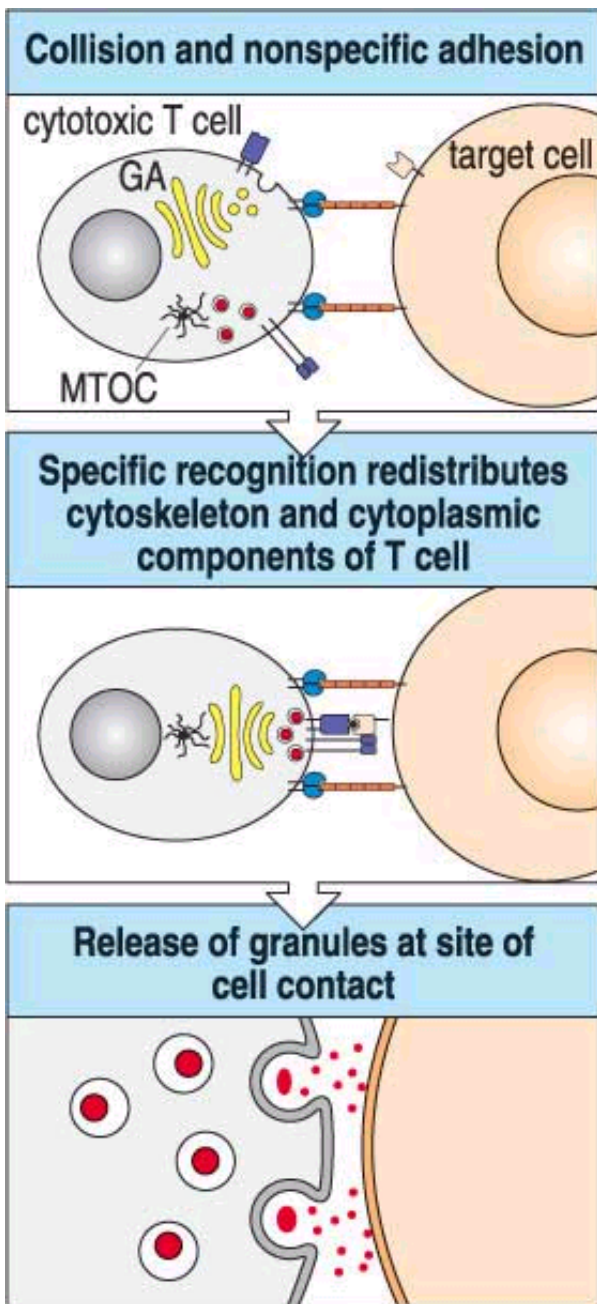
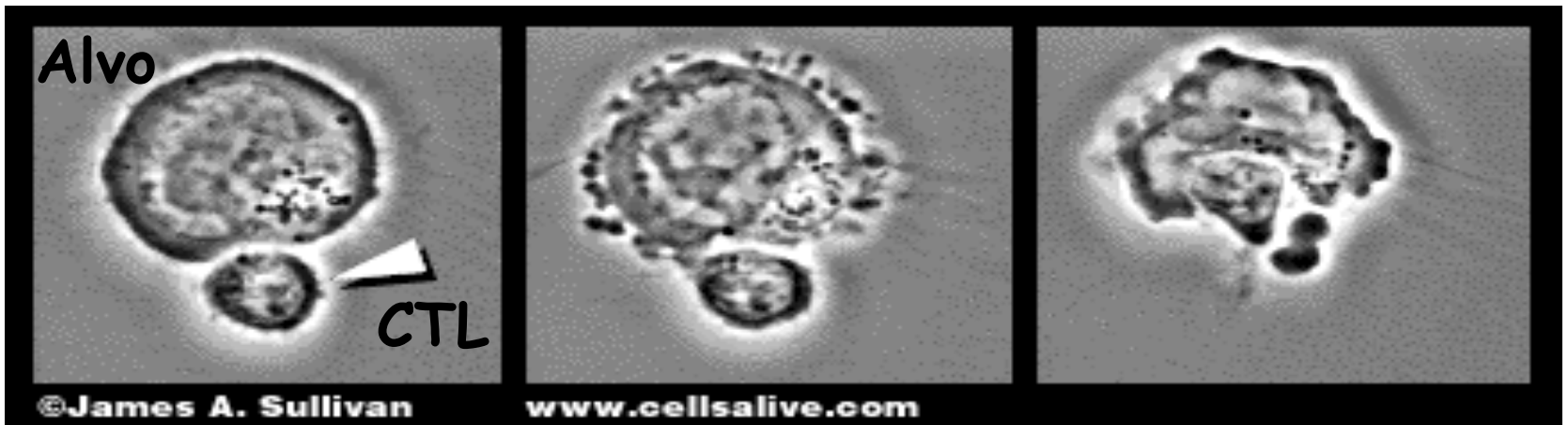
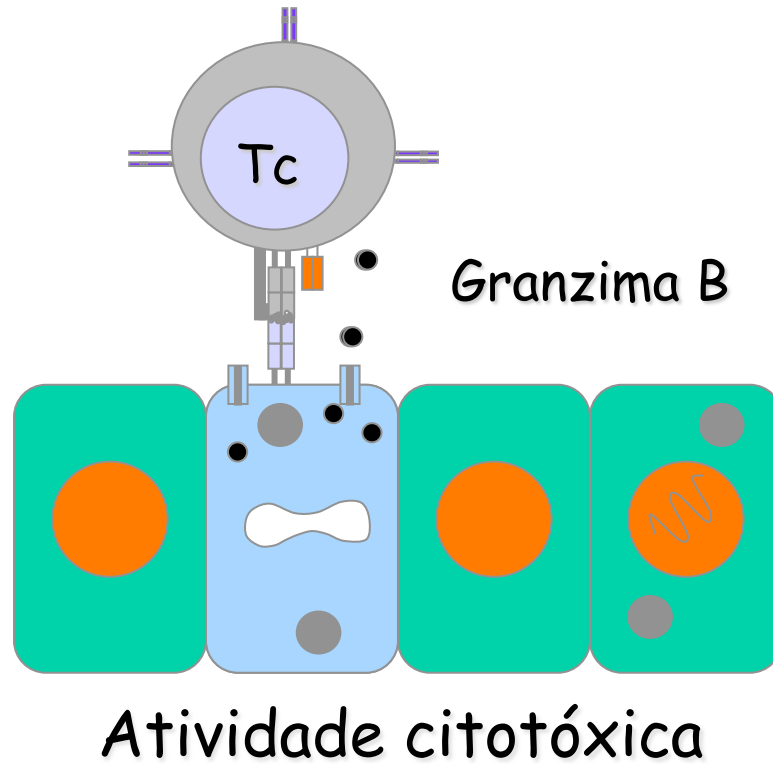
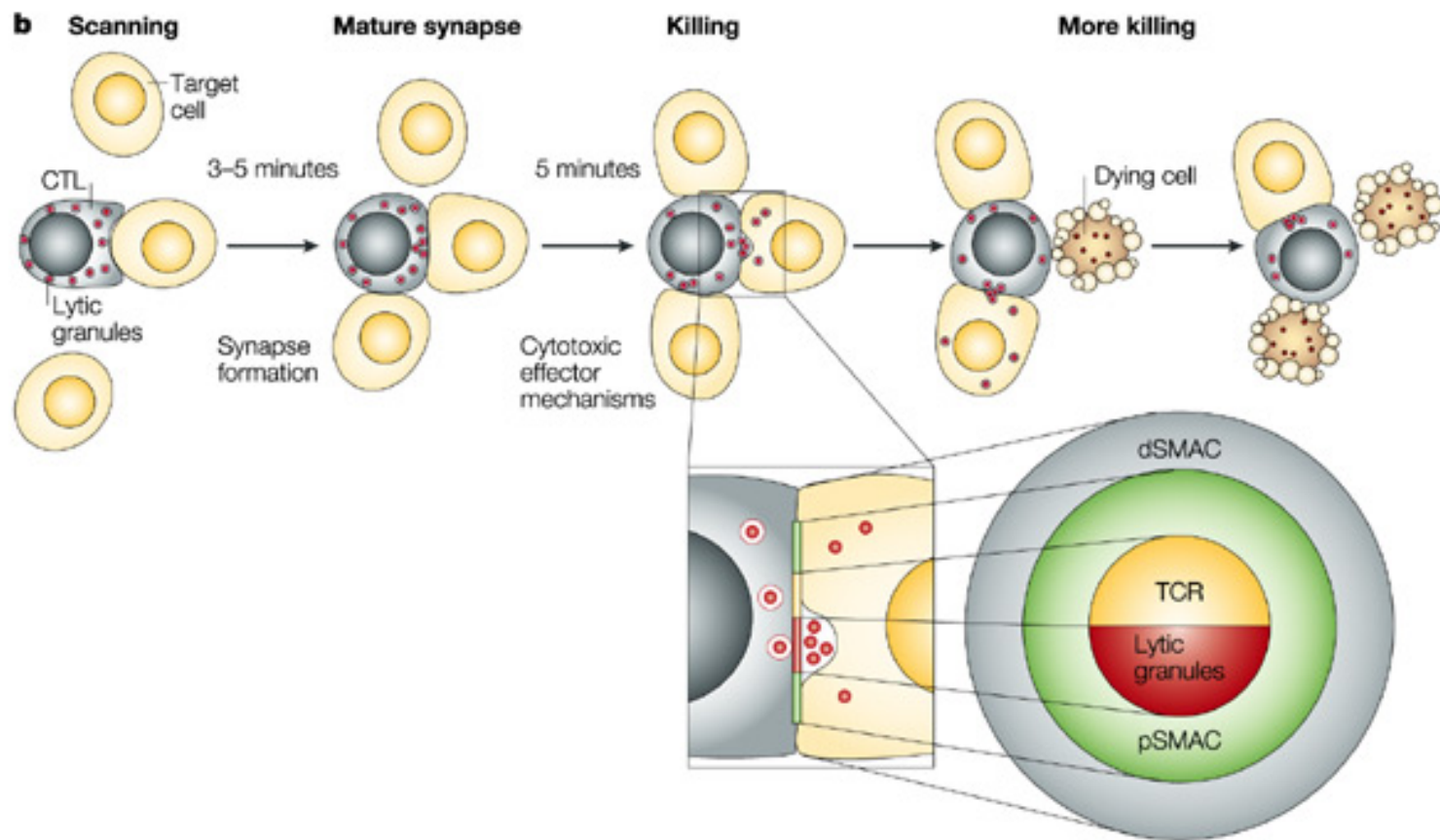
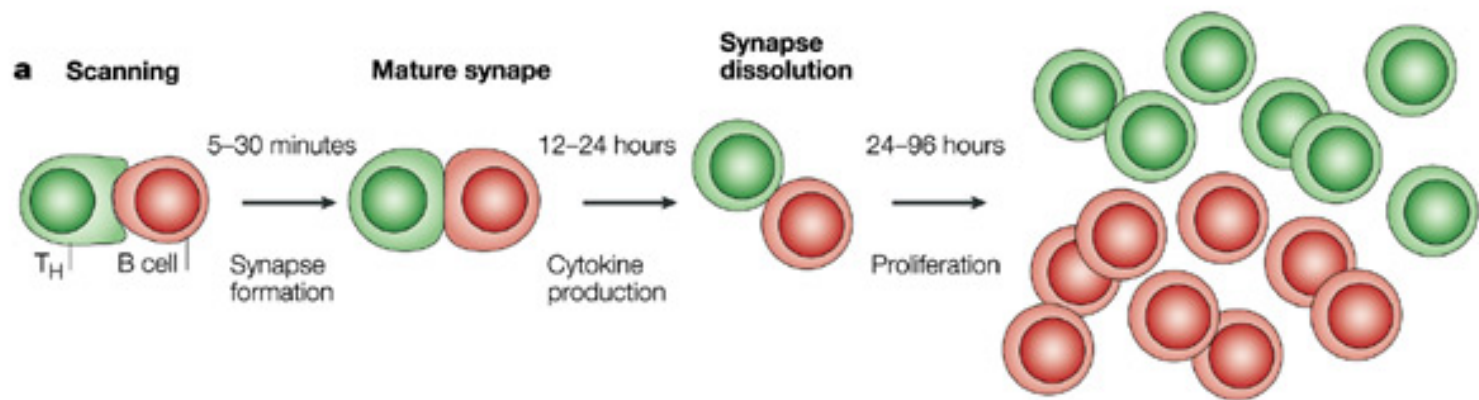


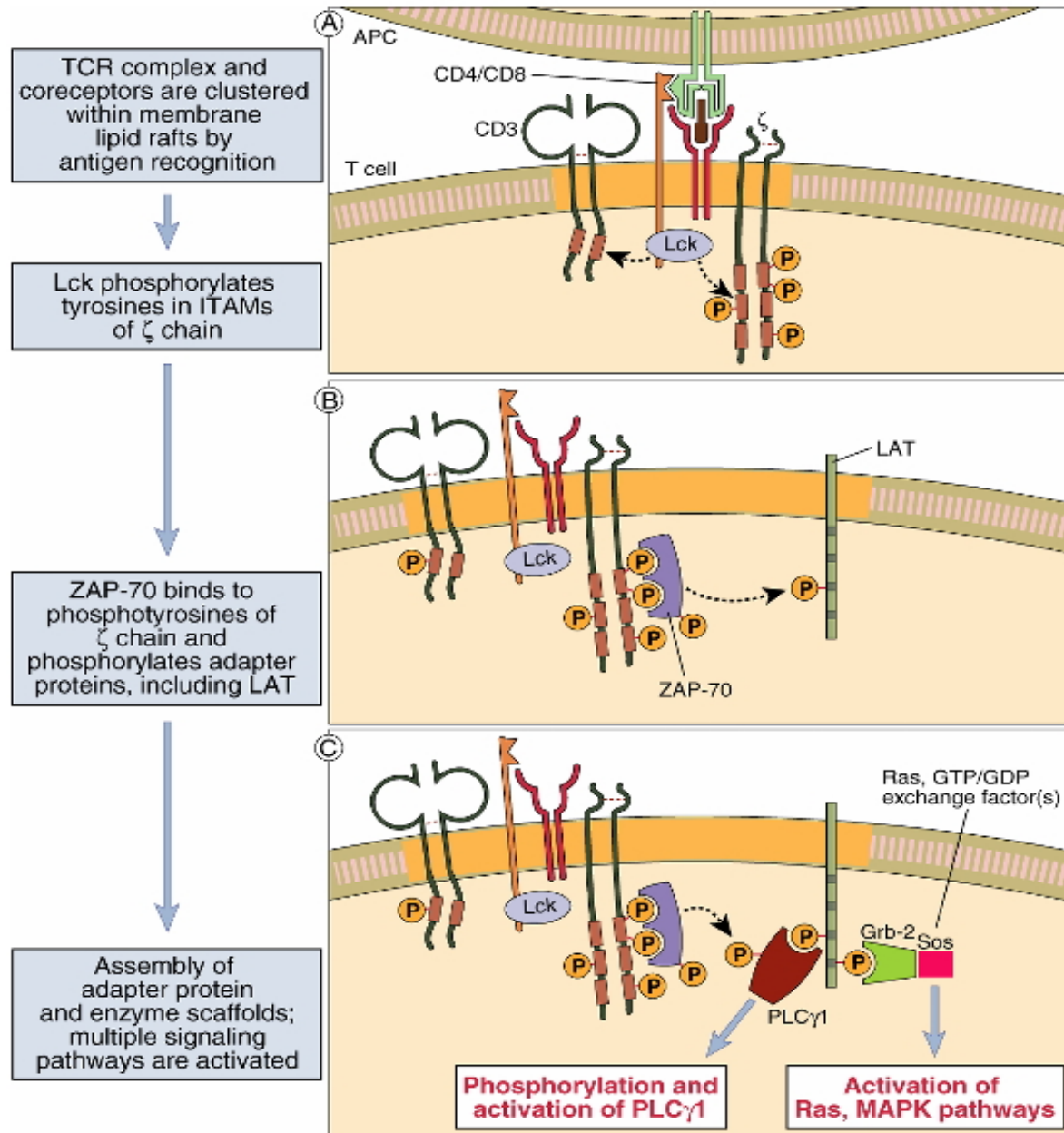
Fig 8.29 © 2001 Garland Science

Apoptose mediada por GrzB

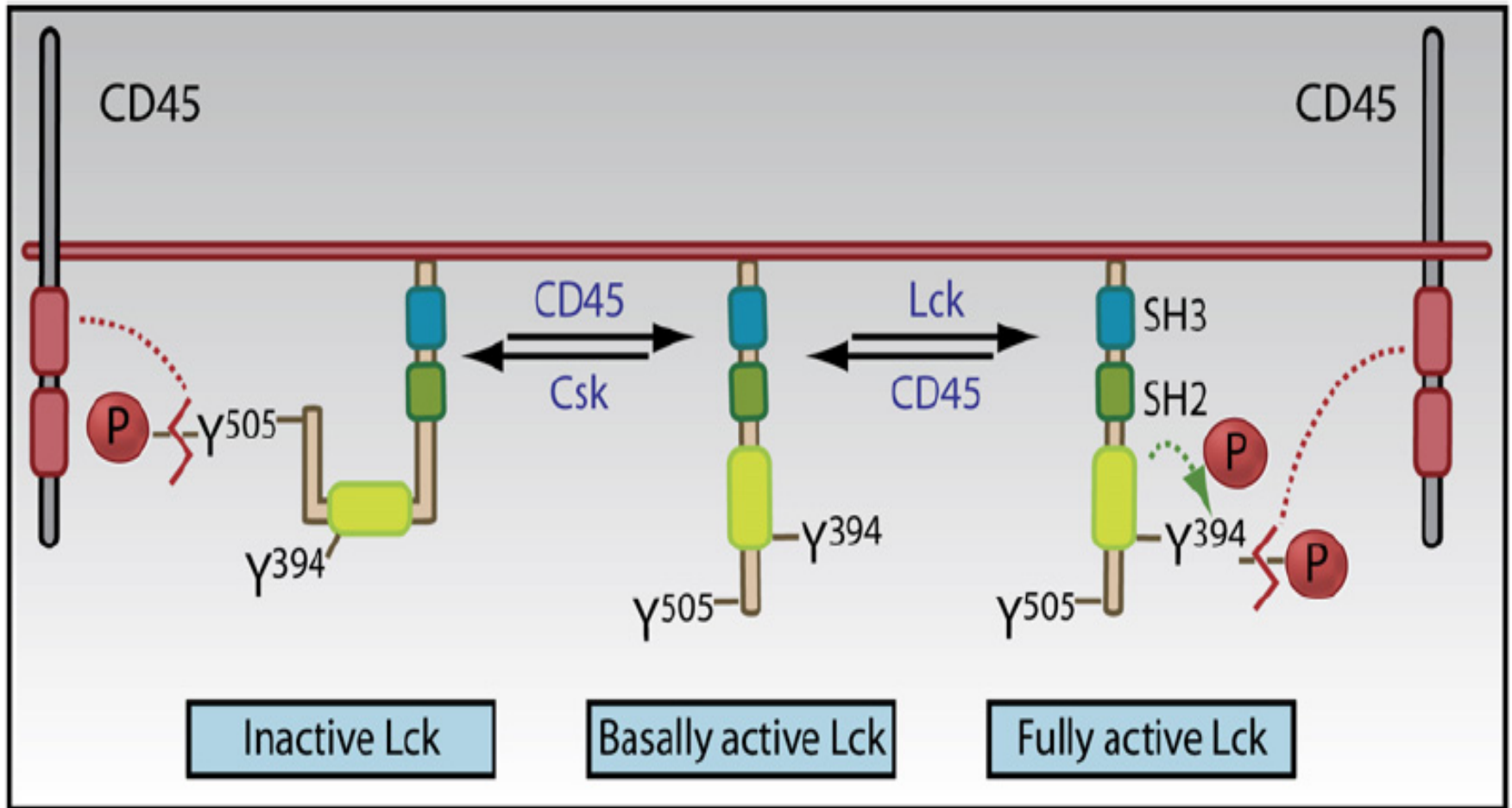




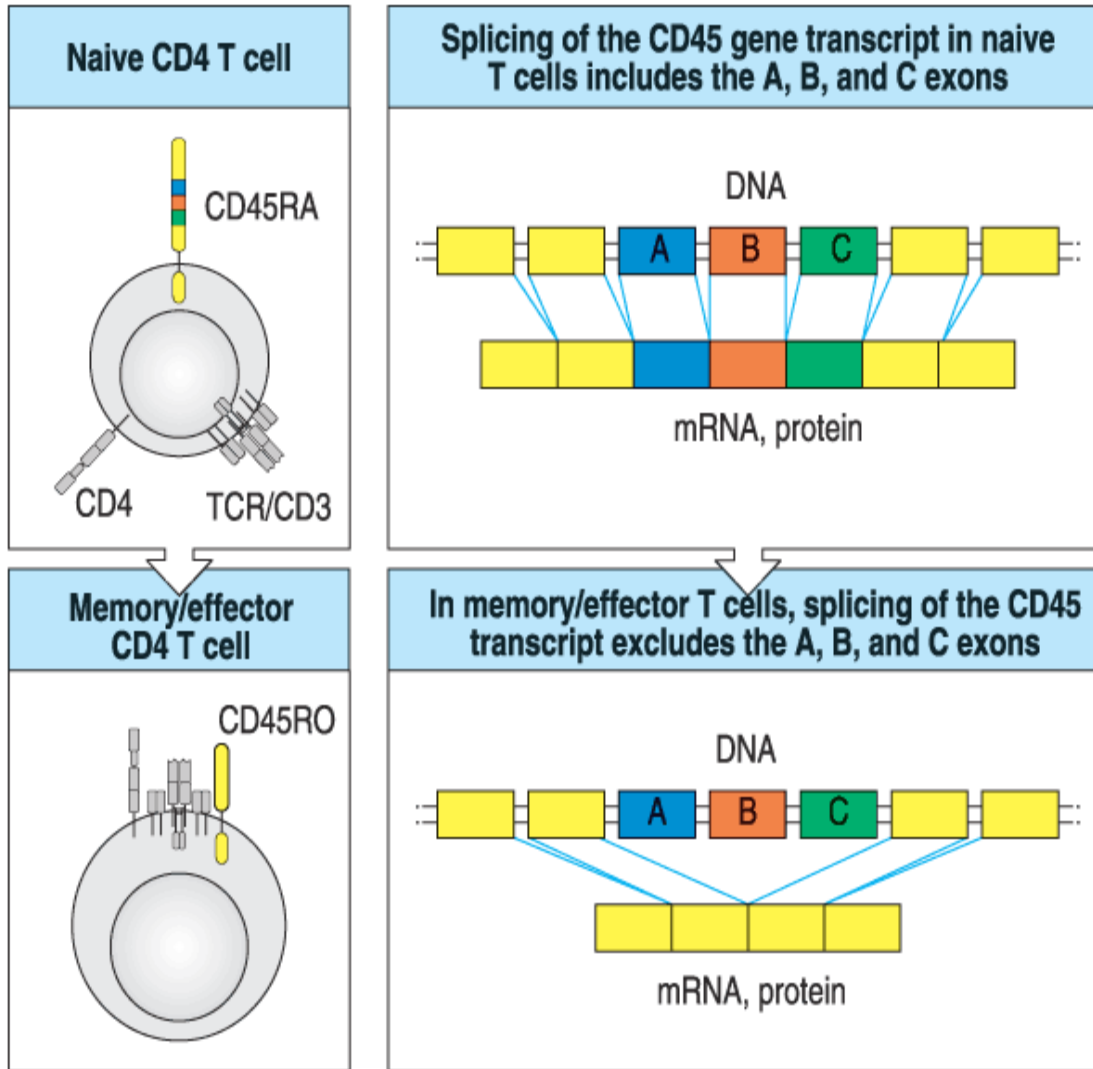
Sinalização intracelular



Papel da fosfatase CD45



Isoformas de CD45

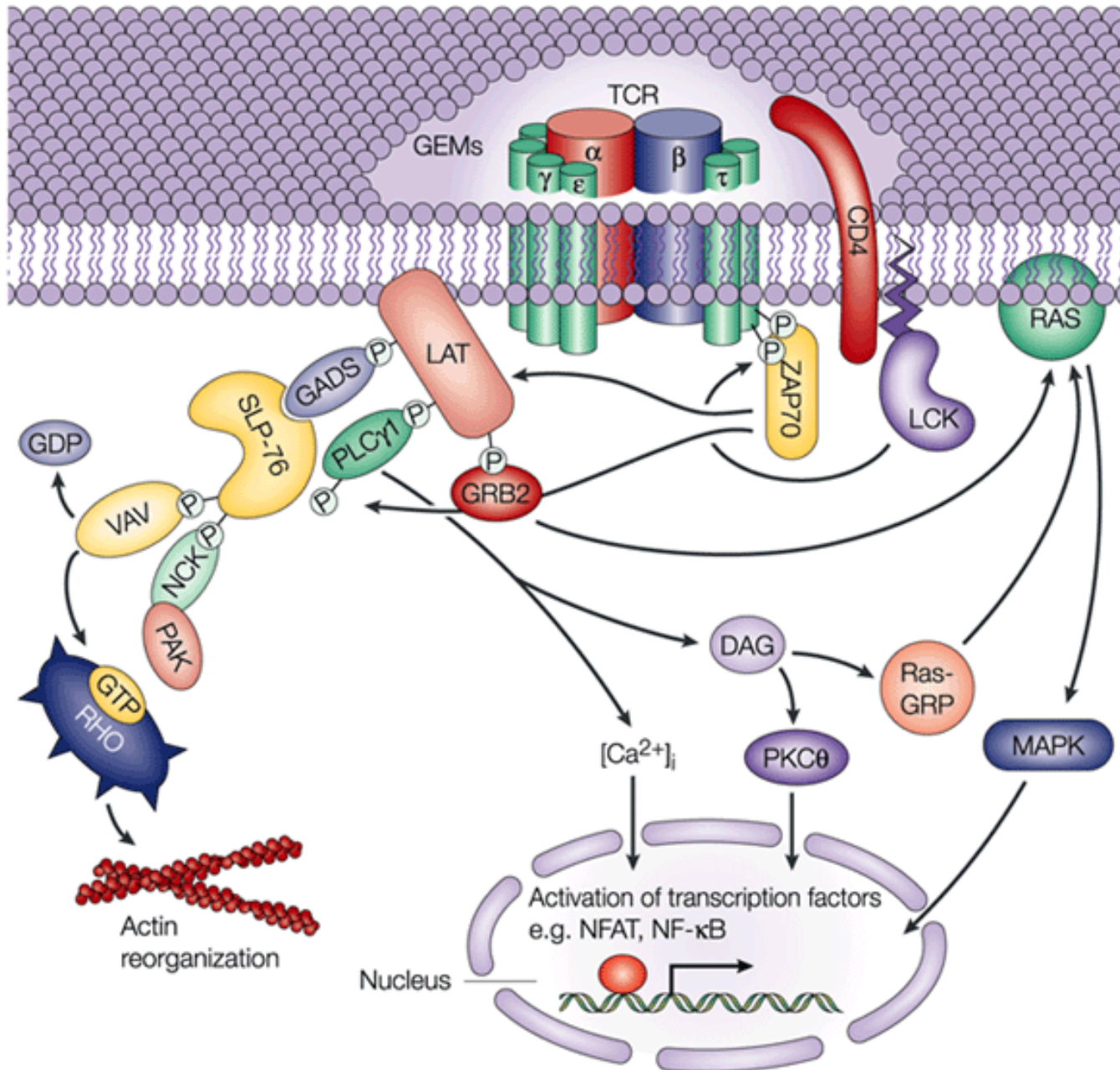


CD45

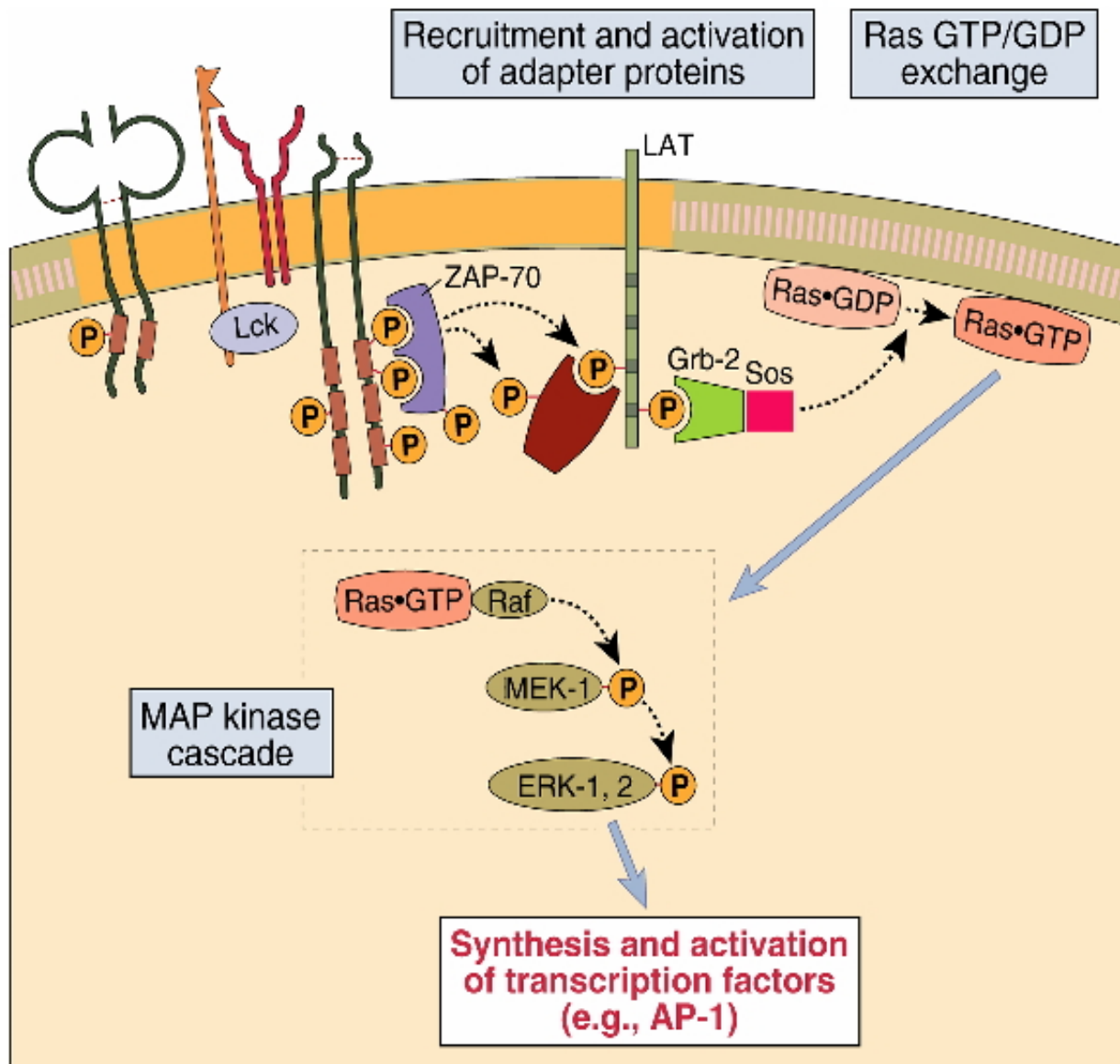
High molecular weight isoforms do not associate with TcR.

Low MW. Forms do associate and helps transduce signals effectively

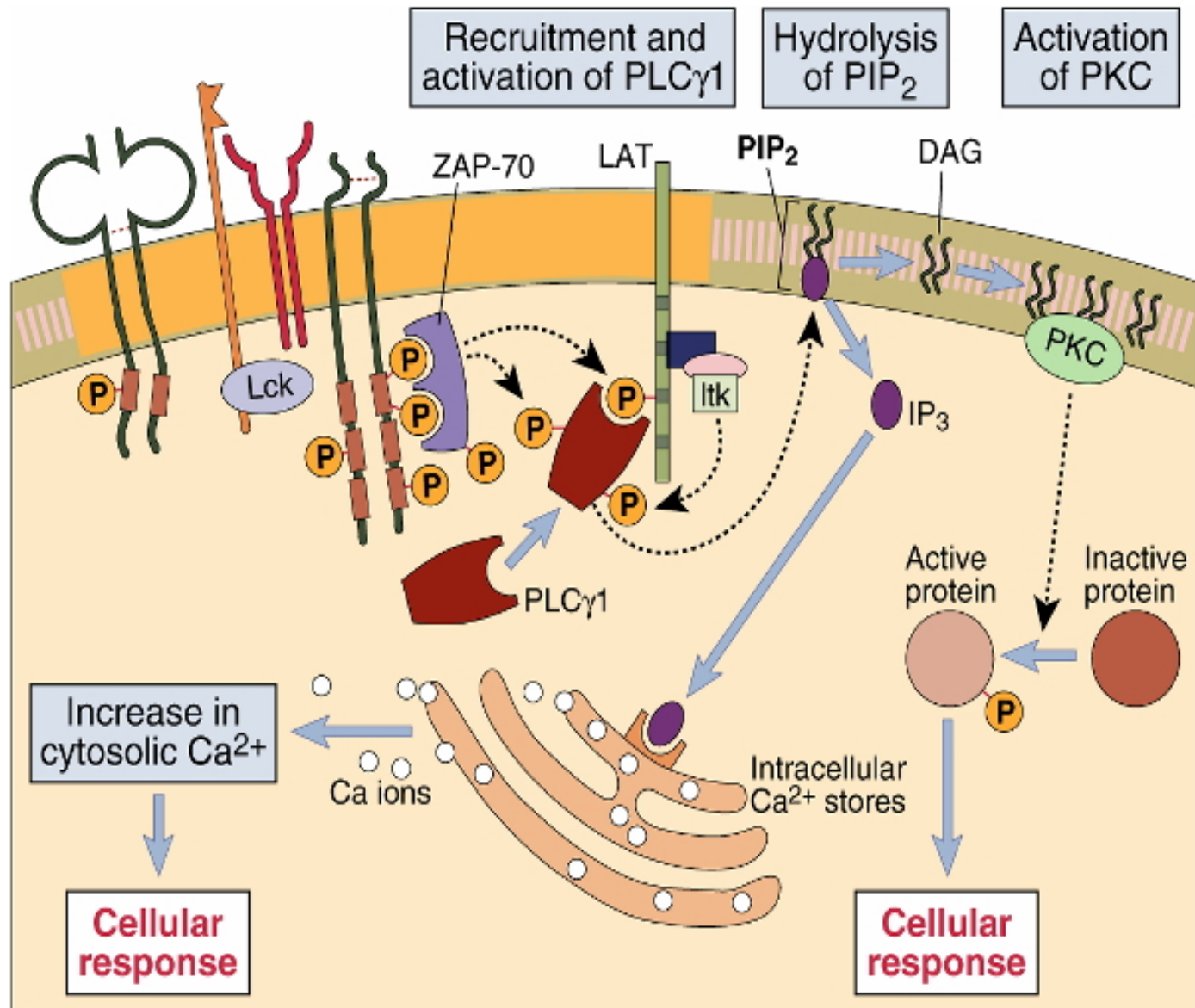
Recrutamiento de moléculas adaptadoras



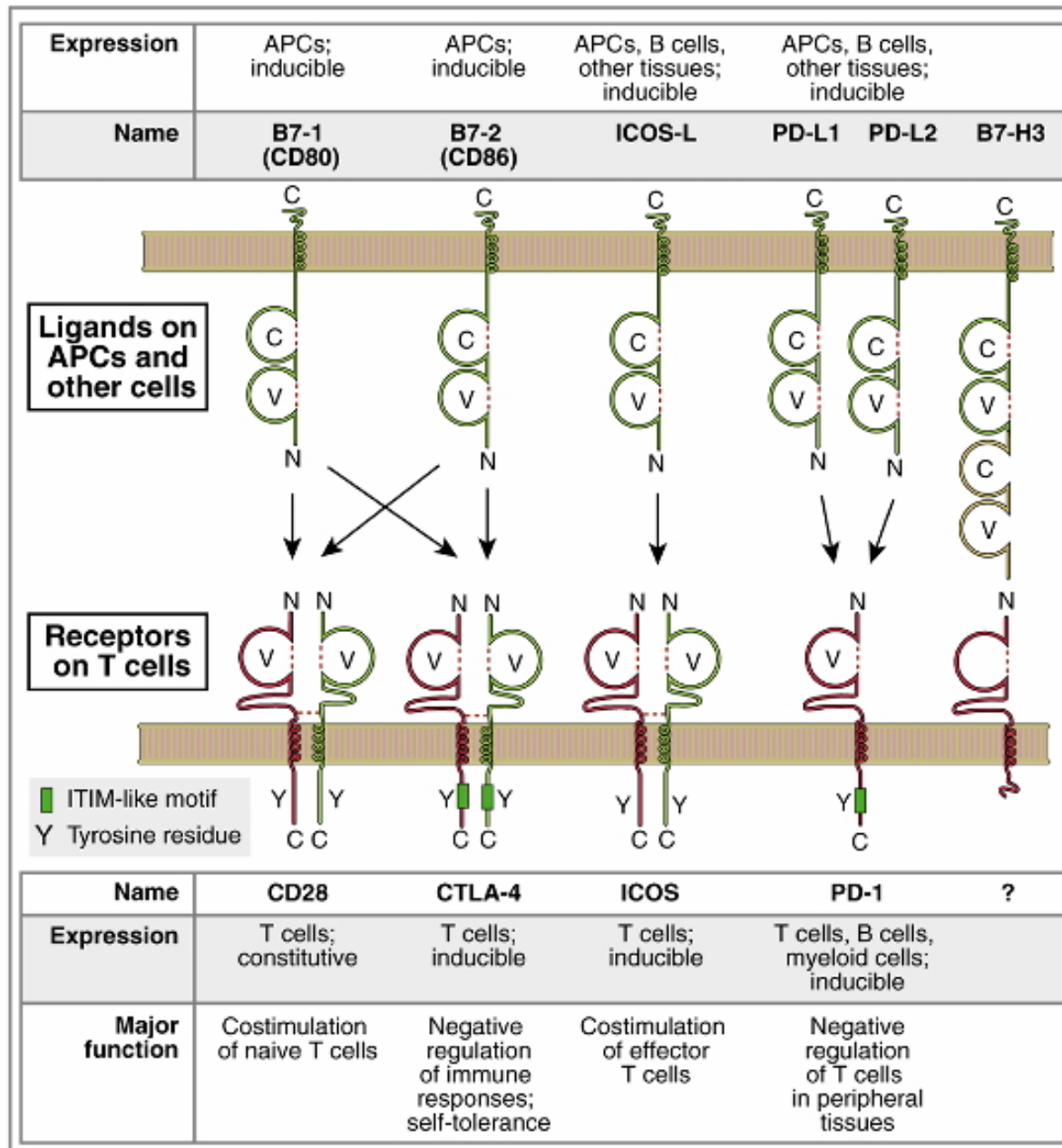
Via Ras-MAP kinase



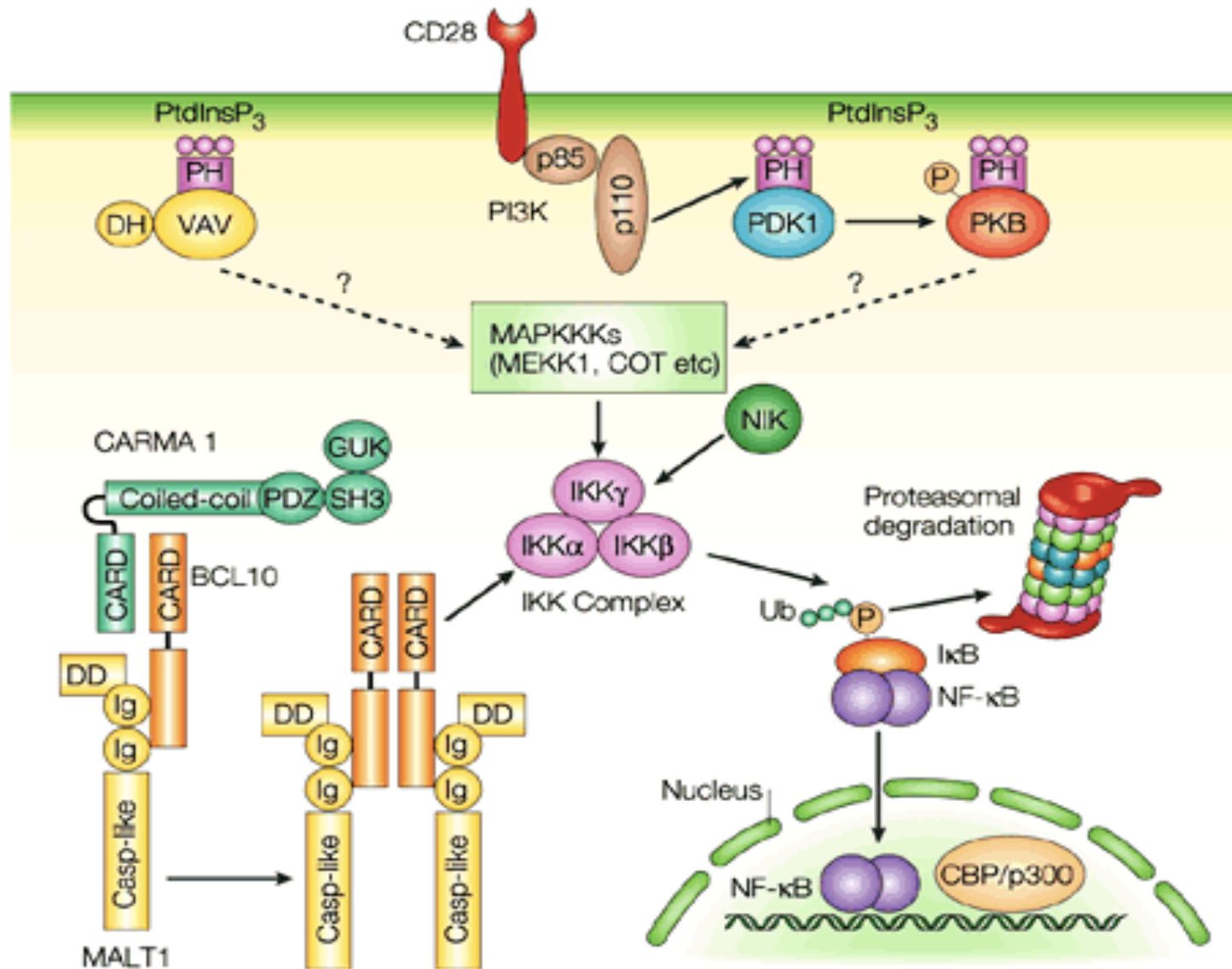
PLC γ 1 e fosfolipides de membrana



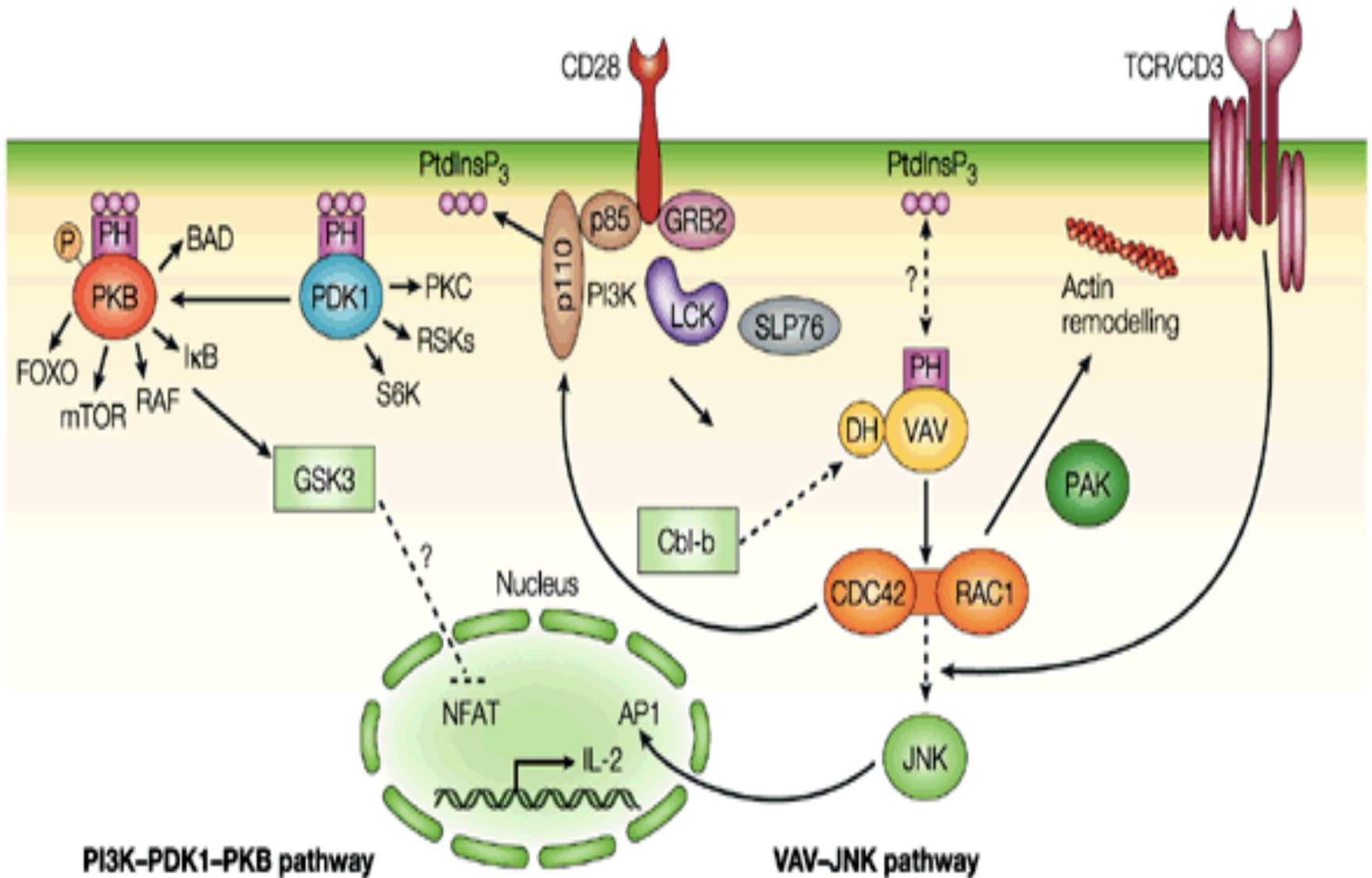
Moléculas Co-estimuladoras



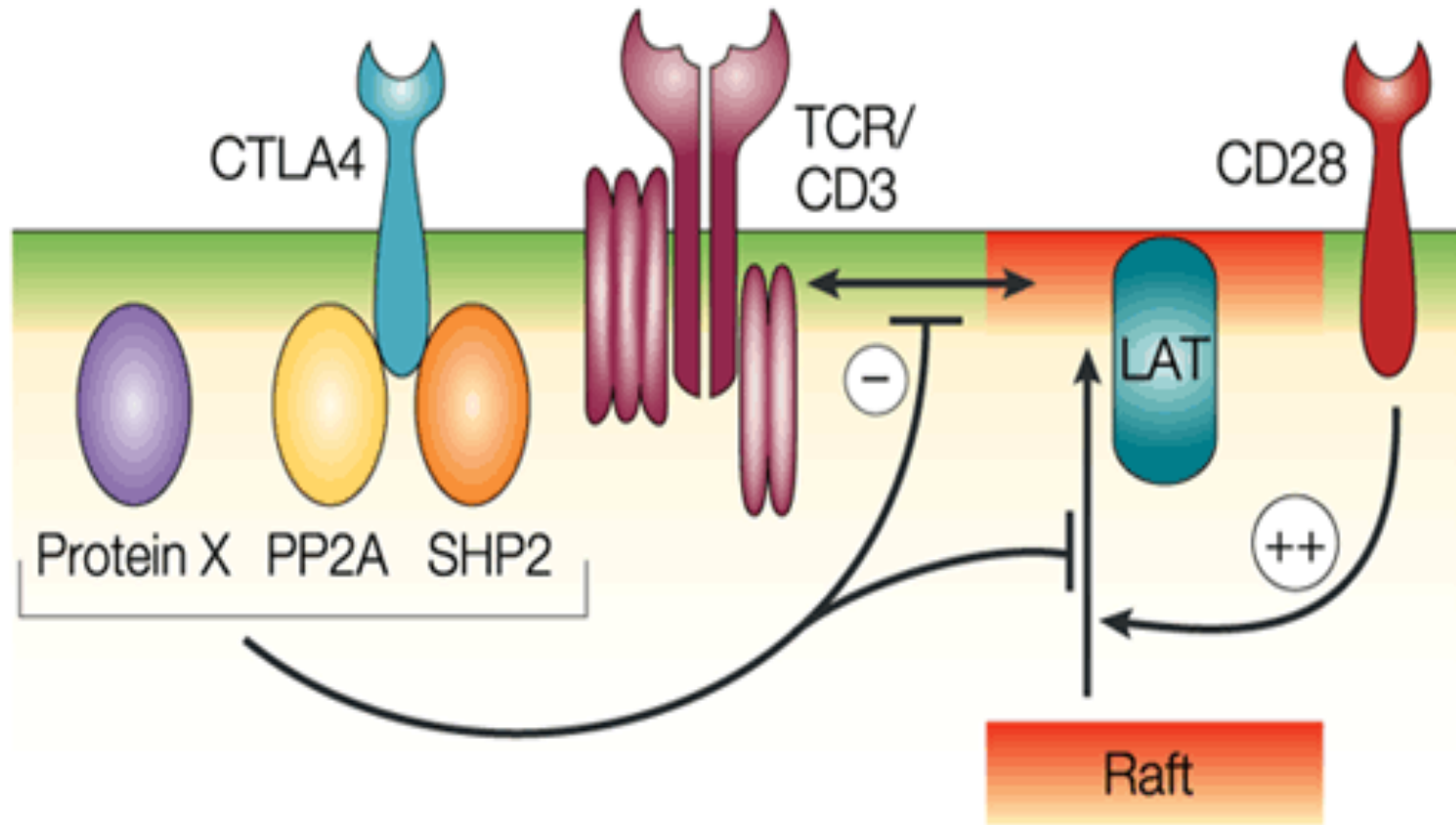
CD28 e a via do NF κ B



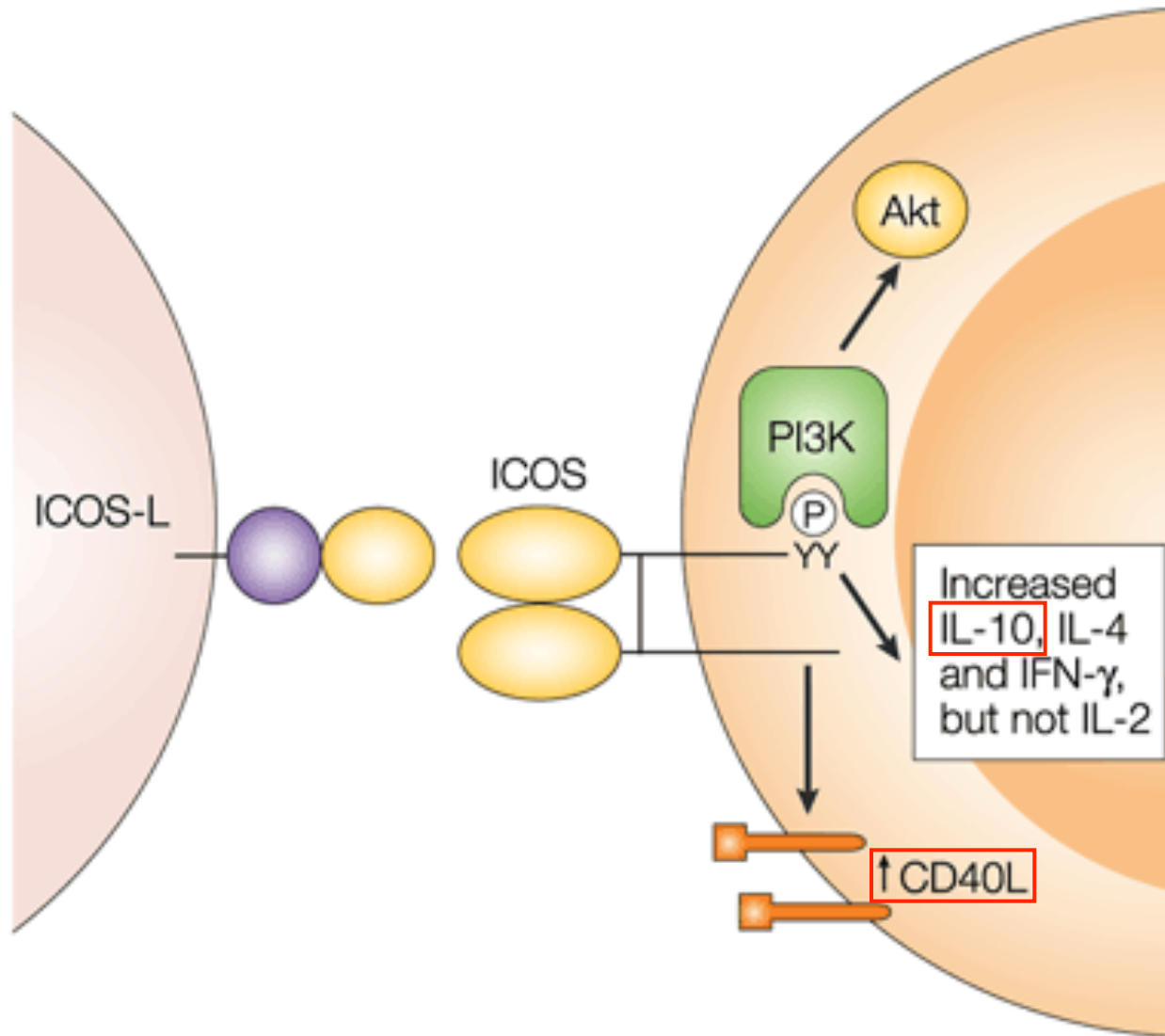
CD28-PI3K-PDK1-PKB



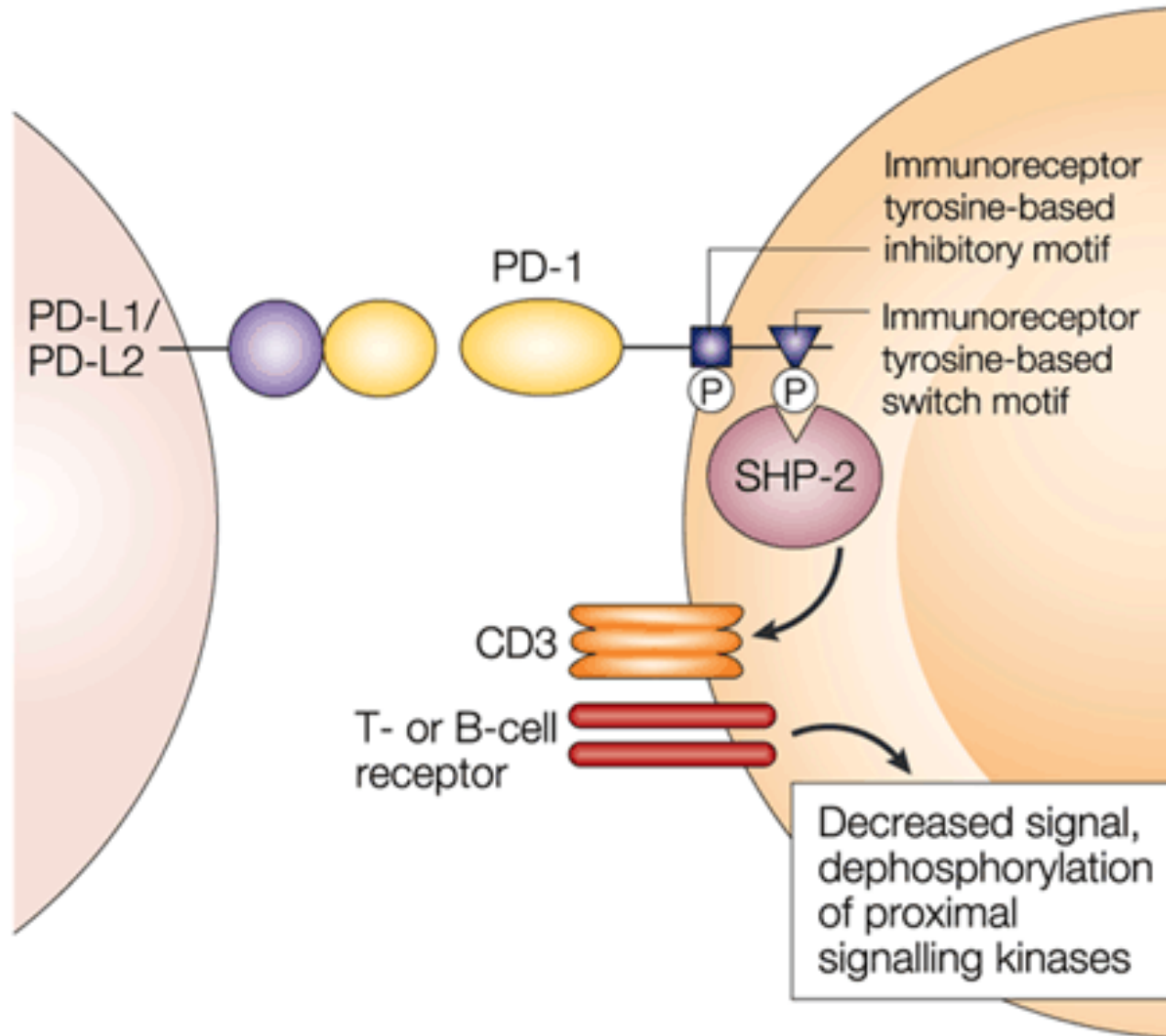
Regulação negativa por CTLA-4



Papel da interação ICOS/ICOS-L



Sinalização negativa por PD-1



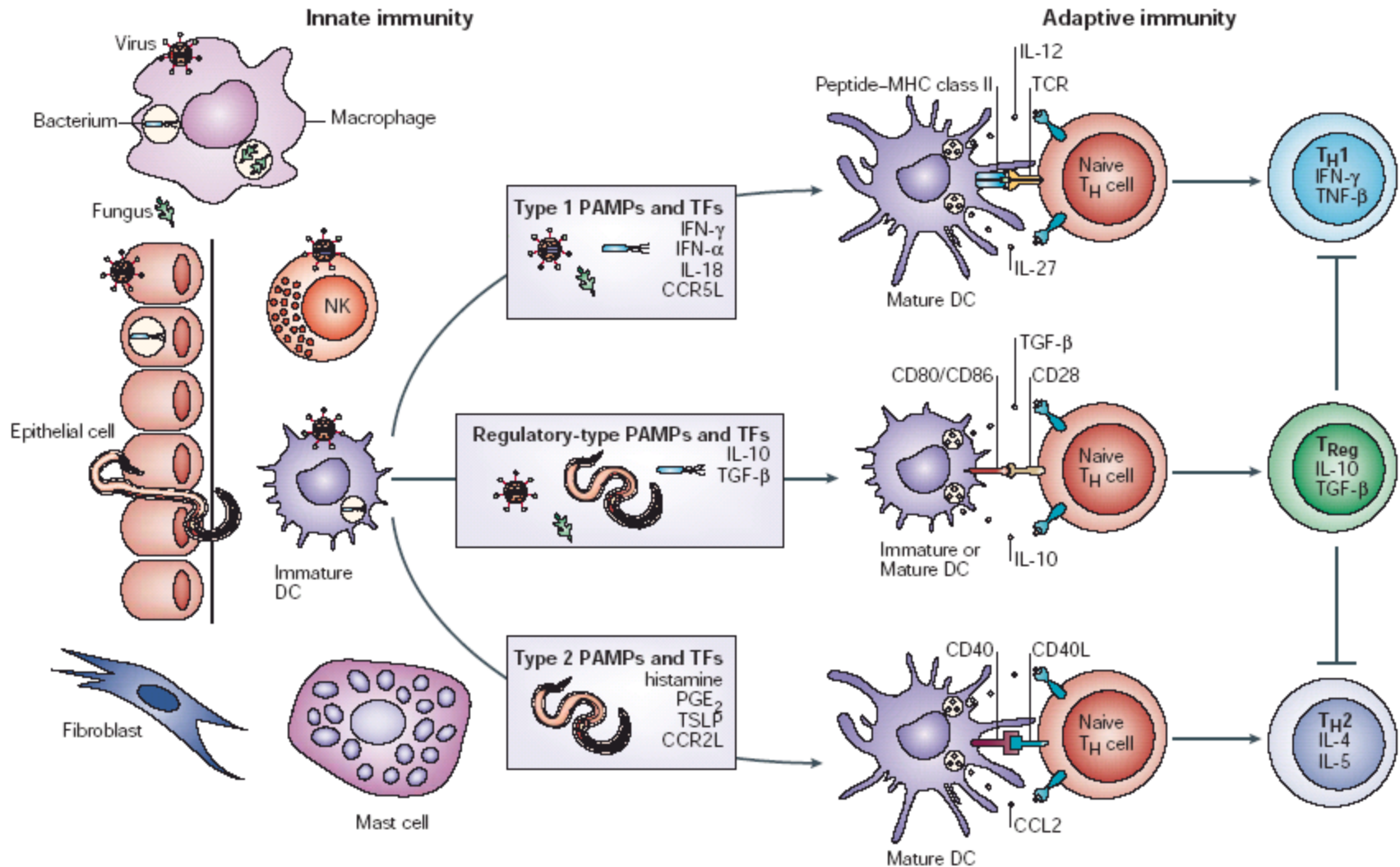
Eventos intracelulares na ativação dos linfócitos T CD4

- One of the earliest detectable events after binding to the TCR is the activation within seconds of “tyrosine kinases”
 - Enzymes which activate proteins by adding phosphate groups to tyrosine residues
 - ITAM (immunoreceptor tyrosine-based activation motif) on CD3 and zeta chains
 - Fyn and Lck (family of Src protein kinases)
 - Associate with CD3+zeta and with CD4 molecule
 - CD45 activates Fyn and Lck
 - Phosphorylated ITAMs then acts as a docking site for ZAP-70 (tyrosine kinase of the Syk family)

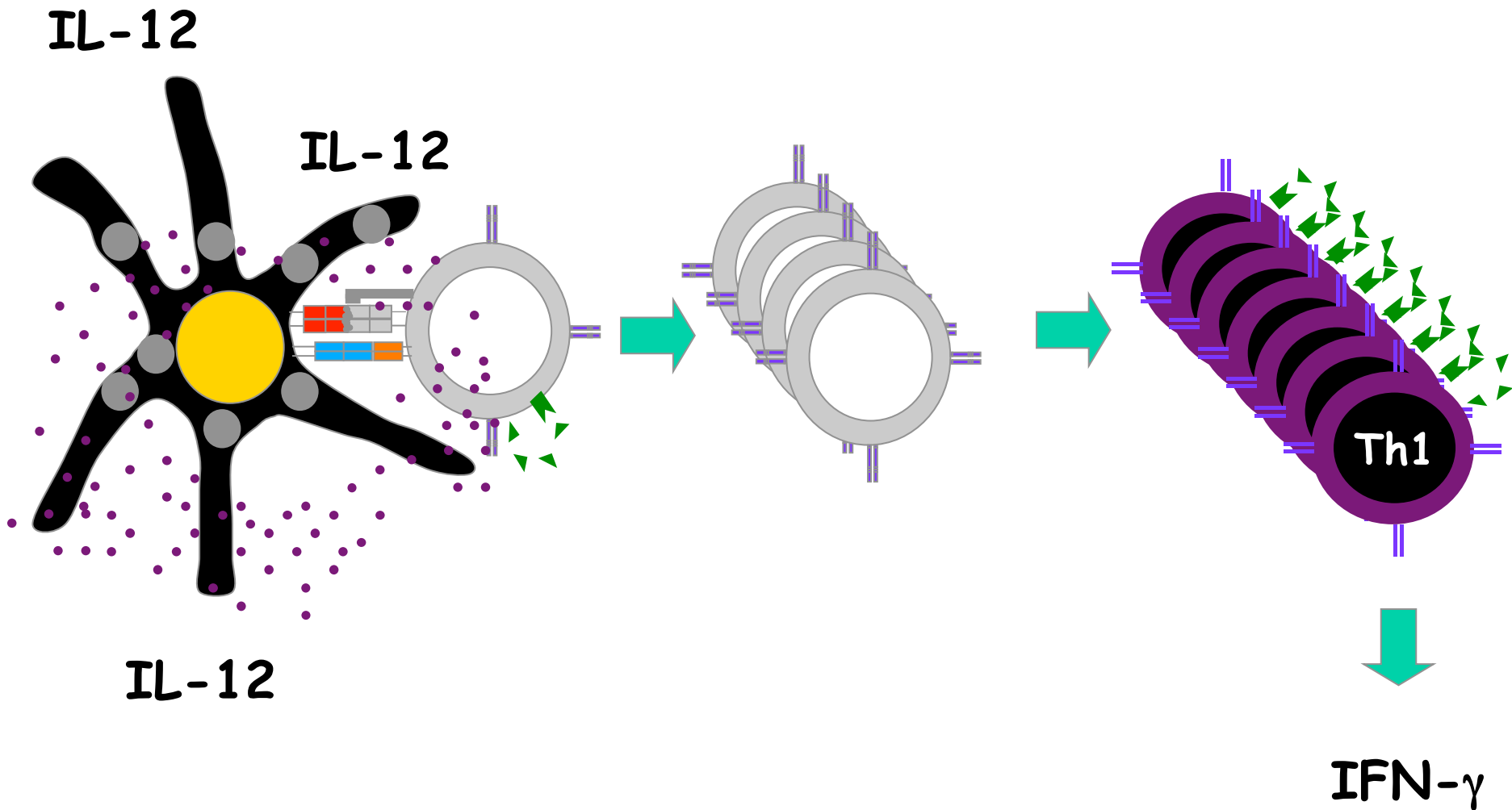
Eventos intracelulares na ativação dos linfócitos T CD4

- Activation of ZAP-70 is critical for T cell activation
- ZAP-70 activated phospholipase C-gamma (PLC-g)
 - PLC-g splits phosphatidylinositol biphosphate (PIP₂) to
 - Diacylglycerol (DAG)
 - Inositol triphosphate (IP₃)
 - DAG → activates protein kinase C (PKC) → kinase cascade
 - IP₃ → increases intracellular Ca⁺⁺ → calcineurin
 - Activation of transcription factors → NF-κB and NF-AT
 - Transcription on cytokine and cytokine receptor genes
 - mRNA → translated into protein
 - Production of IL-2 and IL-2 receptors
 - Stop expressing homing factor CD62L
 - Induce expression of CD49d and CD44 → trafficking to tissues

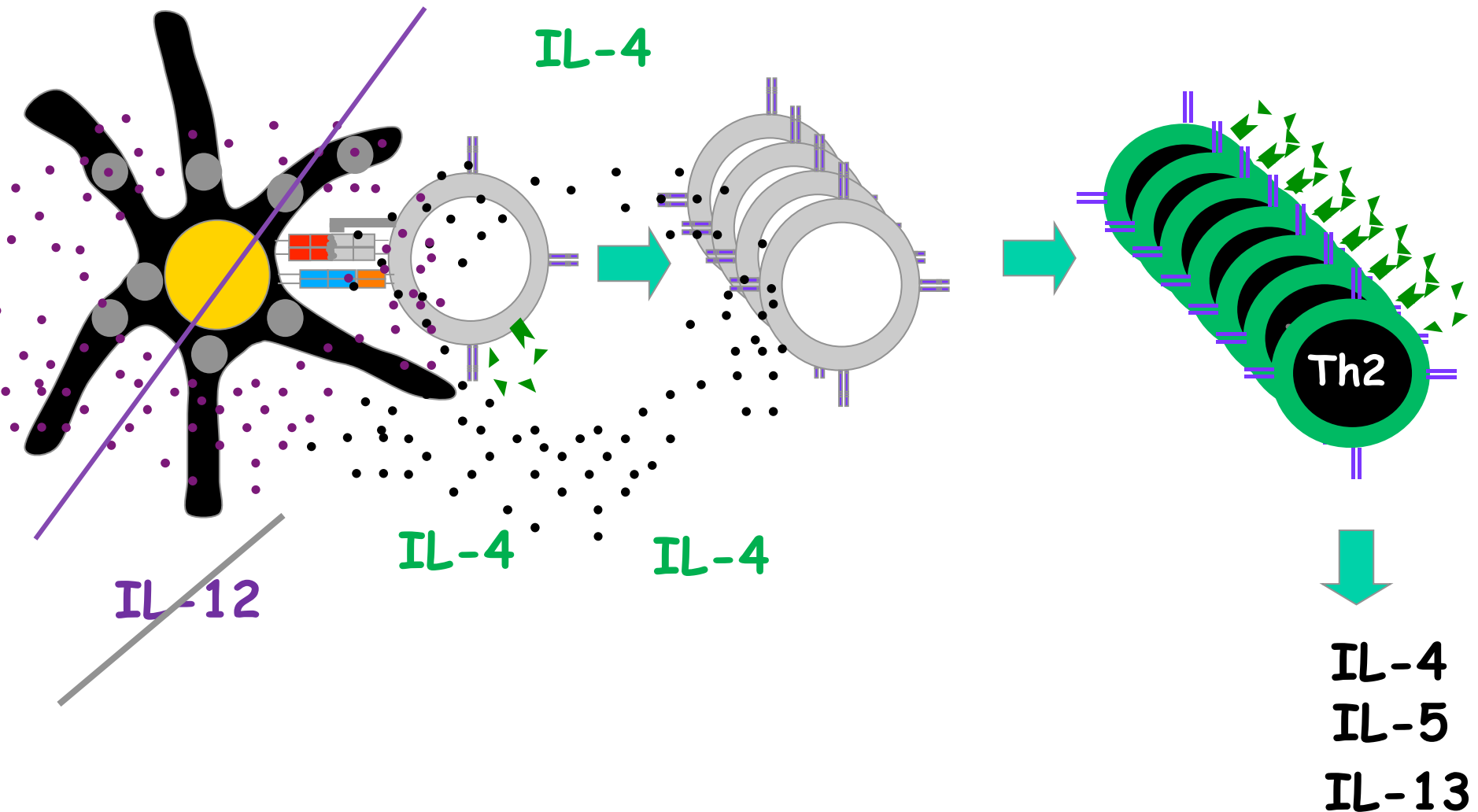
Diferenciação final é dirigida por fatores solúveis produzidos por APCs



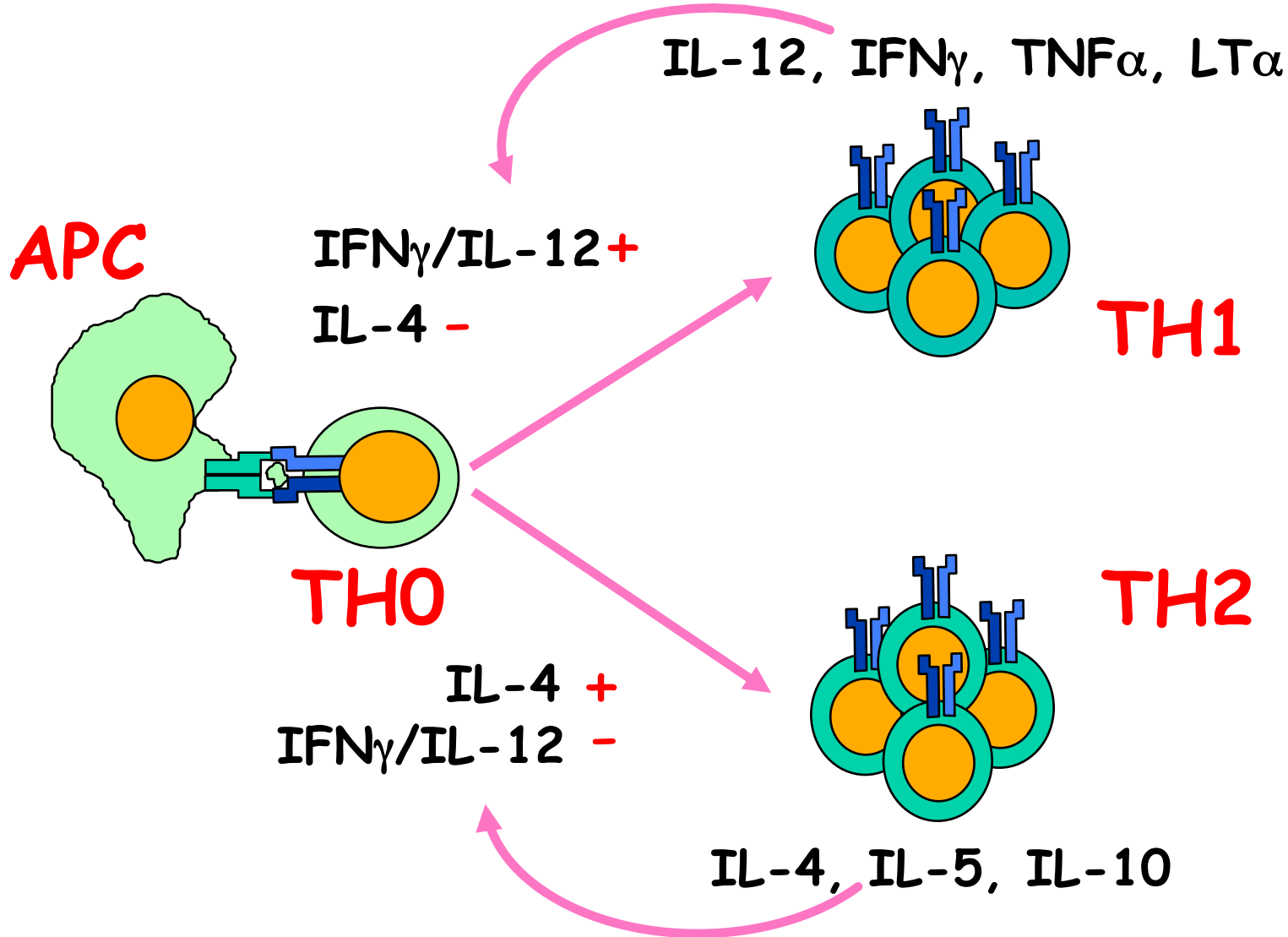
Na presença de IL-12, os linfócitos T se diferenciam para o perfil Th1



Na ausência de IL-12 e presença de IL-4, os linfócitos T se diferenciam para o perfil Th2

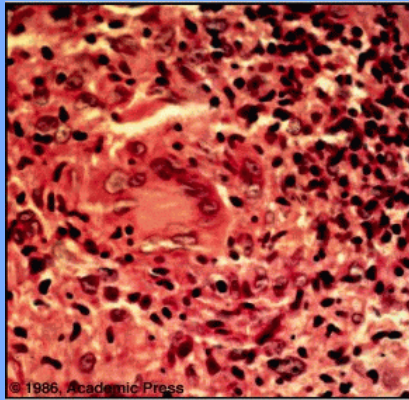


Diferenciação de células T auxiliares em linhagens TH1 e TH2

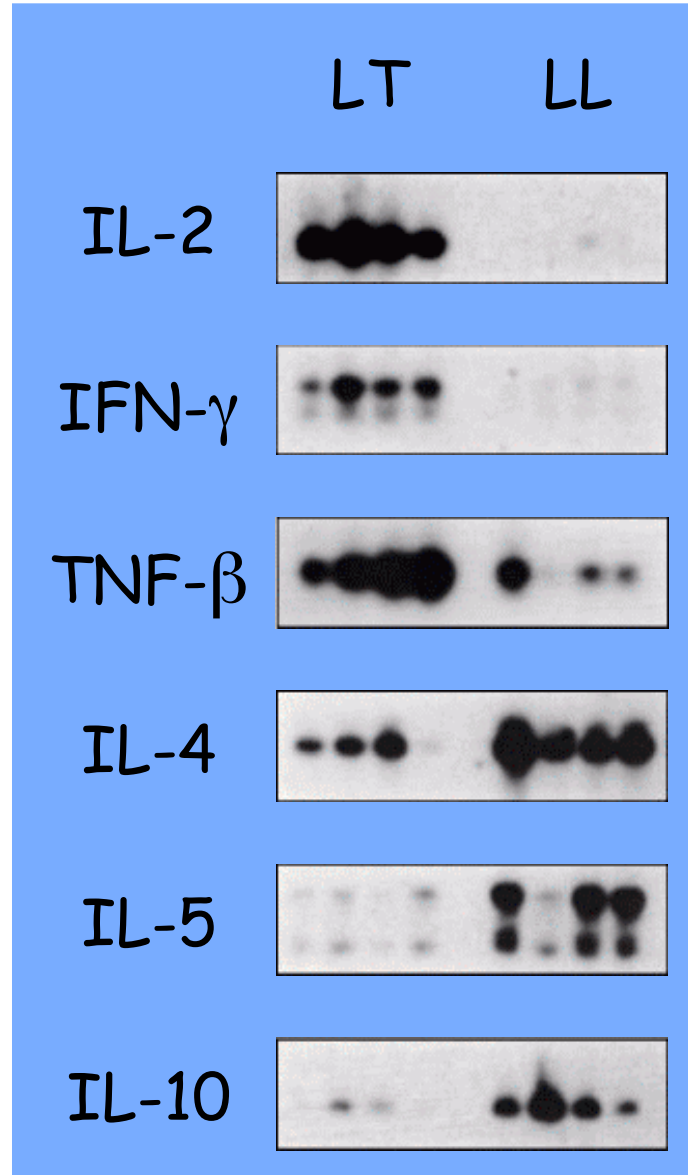
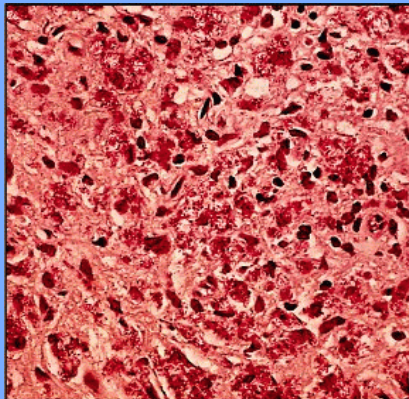


Balanco Th1/Th2 e o desenvolvimento de doenças infecciosas.

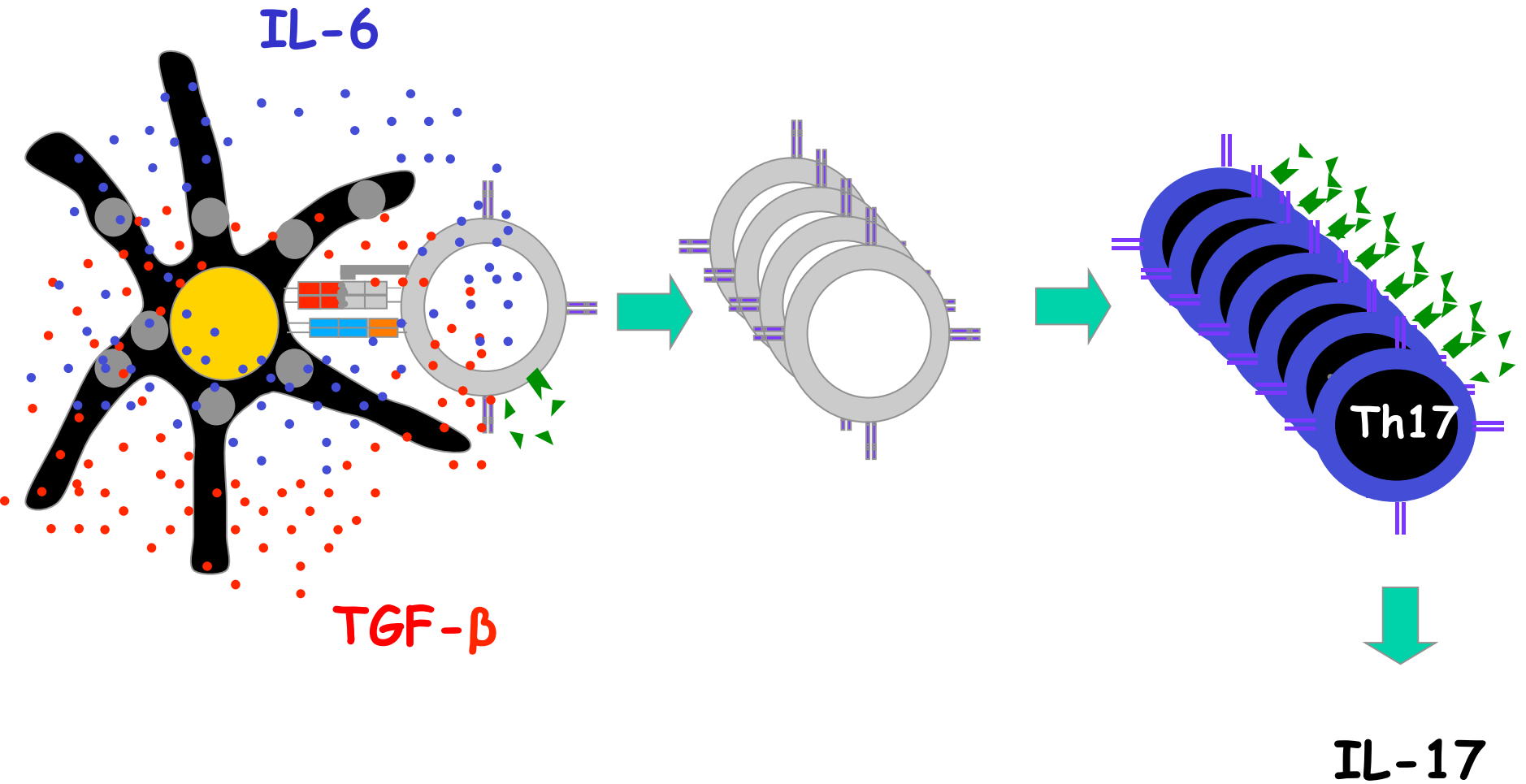
Lepra tuberculóide (restrita)



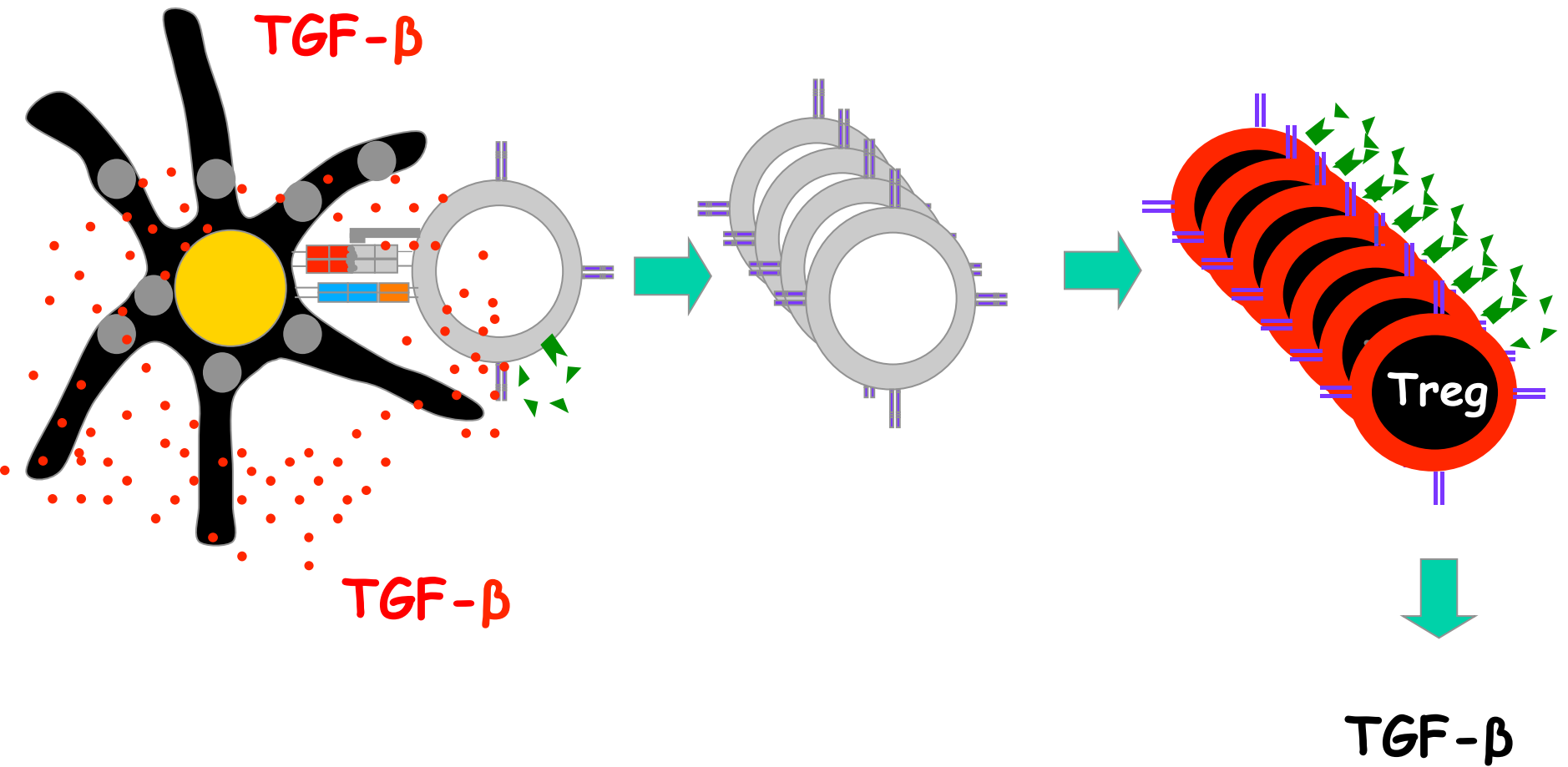
Lepra lepromatosa (disseminada)



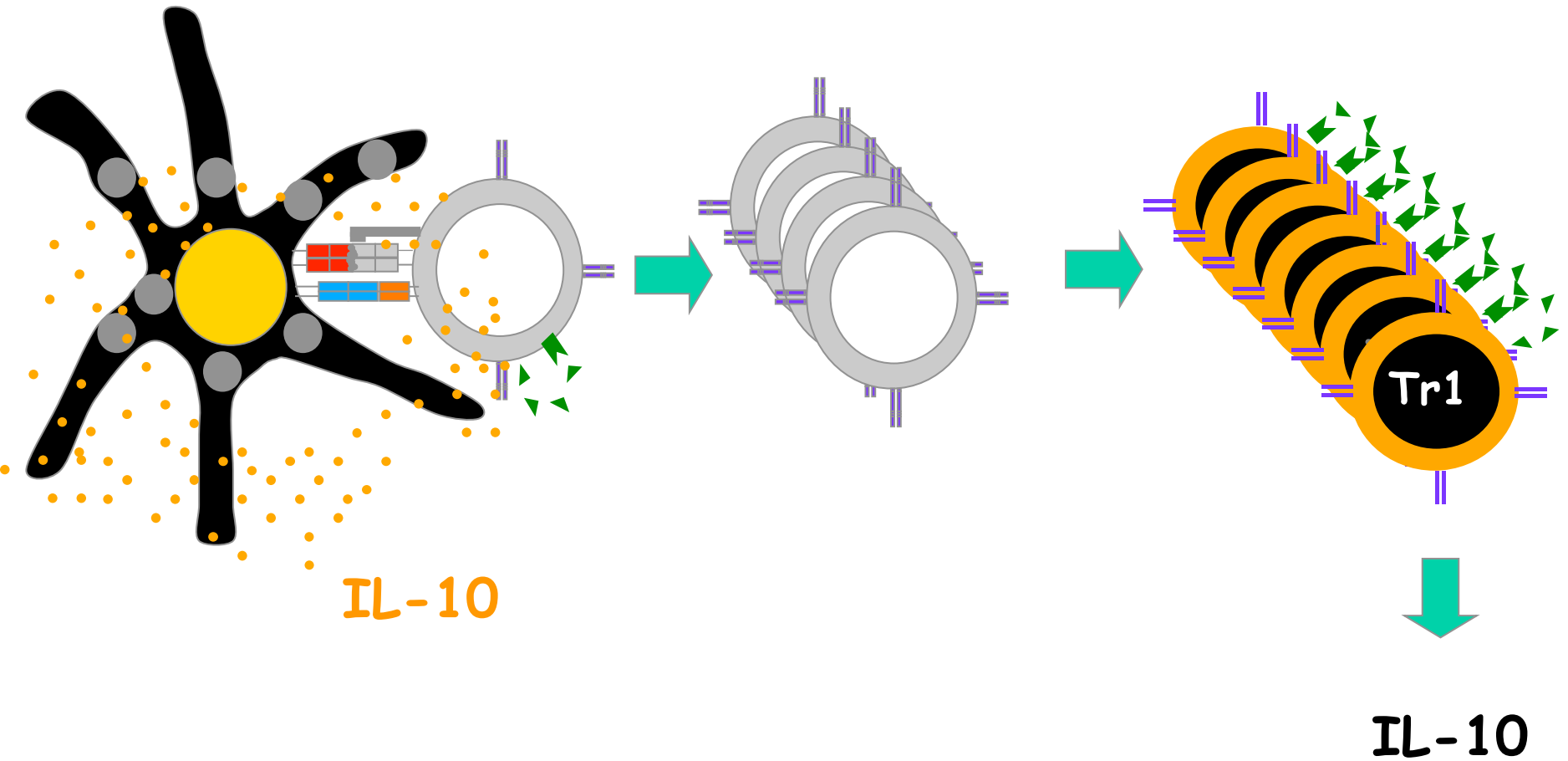
Diferenciação de linfócitos TH17



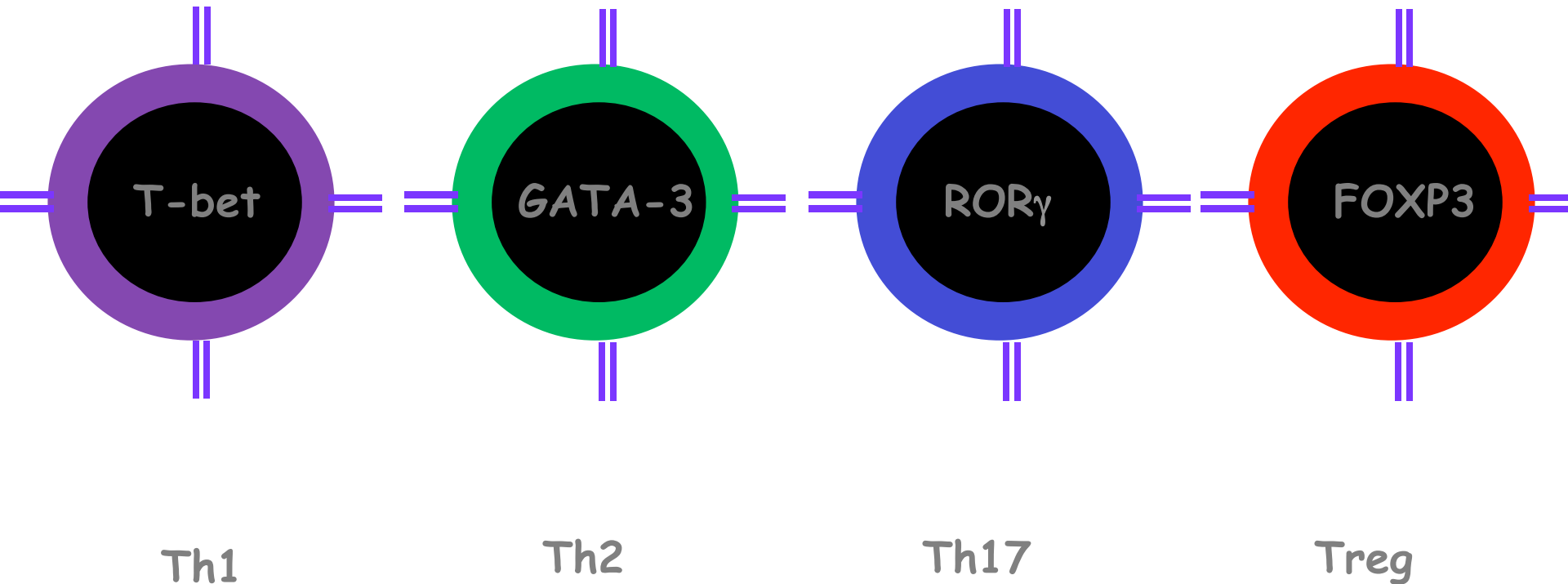
Diferenciação de Treg



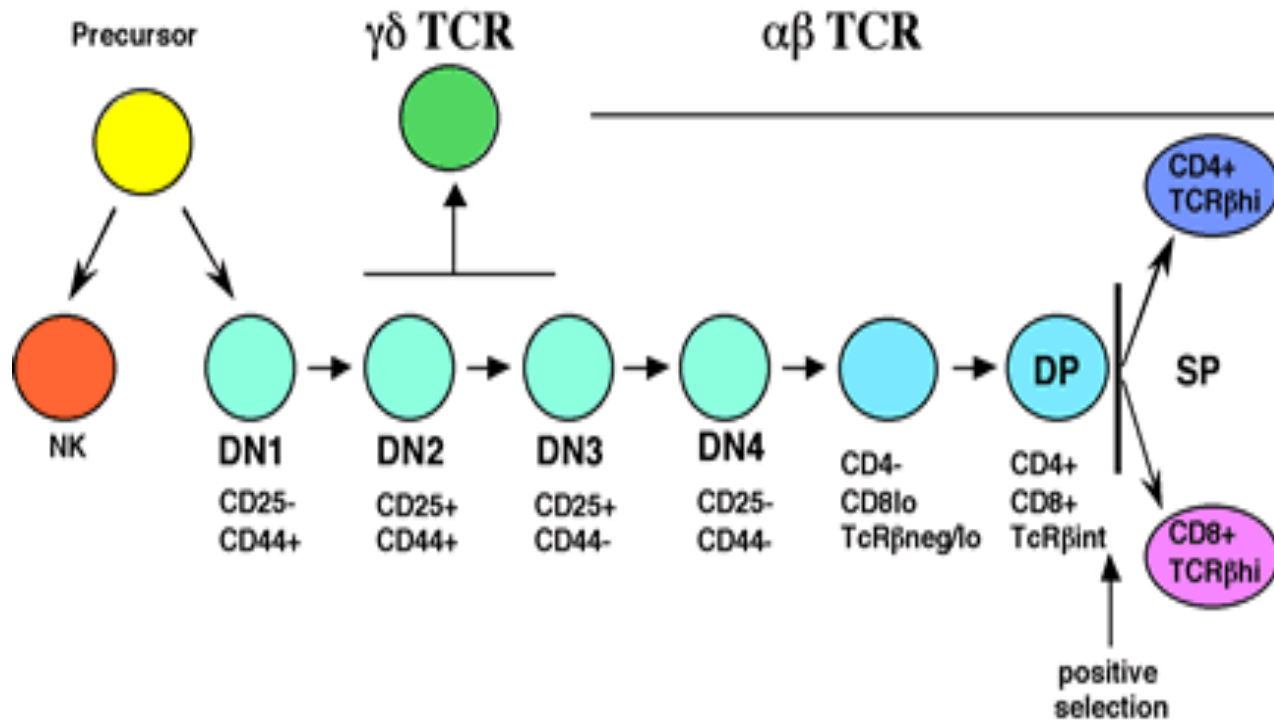
Diferenciação de células Tr1



Os linfócitos T diferenciados expressam fatores de transcrição particulares

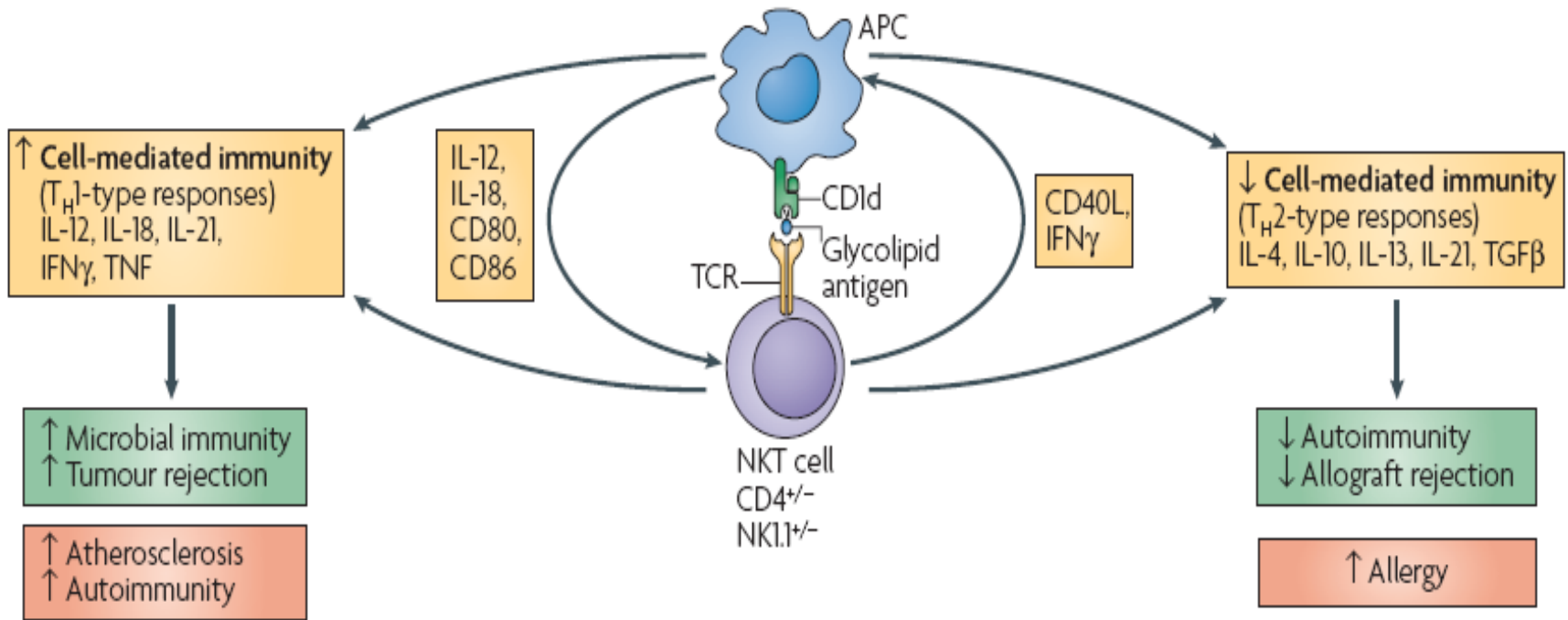


Linfócitos $T\gamma\delta$



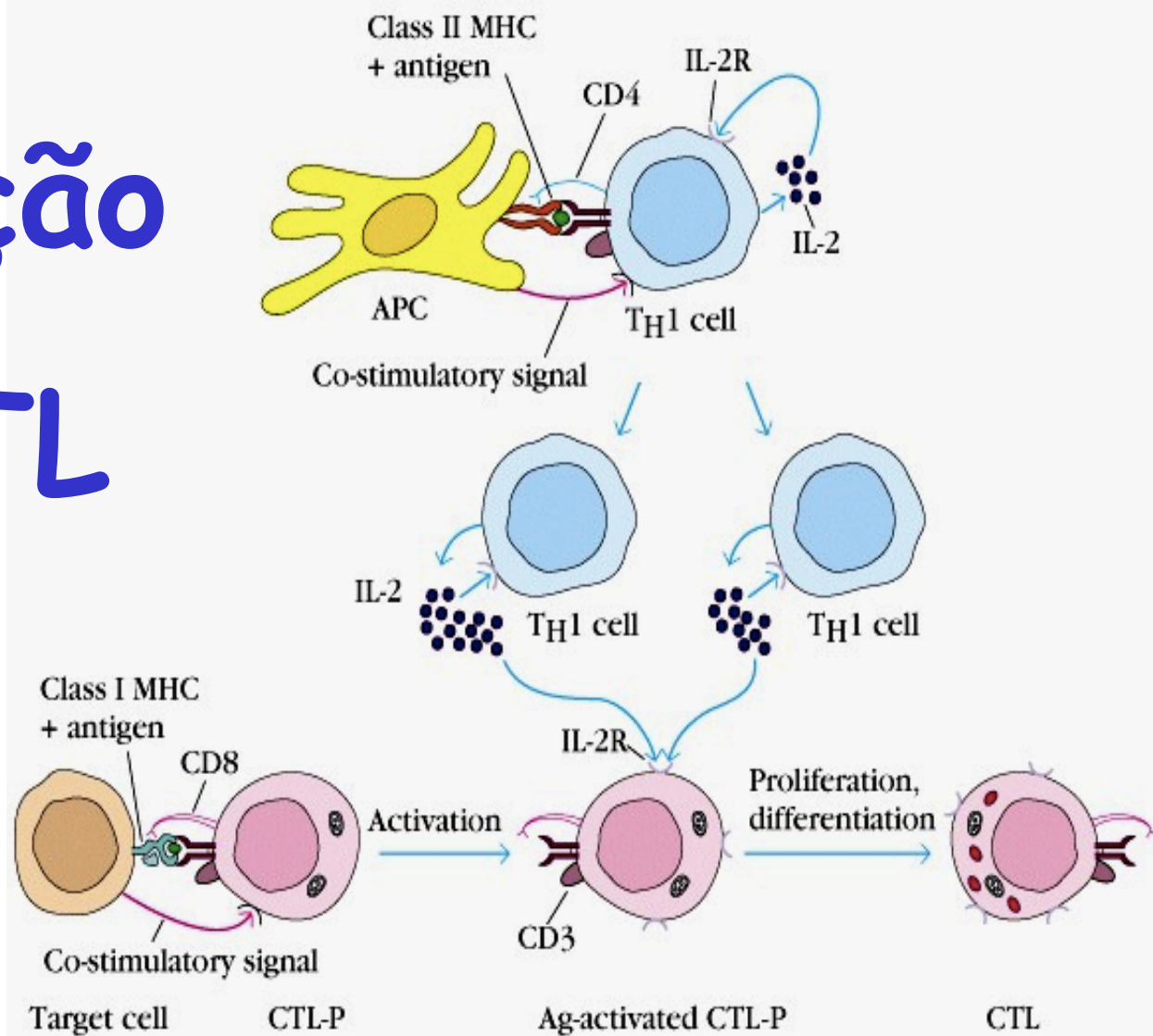
Os linfócitos $T\gamma\delta$ rearranjam seus TCRs no timo ou fora dele. Estas células também utilizam apenas algumas regiões V definidas (repertório restrito) e não passam pela seleção positiva e negativa. Elas são encontradas preferencialmente na pele e mucosas do trato gastrointestinal, respiratório e órgãos sexuais.

Linfócitos NKT

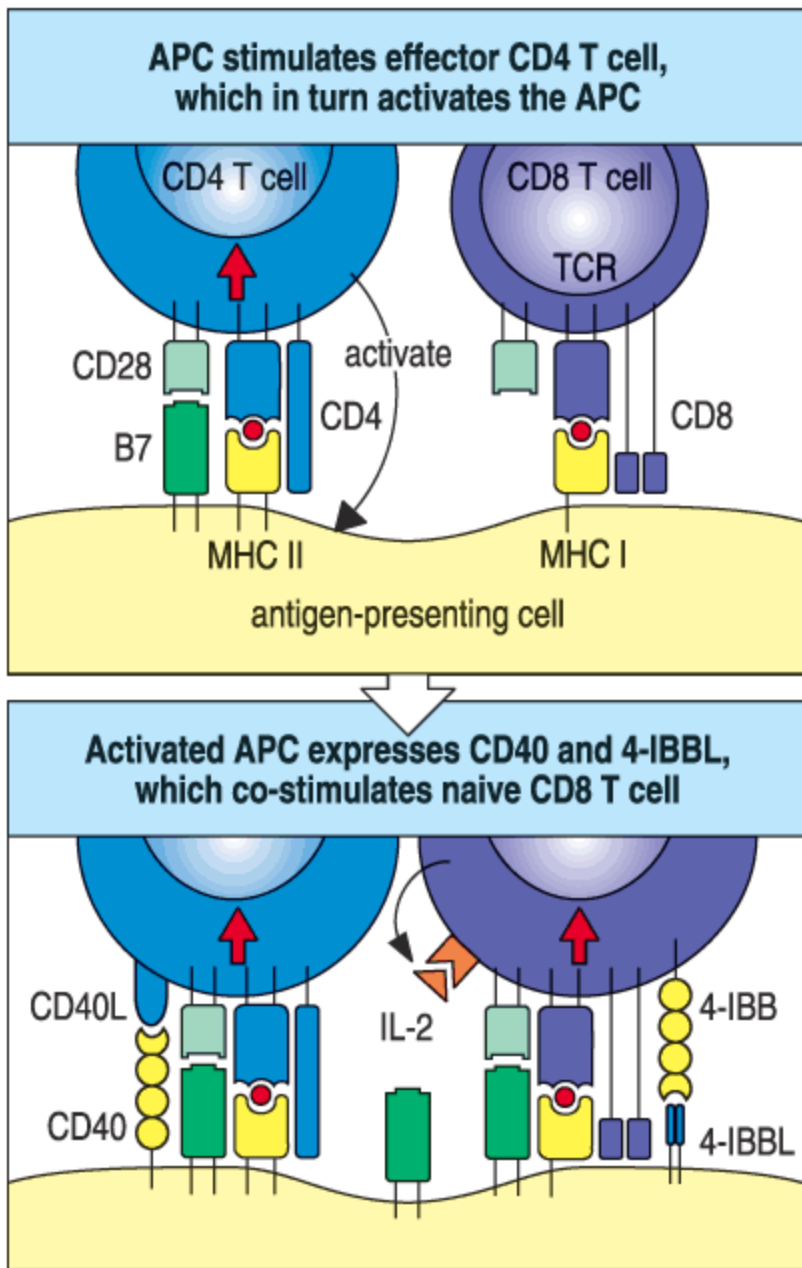


Os linfócitos NK T $\alpha\beta$ rearranjam seus TCRs no timo, mas utilizam apenas algumas regiões V definidas (repertório restrito) e passam apenas pela seleção positiva. Esses linfócitos localizam-se principalmente no tecido hepático, sangue e baço.

Geração de CTL



IL-2R expression	-	+	+
IL-2 expression	-	±	±
Proliferation	-	-	+
Effector cytotoxic function	-	-	+



LTCD8 podem ser ativados diretamente por APCs *ativadas* que expressem o complexo MHC class I/peptídeo

LTCD8 também podem ser ativados pelos LTCD4 que foram ativados por uma mesma APC

Fig 8.26 © 2001 Garland Science