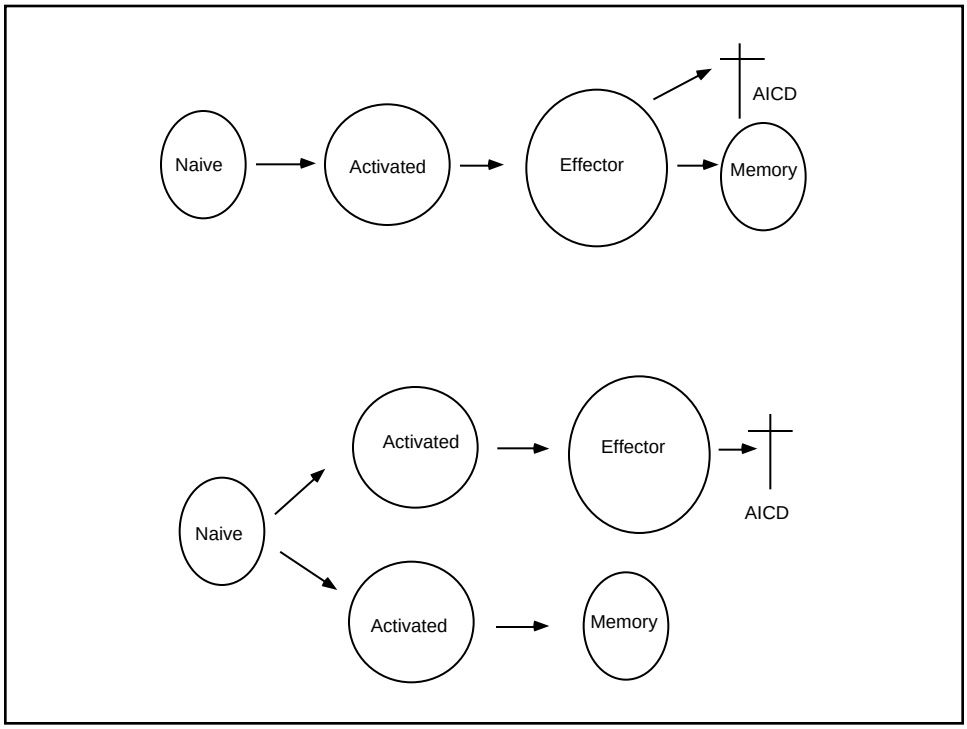


- INNOCUOUS ANTIGEN
- No Danger- very low expression of costimulatory ligands
- Signal One Only
  - Non-responsiveness
  - Tolerance (anergy)

- PATHOGEN
- Danger induces costimulatory ligands
- Signal One + Signal Two
  - Lymphocyte Activation

INNOCUOUS ANTIGENS

- Commensal microbes
- Food antigens
- Host cell proteins that have not induced thymic deletion
- Fetal antigens?

Choices during T cell activation

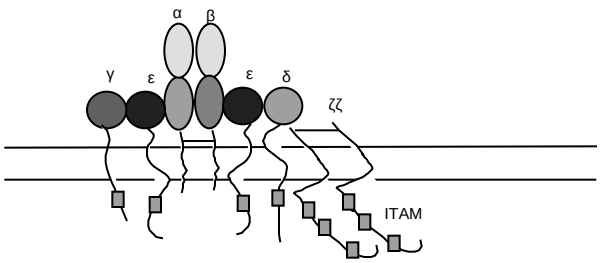
- INITIALLY:
  - Anergy OR
  - Activation into Effector state
- RESTIMULATION OF EFFECTORS
  - Activation Induced Cell death OR
  - Memory
    - Differentiation e.g.Th1 versus Th2
    - Other regulatory subsets

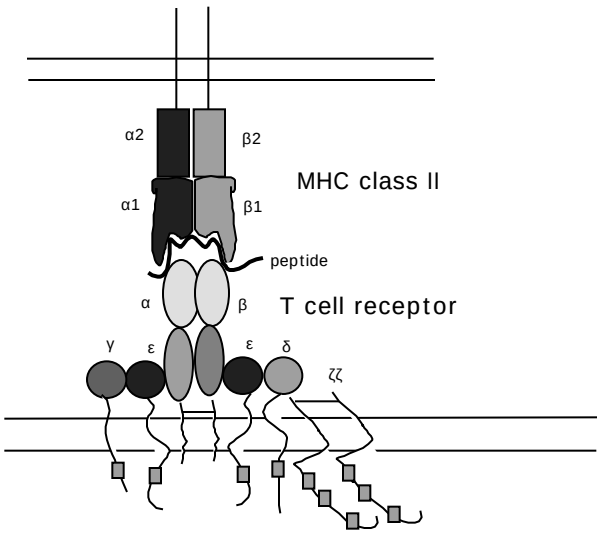
Issues in T cell activation - I

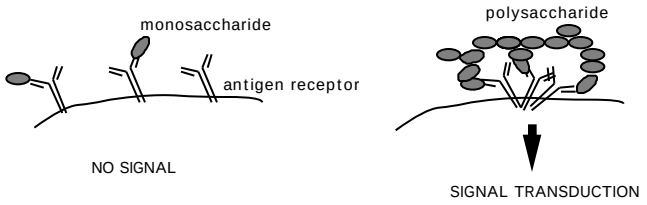
- Crosslinking versus Coreceptors
- Is the Affinity of the TCR for MHC Too Low?
- Lipid rafts and the need for Immunological Synapse generation
- Interfering with Signal One- immunosuppression and how it works

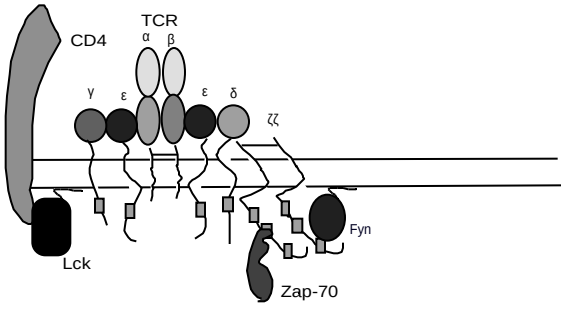
Issues in T cell Activation -II

- Anergy versus Activation
- How is the quality of an immune response modulated - Th1 versus Th2 for instance?


α
β
γ
ε
ε
δ
ζζ
ITAM


α2
β2
MHC class II
α1
β1
peptide
α
β
T cell receptor
γ
ε
ε
δ
ζζ


monosaccharide
antigen receptor
NO SIGNAL
polysaccharide
SIGNAL TRANSDUCTION


CD4
TCR
α
β
γ
ε
ε
δ
ζζ
Fyn
Lck
Zap-70

TCR-MHC interactions

- 1. Few MHC-peptide complexes on an APC specific for a given TCR
- 2. Affinity of TCR for specific MHC-peptide combo is pretty low - 10^{-4} to 10^{-6} M
- 3. How then does a T cell receive specific signals?

It Takes Two to Tango…..?

- SLC (secondary lymphoid chemokine) draws naïve T cells and activated dendritic cells through the HEV into lymph nodes
- Signaling through CCR7 activates integrins for adhesion but also induces T cell polarization via cytoskeletal rearrangements
- “Leading edge” of T cell slides alongside any dendritic cell it sees and a dance begins

Just a brief romance……..?

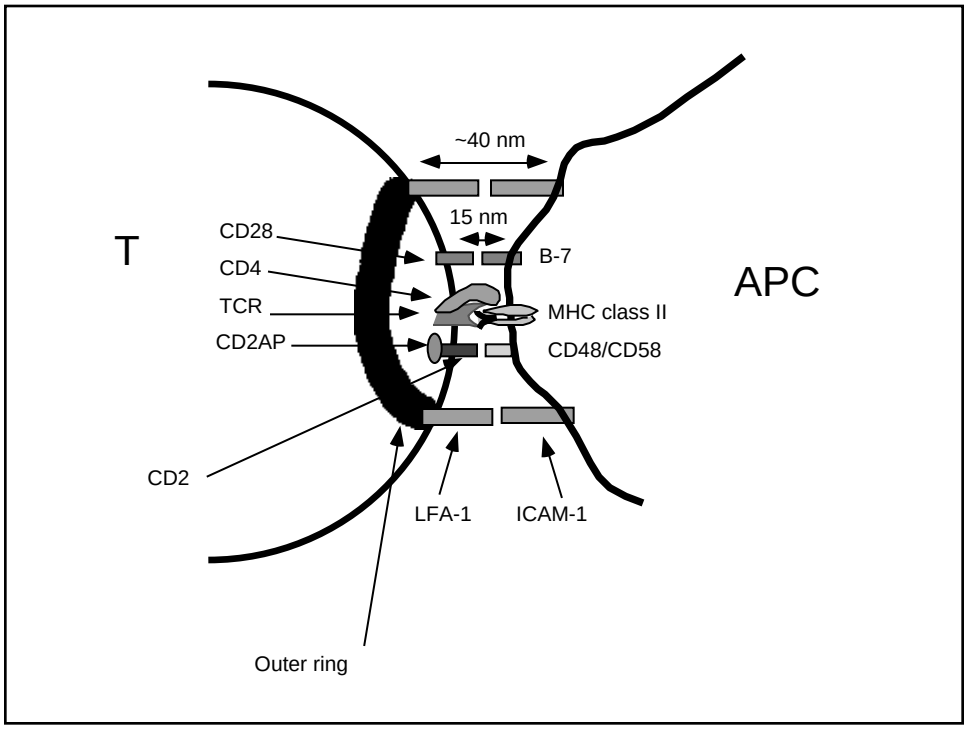
- Adhesion molecules on T cell bind to their counterparts on the DC; LFA-1 to ICAM-1,VLA-4 to VCAM-1 (LFA-1 and VLA-4 are integrins), CD2 to CD48 and so on
- CCR7 mediated adhesion is brief….minutes

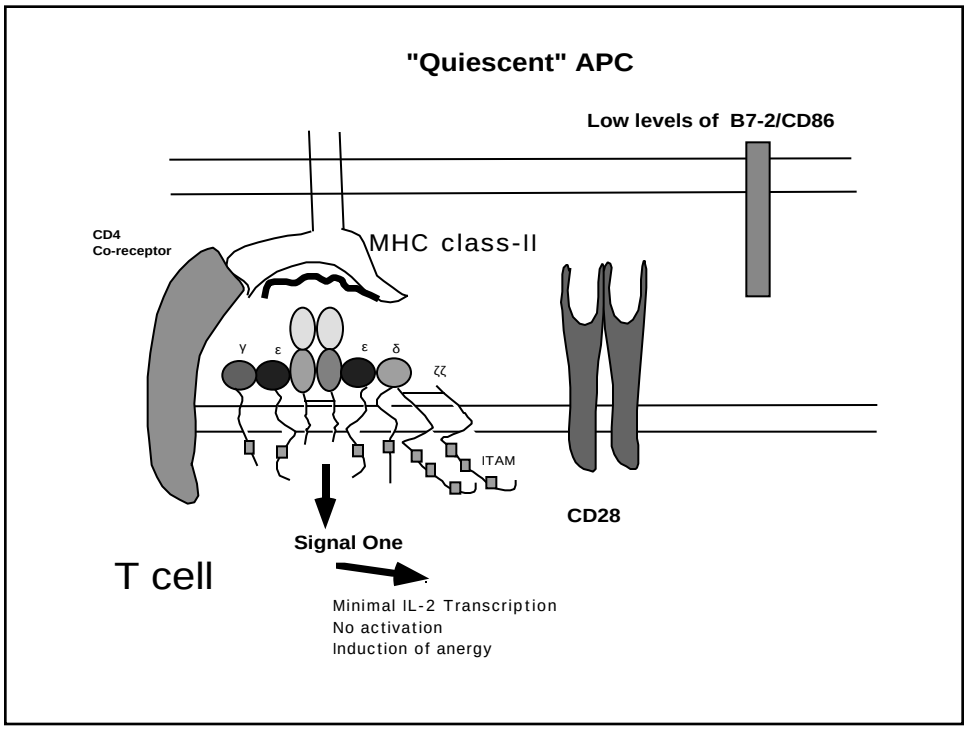
The TCR knows…..

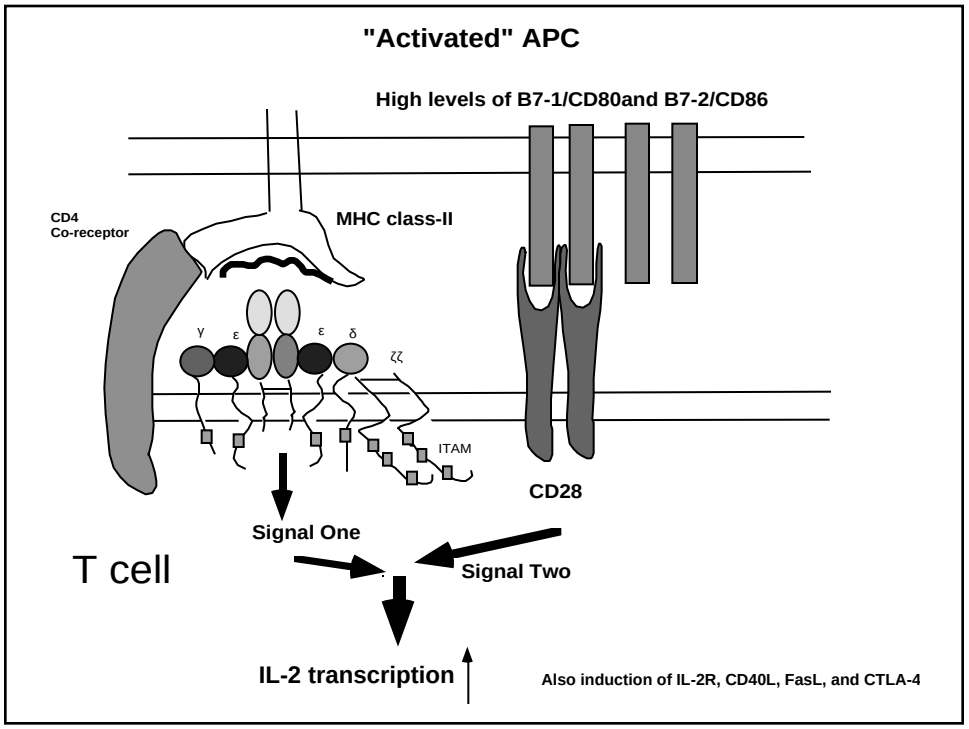
- The right TCR-MHC/peptide interaction sustains a long term relationship…. For a few hours
- True synapse formation is initiated

Lipid rafts and Immunological Synapses

- Signaling initiated from specialized membrane microdomains- lipid rafts aka DIG domains or GEMs (these contain acylated proteins, GPI anchored proteins, PIP2 etc).
- Signaling through TCR induces cytoskeletal rearrangements and fusion of lipid rafts forming immunological synapses.

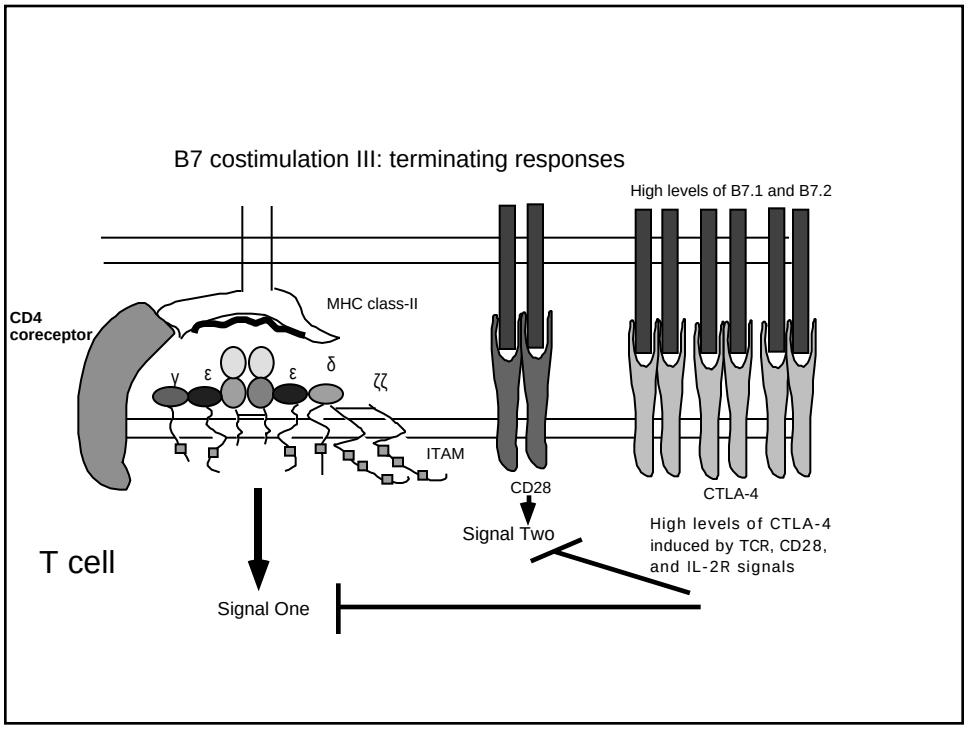

~40 nm
15 nm
T
CD28
CD4
TCR
CD2AP
B-7
MHC class II
CD48/CD58
APC
CD2
LFA-1
ICAM-1
Outer ring

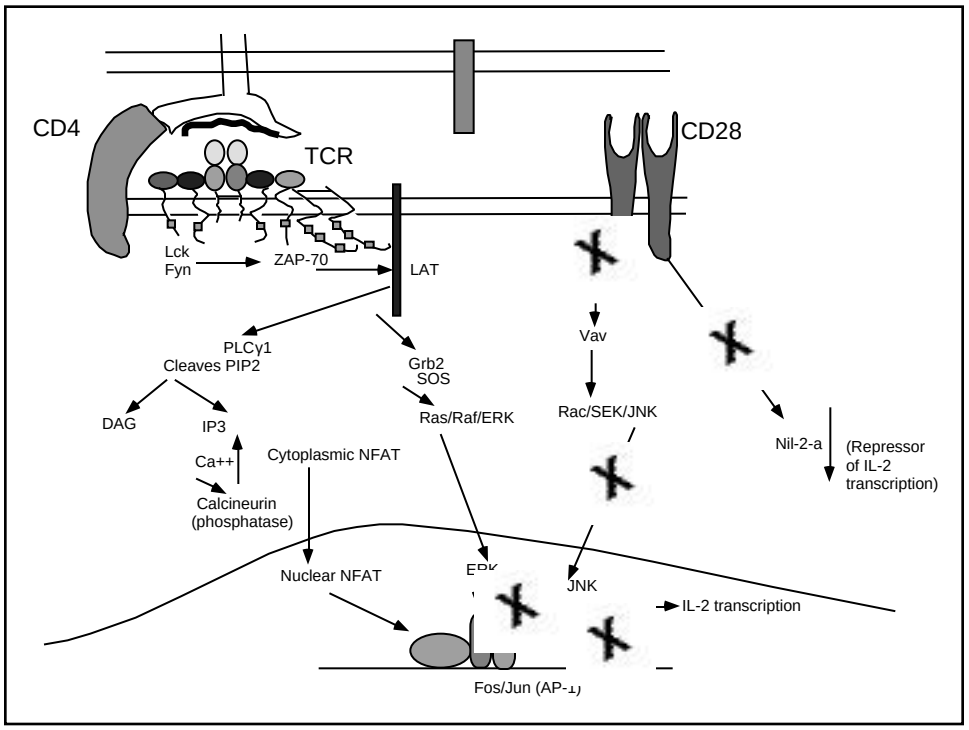

"Quiescent" APC
Low levels of B7-2/CD86
CD4
Co-receptor
MHC class-II
γ
ε
ε
δ
ζζ
ITAM
CD28
Signal One
T cell
Minimal IL-2 Transcription
No activation
Induction of anergy

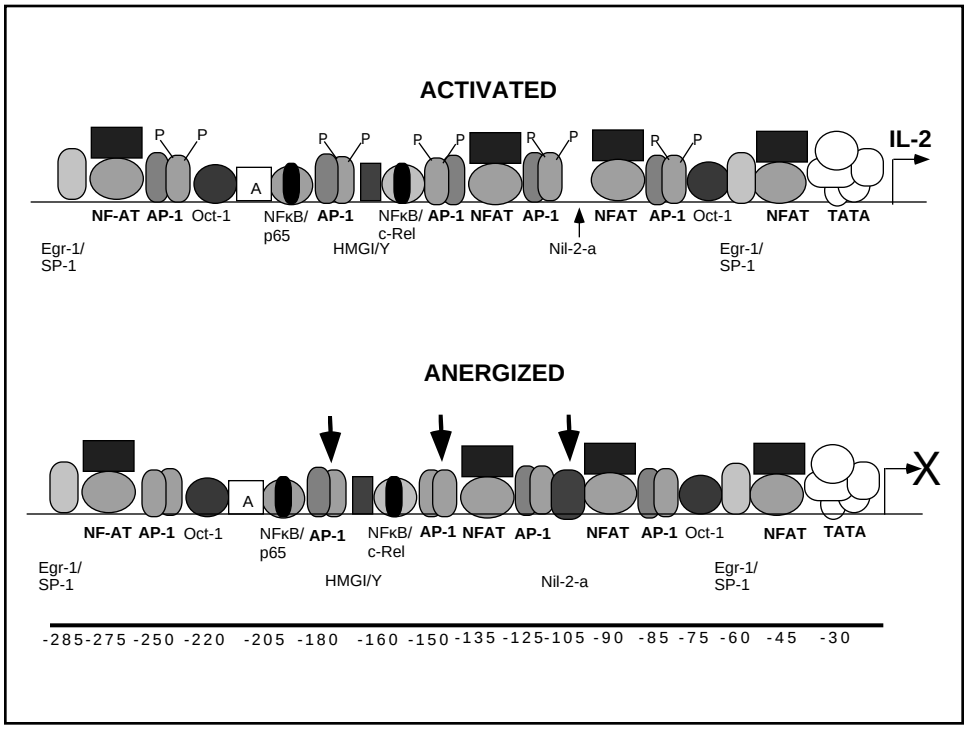


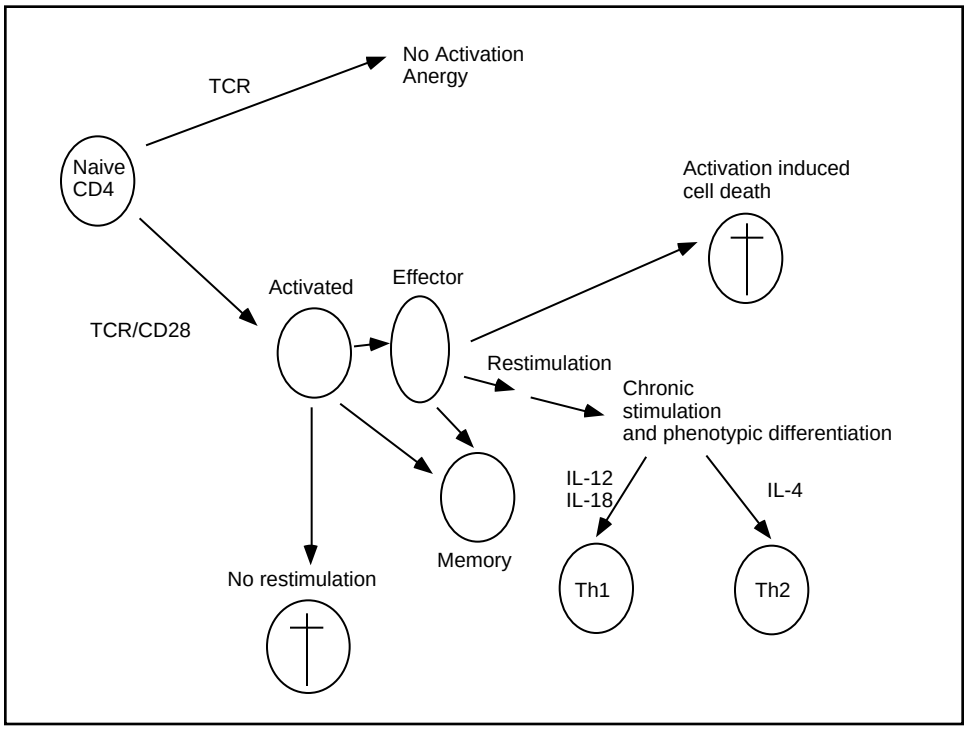
Some of the events turned on by Signals One and Two

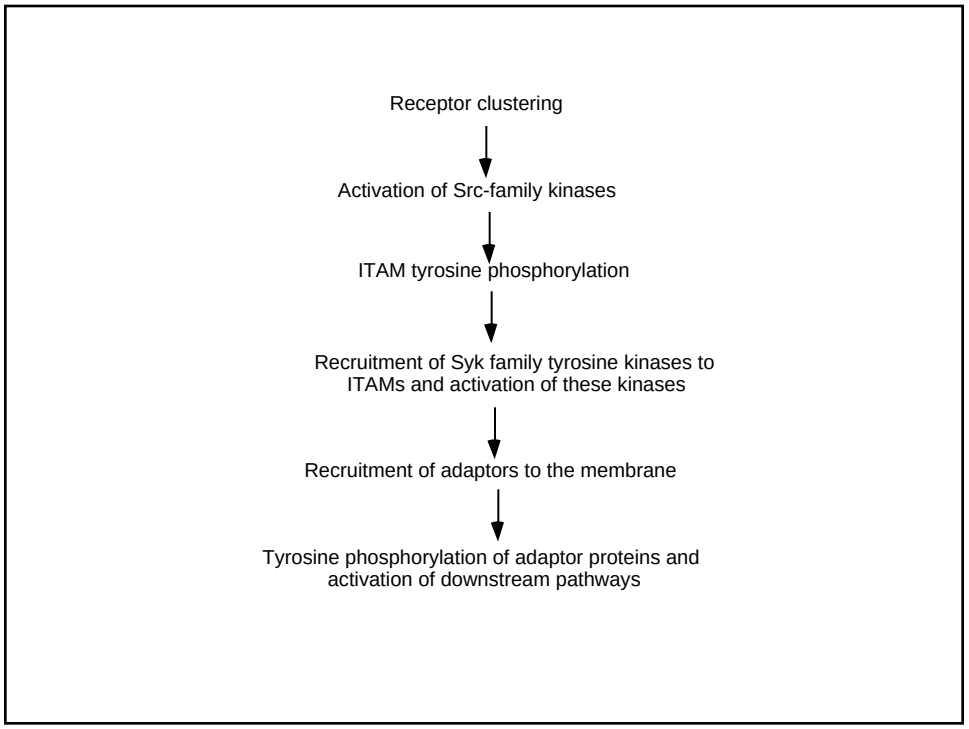
- Induction of IL-2R and IL-2 expression
- Induction of cyclins D2 and D3 and CDK4 and degradation of p27 CDK inhibitor - cells divide
- Induction of CD40L
- Induction of FasL and of higher levels of CTLA-4

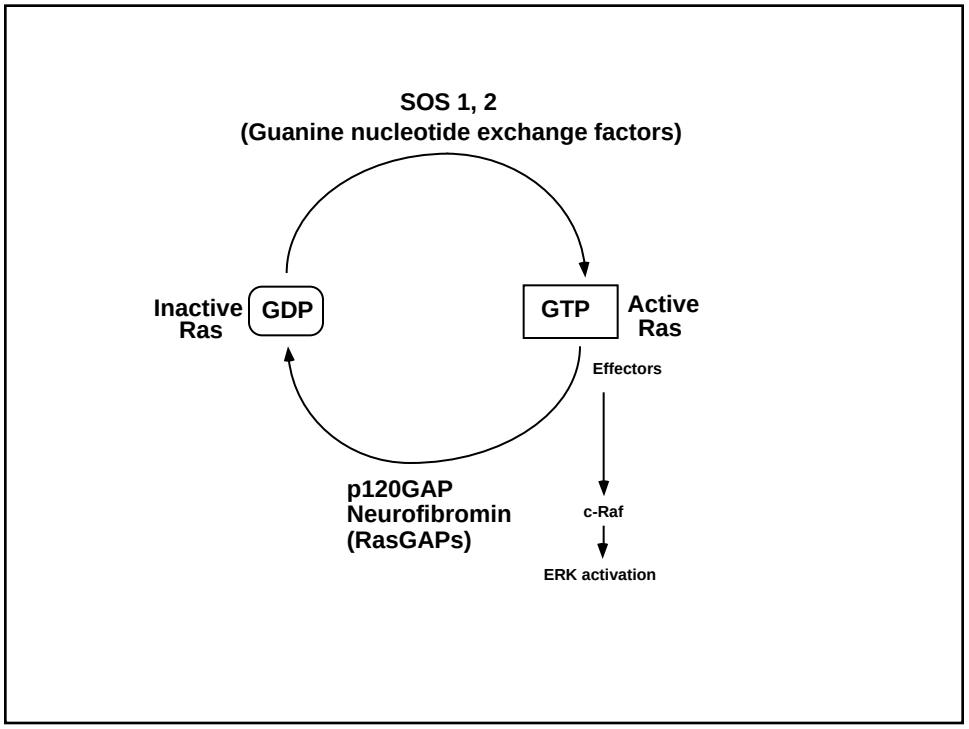

B7 costimulation III: terminating responses
High levels of B7.1 and B7.2
MHC class-II
CD4
coreceptor
γ
ε
ε
δ
ζζ
ITAM
CD28
CTLA-4
Signal Two
High levels of CTLA-4
induced by TCR, CD28,
and IL-2R signals
T cell
Signal One

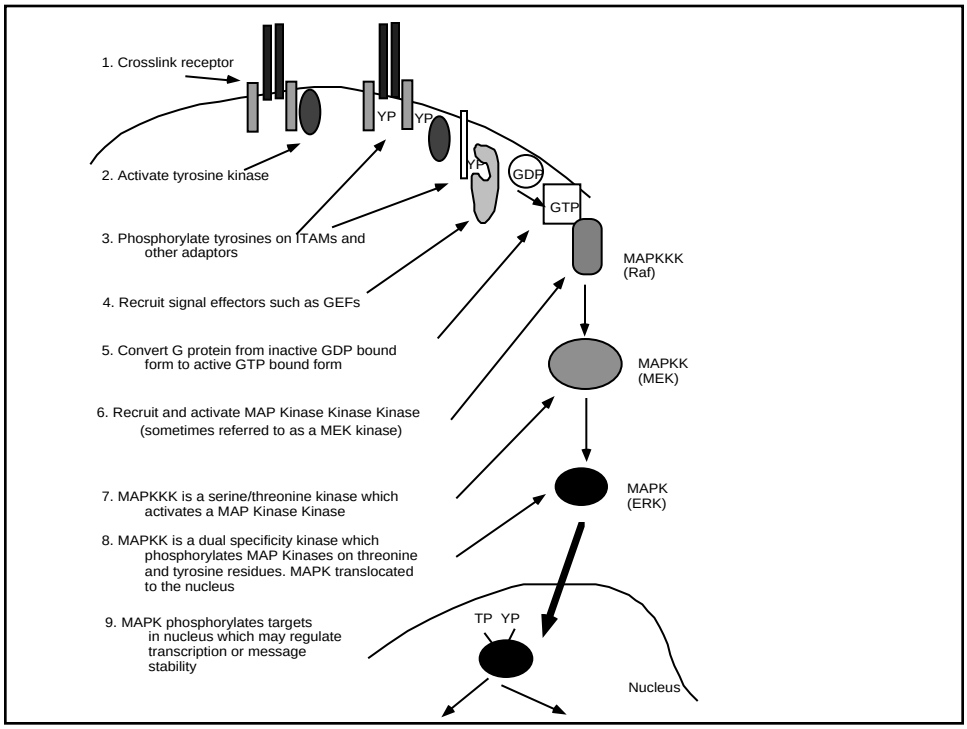

CD4
TCR
CD28
Lck
Fyn
ZAP-70
LAT
PLCγ1
Cleaves PIP2
Grb2
SOS
Vav
DAG
IP3
Ras/Raf/ERK
Rac/SEK/JNK
Nil-2-a
(Repressor
of IL-2
transcription)
Ca++
Calcineurin
(phosphatase)
Cytoplasmic NFAT
Nuclear NFAT
JNK
IL-2 transcription
Fos/Jun (AP-1)

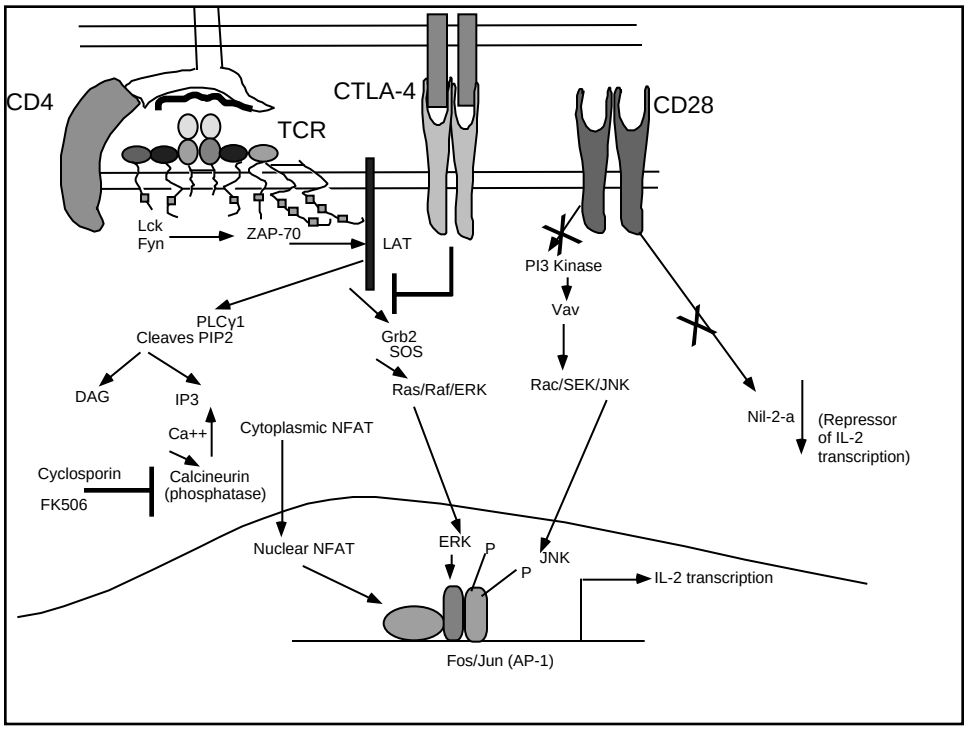

ACTIVATED
IL-2
NF-AT AP-1 Oct-1
NFκB/ p65
AP-1
NFκB/ c-Rel
AP-1 NFAT AP-1
NFAT AP-1 Oct-1
NFAT TATA
Egr-1/ SP-1
HMGI/Y
Nil-2-a
Egr-1/ SP-1
ANERGIZED
NF-AT AP-1 Oct-1
NFκB/ p65
AP-1
NFκB/ c-Rel
AP-1 NFAT AP-1
NFAT AP-1 Oct-1
NFAT TATA
Egr-1/ SP-1
HMGI/Y
Nil-2-a
Egr-1/ SP-1
-285 -275 -250 -220 -205 -180 -160 -150 -135 -125 -105 -90 -85 -75 -60 -45 -30

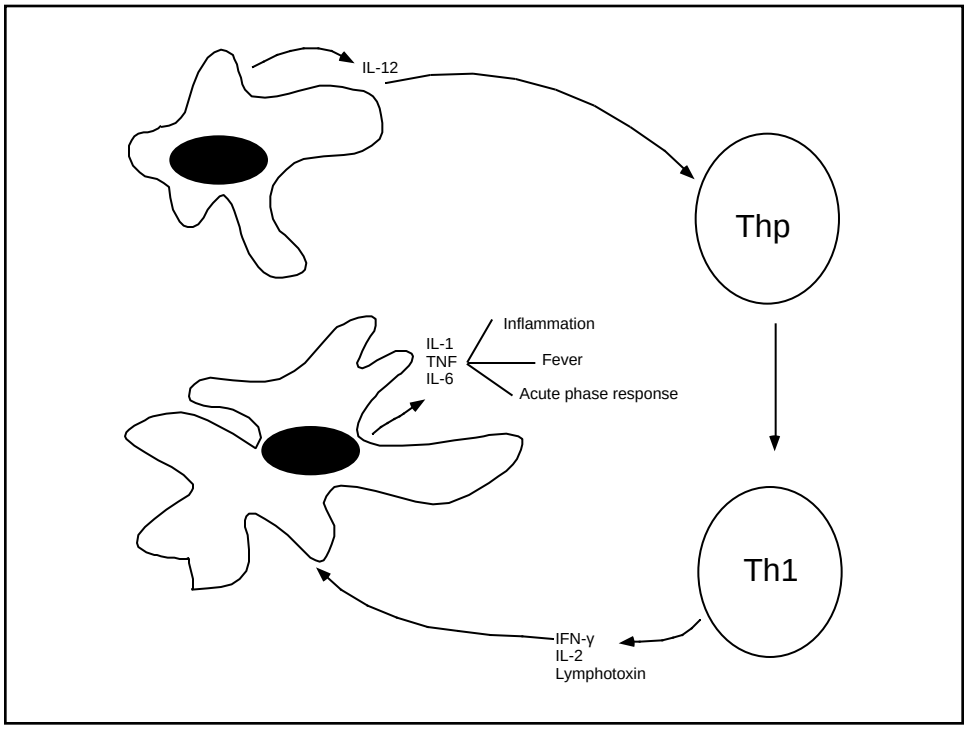

TCR
No Activation
Anergy
Naive CD4
Activation induced cell death
Activated
Effector
TCR/CD28
Restimulation
Chronic stimulation and phenotypic differentiation
IL-12 IL-18
IL-4
Memory
No restimulation
Th1
Th2


Receptor clustering
Activation of Src-family kinases
ITAM tyrosine phosphorylation
Recruitment of Syk family tyrosine kinases to ITAMs and activation of these kinases
Recruitment of adaptors to the membrane
Tyrosine phosphorylation of adaptor proteins and activation of downstream pathways


SOS 1, 2
(Guanine nucleotide exchange factors)
Inactive Ras
GDP
GTP
Active Ras
Effectors
p120GAP
Neurofibromin
(RasGAPs)
c-Raf
ERK activation


1. Crosslink receptor
YP
YP
2. Activate tyrosine kinase
YP
GDP
GTP
3. Phosphorylate tyrosines on ITAMs and other adaptors
MAPKKK (Raf)
4. Recruit signal effectors such as GEFs
5. Convert G protein from inactive GDP bound form to active GTP bound form
MAPKK (MEK)
6. Recruit and activate MAP Kinase Kinase Kinase (sometimes referred to as a MEK kinase)
7. MAPKKK is a serine/threonine kinase which activates a MAP Kinase Kinase
MAPK (ERK)
8. MAPKK is a dual specificity kinase which phosphorylates MAP Kinases on threonine and tyrosine residues. MAPK translocated to the nucleus
9. MAPK phosphorylates targets in nucleus which may regulate transcription or message stability
TP YP
Nucleus


CD4
CTLA-4
CD28
TCR
Lck
Fyn
ZAP-70
LAT
PI3 Kinase
Vav
PLCγ1
Cleaves PIP2
Grb2
SOS
DAG
IP3
Ras/Raf/ERK
Rac/SEK/JNK
Nil-2-a
(Repressor of IL-2 transcription)
Ca++
Cytoplasmic NFAT
Cyclosporin
FK506
Calcineurin (phosphatase)
Nuclear NFAT
ERK
P
P
JNK
IL-2 transcription
Fos/Jun (AP-1)



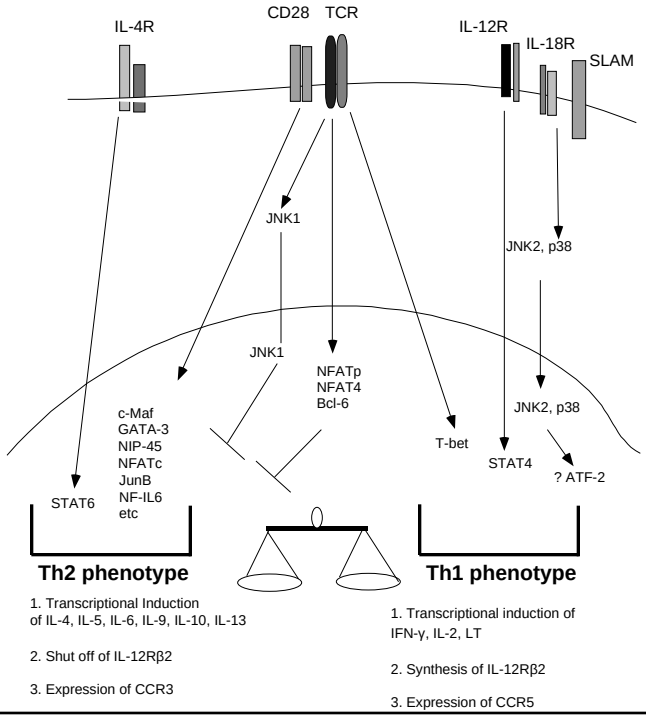
Th1/Th2

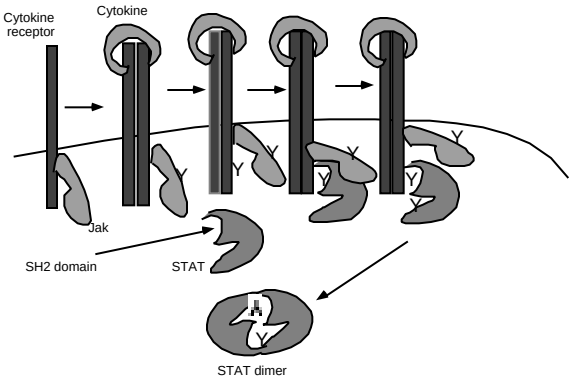
1. TCR Signal strength - nature of antigen and amount

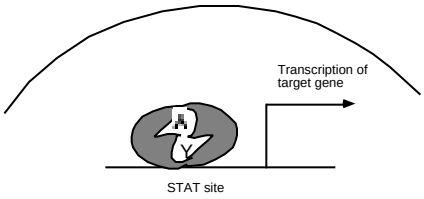
2. Route of immunization and cytokine milieu

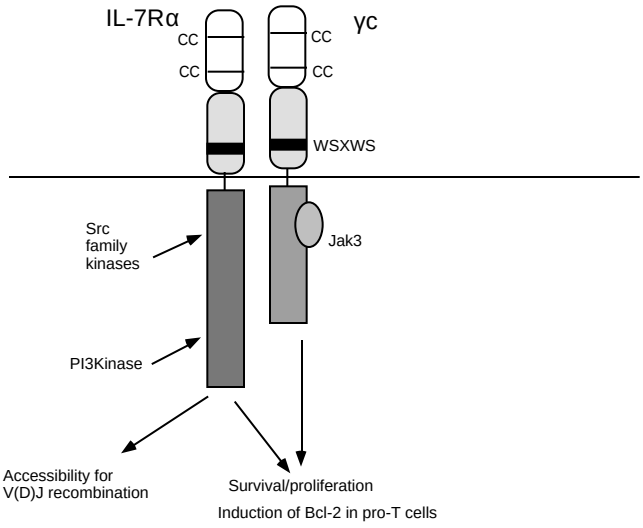
3. Signals induce transcriptional patterns that influence differentiation

The lists of transcription factors on the next image are included to paint the picture more completely - you do NOT need to memorize them




Cytokine receptor
Cytokine
Y
Jak
SH2 domain
STAT
STAT dimer

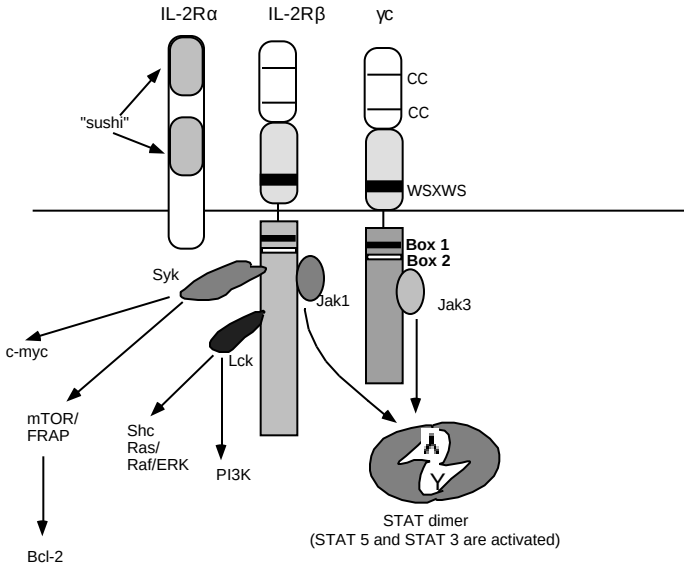

Transcription of target gene
STAT site

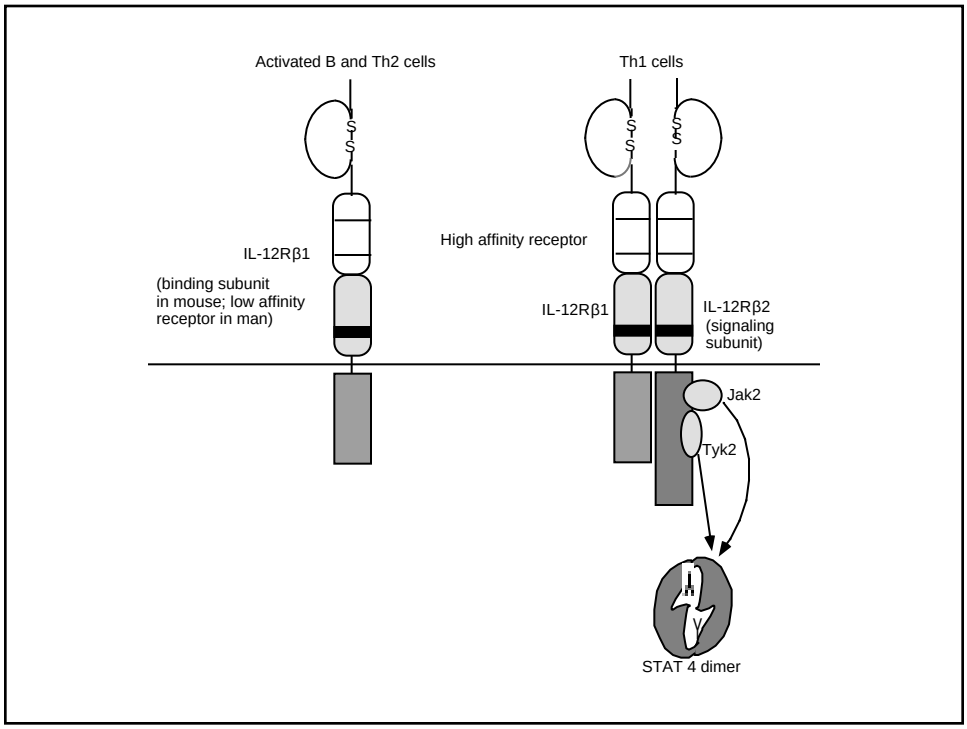

IL-7Rα
γc
CC
CC
CC
CC
WSXWS
Src family kinases
Jak3
PI3Kinase
Accessibility for V(D)J recombination
Survival/proliferation
Induction of Bcl-2 in pro-T cells

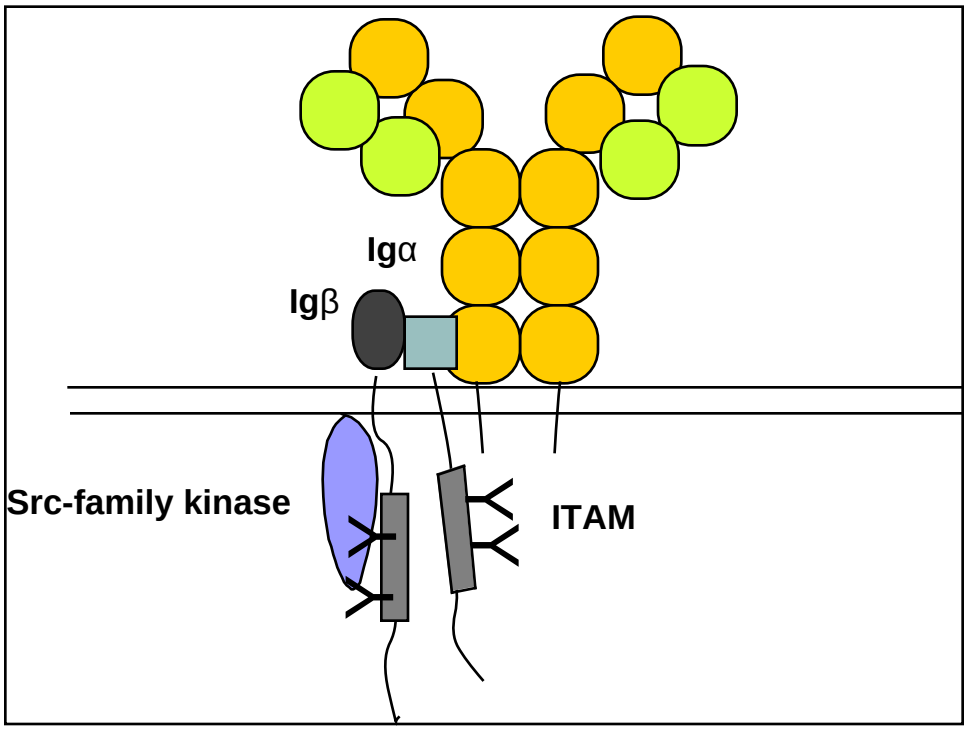
γc and X-linked SCID

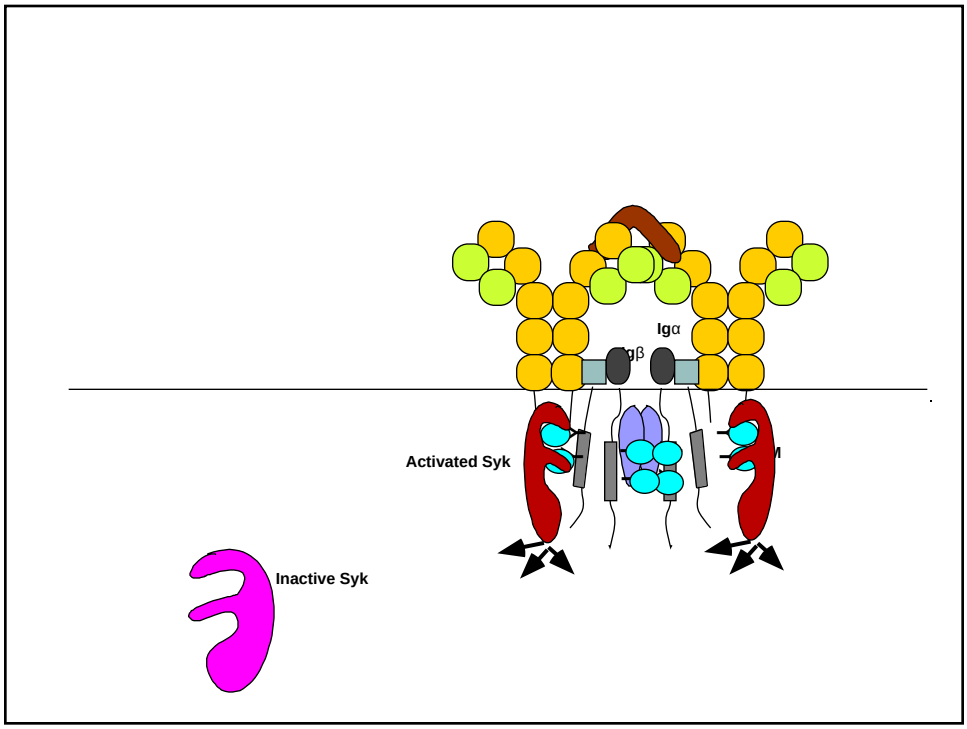
γc or the common gamma chain is a component of the IL-2, IL-4, IL-7, IL-9, and IL-15 receptors

The γc gene is encoded on the X-chromosome

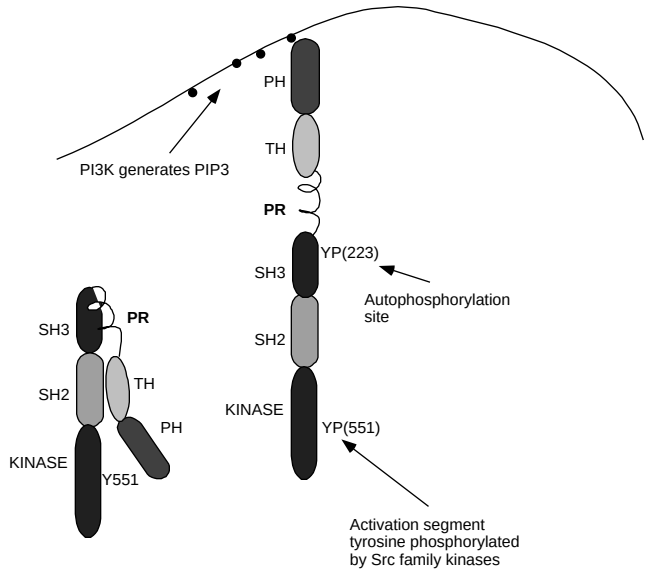



Activated B and Th2 cells
Th1 cells
IL-12Rβ1
(binding subunit
in mouse; low affinity
receptor in man)
High affinity receptor
IL-12Rβ1
IL-12Rβ2
(signaling
subunit)
Jak2
Tyk2
STAT 4 dimer


Igα
Igβ
Src-family kinase
ITAM

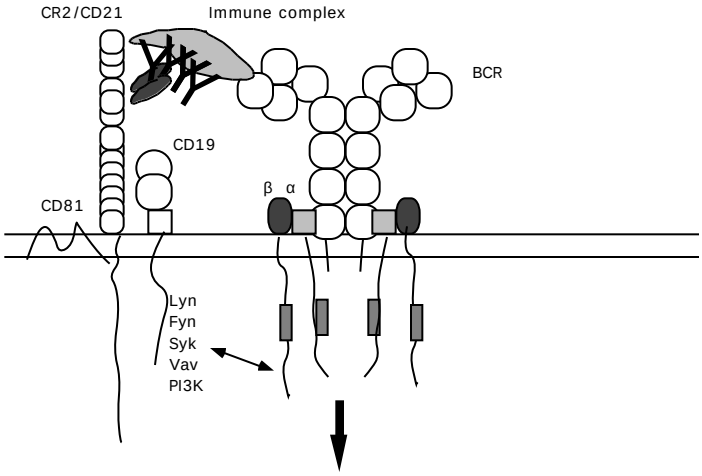


Btk is recruited to the membrane by PI3K

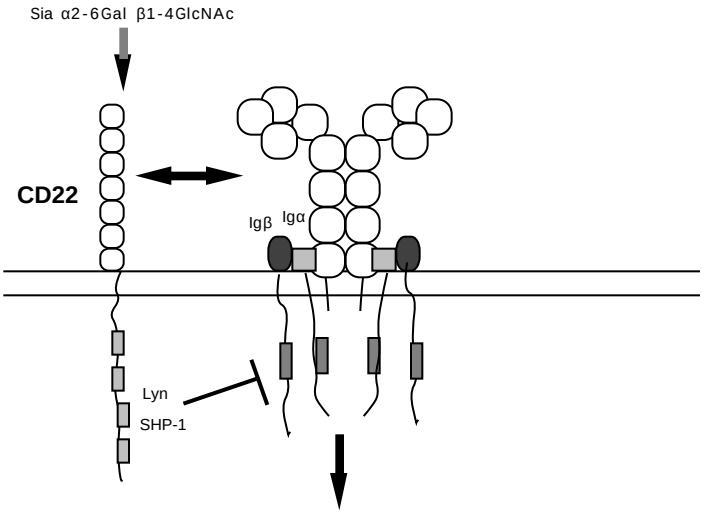


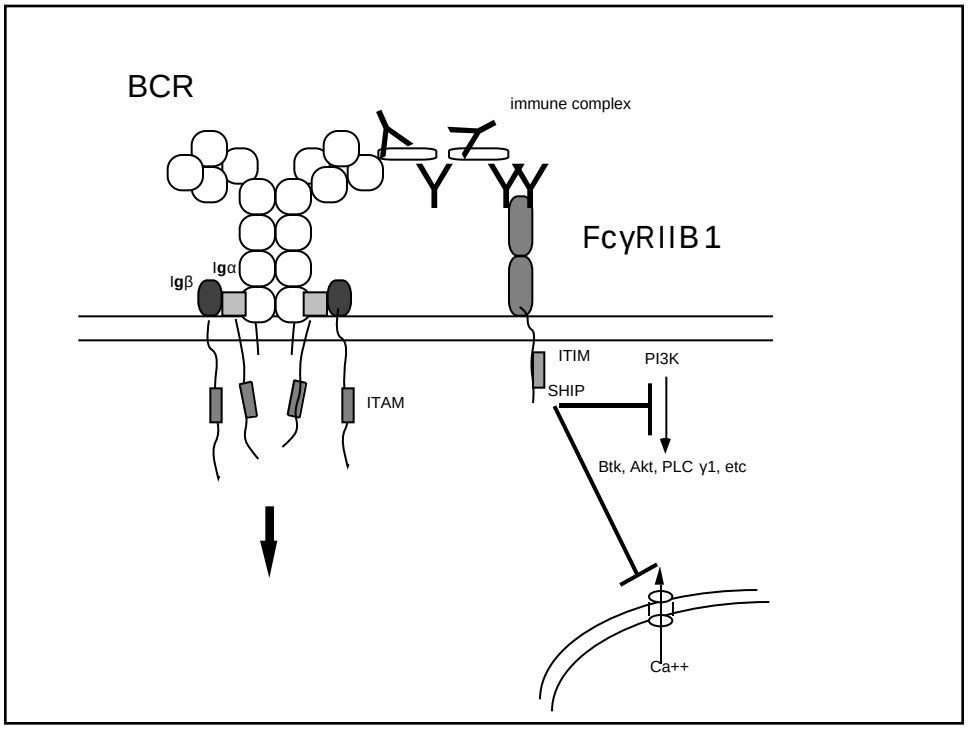
CR2/CD21 is a coreceptor and positive regulator of BCR signaling

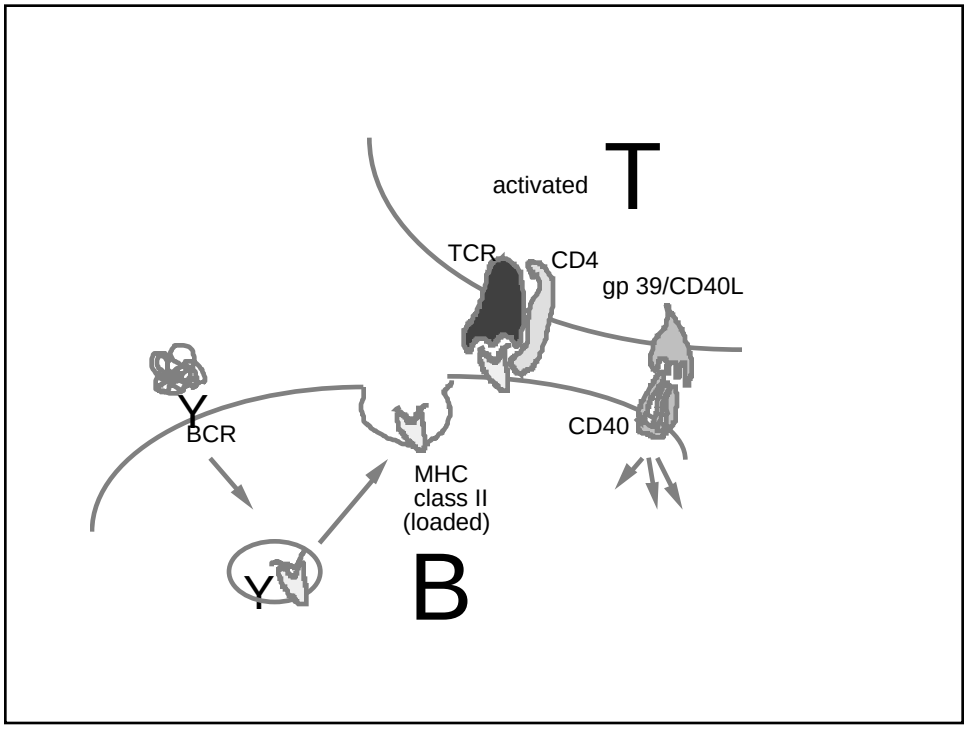
Receptor for EBV

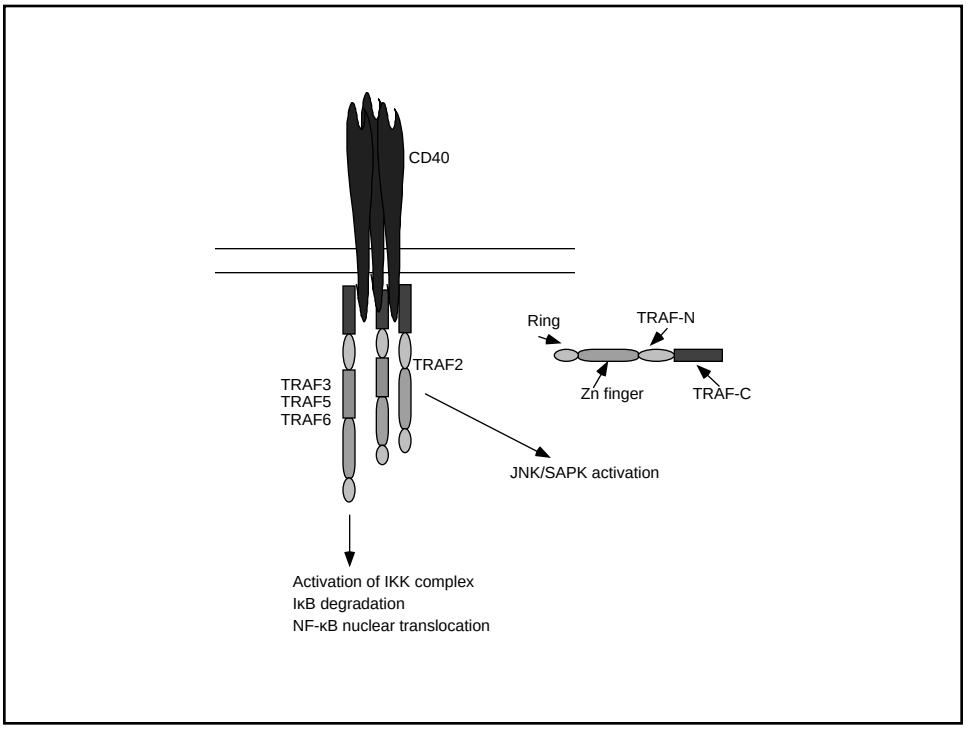


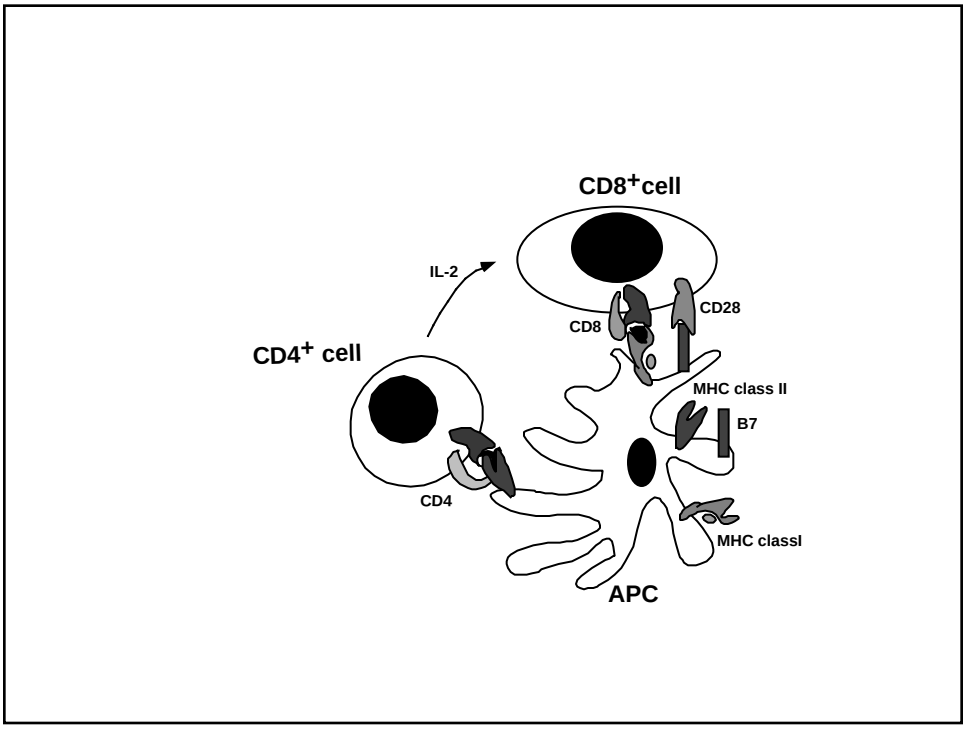
CD22 is a negative regulator of BCR signaling

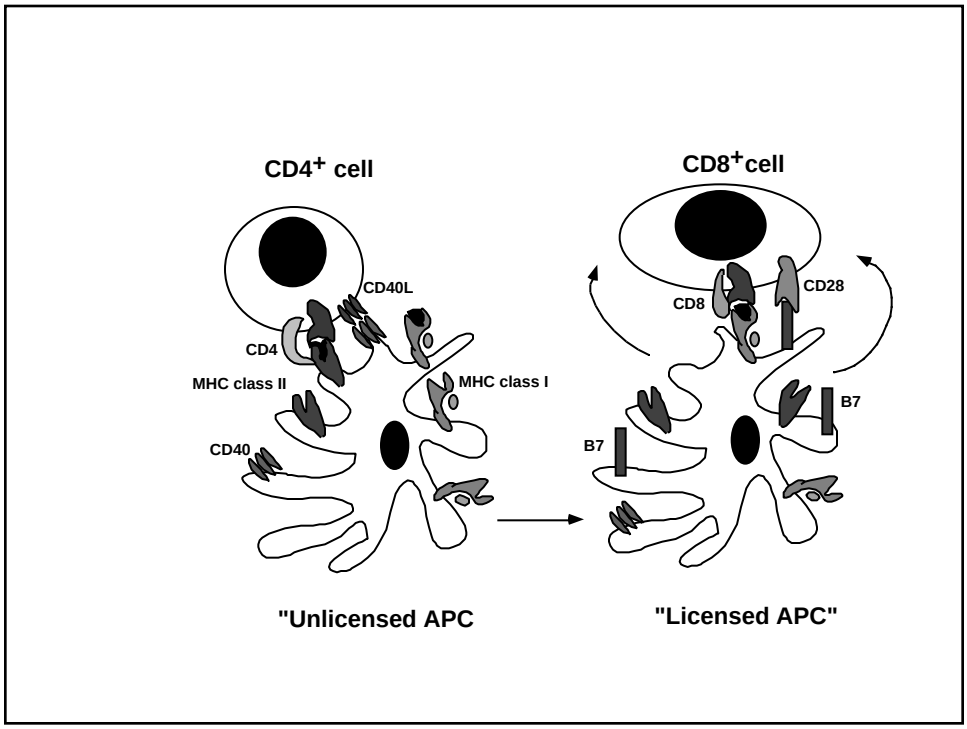



BCR
immune complex
FcγRIIB1
Igβ
Igα
ITAM
ITIM
SHIP
PI3K
Btk, Akt, PLC γ1, etc
Ca++


activated
T
TCR
CD4
gp 39/CD40L
BCR
CD40
MHC
class II
(loaded)
B


CD40
Ring
TRAF-N
TRAF2
Zn finger
TRAF-C
TRAF3
TRAF5
TRAF6
JNK/SAPK activation
Activation of IKK complex
IκB degradation
NF-κB nuclear translocation


CD8+cell
IL-2
CD28
CD8
CD4+ cell
MHC class II
B7
CD4
MHC classI
APC


CD4+ cell
CD8+cell
CD40L
CD4
MHC class II
MHC class I
CD40
CD8
CD28
B7
B7
"Unlicensed APC
"Licensed APC"