- 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?

Choice 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?
NO SIGNAL
monosaccharide
antigen receptor

SIGNAL TRANSDUCTION
polysaccharide

CD4
Lck
TCR
γεδ
ζζ
Fyn
Zap-70

αβ
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.
**Quiescent** APC

- Low levels of B7-2/CD86
- Minimal IL-2 Transcription
- No activation
- Induction of anergy

Diagram:
- T cell
- APC
- CD4
- Co-receptor
- MHC class II
- ITAM
- Signal One
- CD28
- B-7
- ICAM-1
- LFA-1
- CD48/CD58
- MHC class II
- CD4/CD58

Text:
- Outer ring
- 15 nm
- ~40 nm
- TCR
- CD4
- CD2
- CD28
- "Quiescent" APC
- No activation
- Induction of anergy
- Minimal IL-2 Transcription
- Low levels of B7-2/CD86
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

High levels of CTLA-4

Signal One

Signal Two

T cell

Signal One induced by TCR, CD28, and IL-2R signals

Signal Two

High levels of CTLA-4 induced by TCR, CD28, and IL-2R signals

CD4 coreceptor

MHC class-II

ITAM

CD28

CTLA-4

T cell

Signal One

Signal Two

CD4

TCR

CD28

Lck

Fyn

ZAP-70

Grb2

SOS

Racl/Rac/ERK

Calcineurin

Cytoplasmic NFAT

Nuclear NFAT

Fos/Jun (AP-1)

(Repressor of IL-2 transcription)

DAG

IP3

Ca++

Calcineurin (phosphatase)

PLC-γ

Cleaves PIP2

Cytoplasmic NFAT

Ras/Raf/ERK

Nuclear NFAT

Fos/Jun (AP-1)

(Repressor of IL-2 transcription)

Rac/SEK/JNK

IL-2 transcription

Grb2

SOS

Racl

ERK

JNK

IL-2 transcription

PI3 Kinase

Vav

Rac/SEK/JNK

IL-2 transcription
**ACTIVATED**

- NFAT
- AP-1
- Oct-1
- \( \kappa B \)
- c-Rel
- \( \kappa B \)
- p65
- HMGI/Y
- Egr-1/
- SP-1

**ANERGIZED**

- NF-AT
- AP-1
- Oct-1
- \( \kappa B \)
- c-Rel
- HMGI/Y
- Egr-1/
- SP-1

**Naive CD4**

- TCR
- No Activation
- Anergy

- Activation induced cell death

- Effector
- Restimulation
- Chronic stimulation
- and phenotypic differentiation

- Th1
- Th2

- IL-12
- IL-18
- IL-4

- No restimulation

- Memory

- IL-12
- IL-18

- 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

c-Raf

ERK activation

p120GAP

Neurofibromin
(RasGAPs)
1. Crosslink receptor
2. Activate tyrosine kinase
3. Phosphorylate tyrosines on ITAMs and other adaptors
4. Recruit signal effectors such as GEFs
5. Convert G protein from inactive GDP bound form to active GTP bound form
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
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 translation stability
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 → Jak → SH2 domain → STAT → STAT dimer → Transcription of target genes → STAT site → Access for V(D)J recombination → PI3Kinase → Survival/proliferation

IL-7Rα CC CC γc CC CC WLRWS → Src family kinases → Jak3 → IL-7Rα CC CC γc CC CC WLRWS → Entry into pro-T cells
\( \gamma_c \) and X-linked SCID

\( \gamma_c \) or the common gamma chain is a component of the IL-2, IL-4, IL-7, IL-9, and IL-15 receptors

The \( \gamma_c \) gene is encoded on the X-chromosome
Activated B and Th2 cells

IL-12Rβ1
(binding subunit in mouse; low affinity receptor in man)

Th1 cells

High affinity receptor

IL-12Rβ1

IL-12Rβ2
(signaling subunit)

Jak2

Tyk2

STAT 4 dimer

Src-family kinase

ITAM
Igα Igβ ITAM

Inactive Syk

Activated Syk

PI3K generates PIP3

SH3 SH2 KINASE

Y551 PR

Activation segment tyrosine phosphorylated by Src family kinases

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
ITAM

Ca++

γY

γY

ITIM

SHIP

FcγRIIB1

B

BCR

activated T

gp 39/CD40L

CD4

TCR

gp 39/CD40L

MHC class II (loaded)

BCR

CD40

T

B

ITIM

PI3K

Sik, Akt, PLC γ1, etc

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

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

CD40L
CD4
MHC class II
CD40

"Unlicensed APC"

CD8+ cell

CD8
CD28
B7
MHC class I

"Licensed APC"