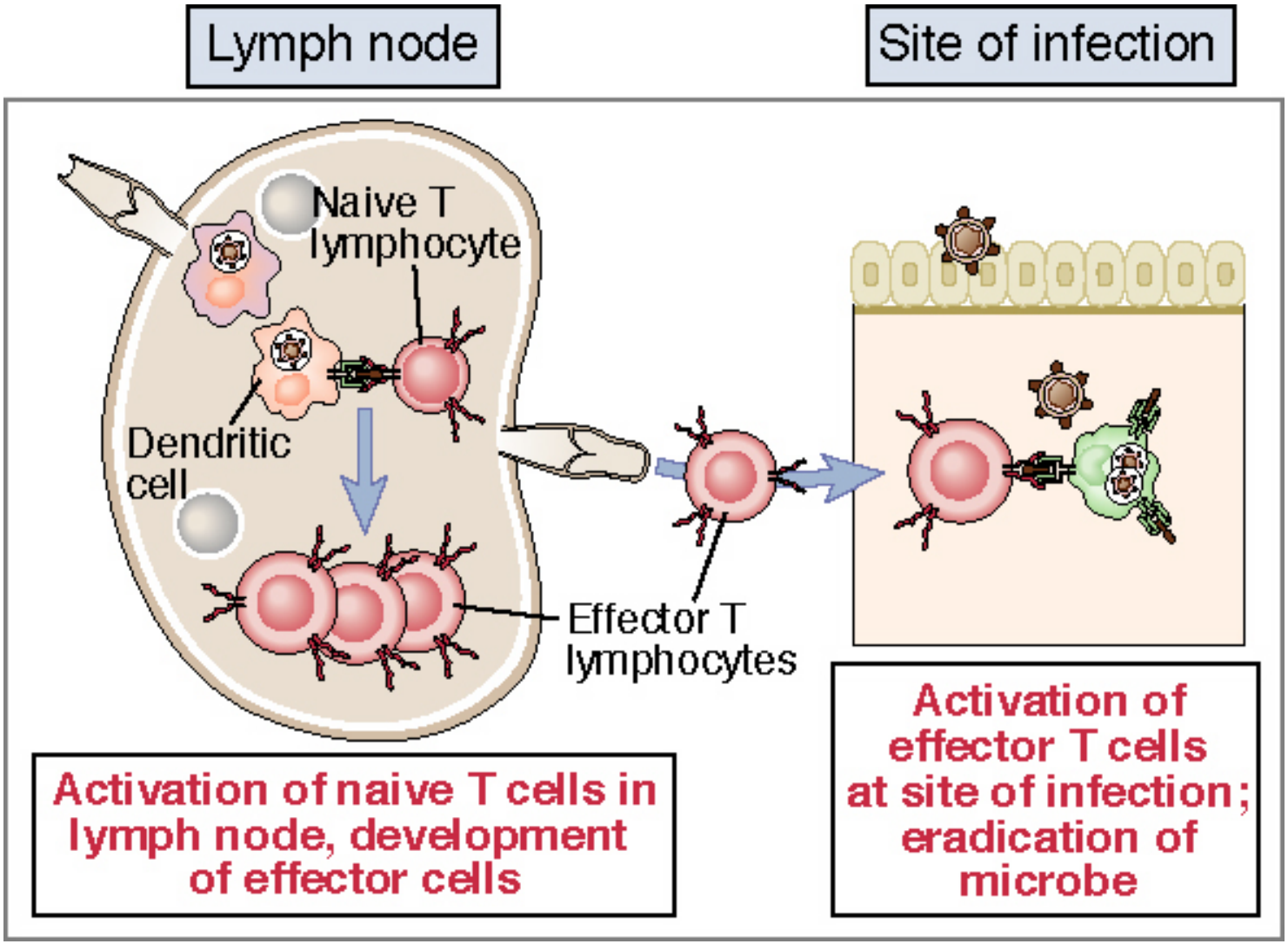
# Lecture: T Cell Activation and Regulation

Mark Anderson, MD,PhD
UCSF Diabetes Center
manderson@diabetes.ucsf.edu
415-502-8052

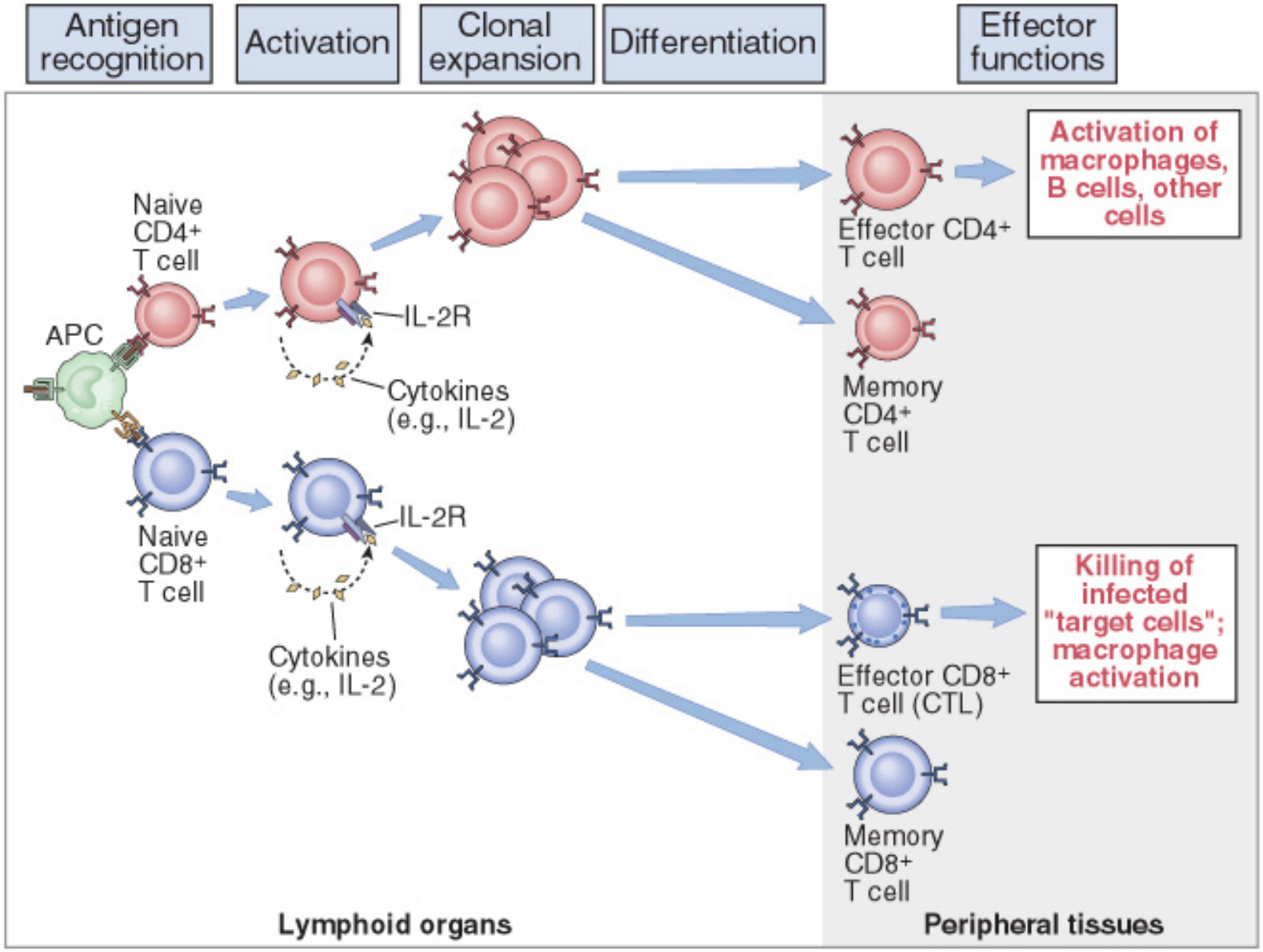
# Lecture Overview

- Anatomical concerns
- "The rules of engagement"
  - T cell activation requires more than the generation of foreign peptide-self MHC complexes on APC's…..
- T cell signaling
- Two signal model and co-stimulation (bulk of the lecture)
- Putting it all together

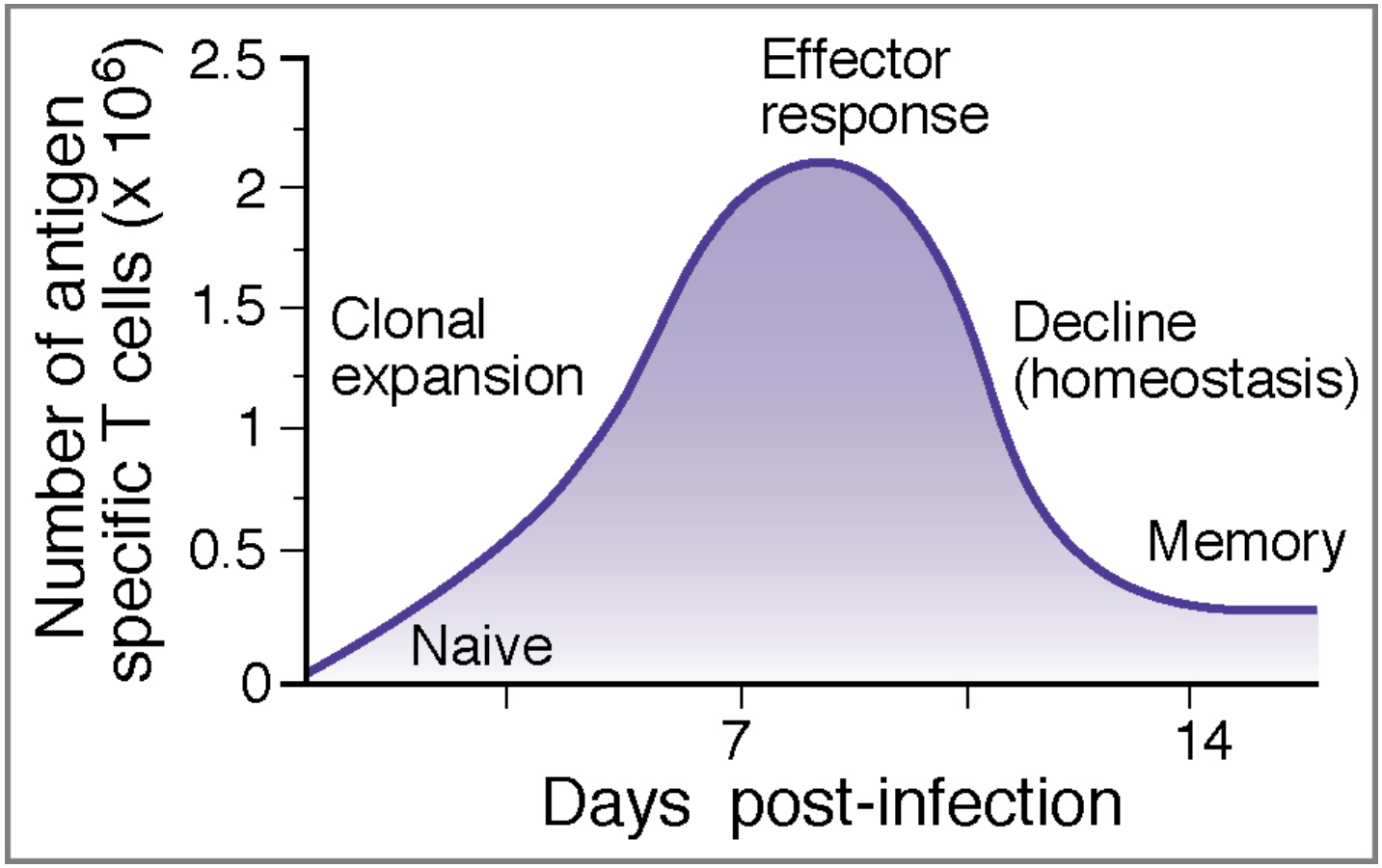
# Functional responses of T lymphocytes



**Steps in the activation of T lymphocytes.**



# Kinetics of a T cell response

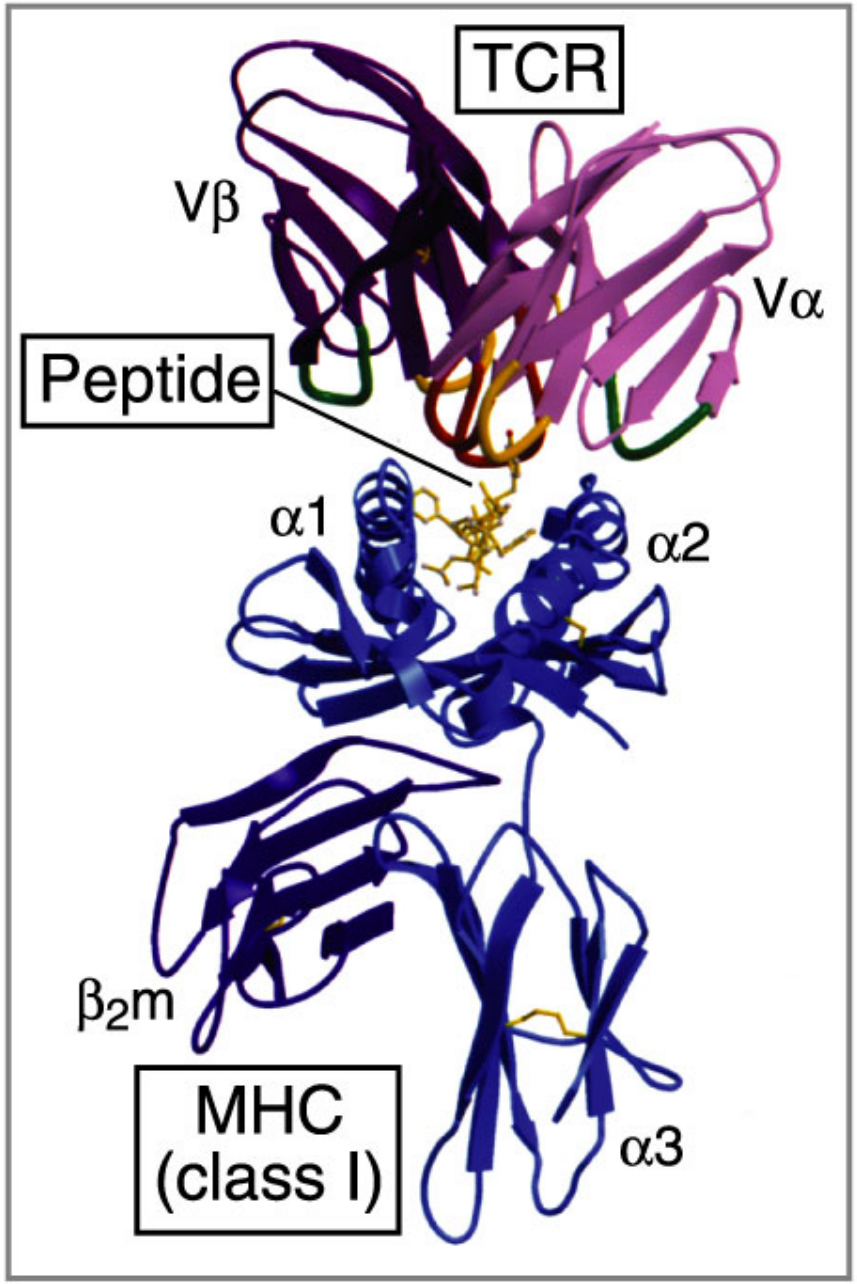



From: Abbas & Lichtman, Cellular & Molecular Immunology, W. B. Saunders, 2003

# Signals for T cell activation

- **Antigen recognition**
  - Regulated movement of signaling receptors and adhesion molecules at (immune synapse)
- **Costimulators** (second signals)
- **Cytokines**
  - Produced by APCs or T cells
  - Stimulate T cell expansion and differentiation into effector cells

**The recognition of a peptide-MHC complex by a T cell antigen receptor.**



# Antigen recognition by T cells

- Each T cell sees an MHC molecule and bound peptide
  - Dual recognition determines specificity and MHC restriction
- Each T cell sees very few (1-3) residues of the peptide antigen
  - T cells distinguish between diverse microbes based on recognition of few amino-acids
- The affinity of TCR-antigen interactions is low
  - Kd on the order of $10^{-5}$ to $10^{-6}$
  - Because T cells are selected by recognition of self MHC in the thymus (the only MHC they can encounter during their lives)
  - T cell-APC contacts need to be stabilized by other molecules
- The activation of T cells may require multiple or prolonged TCR-antigen interactions
  - T cell receptors and signaling proteins assemble in the synapse

# T cells first "stick" to APC's using cell adhesion molecules

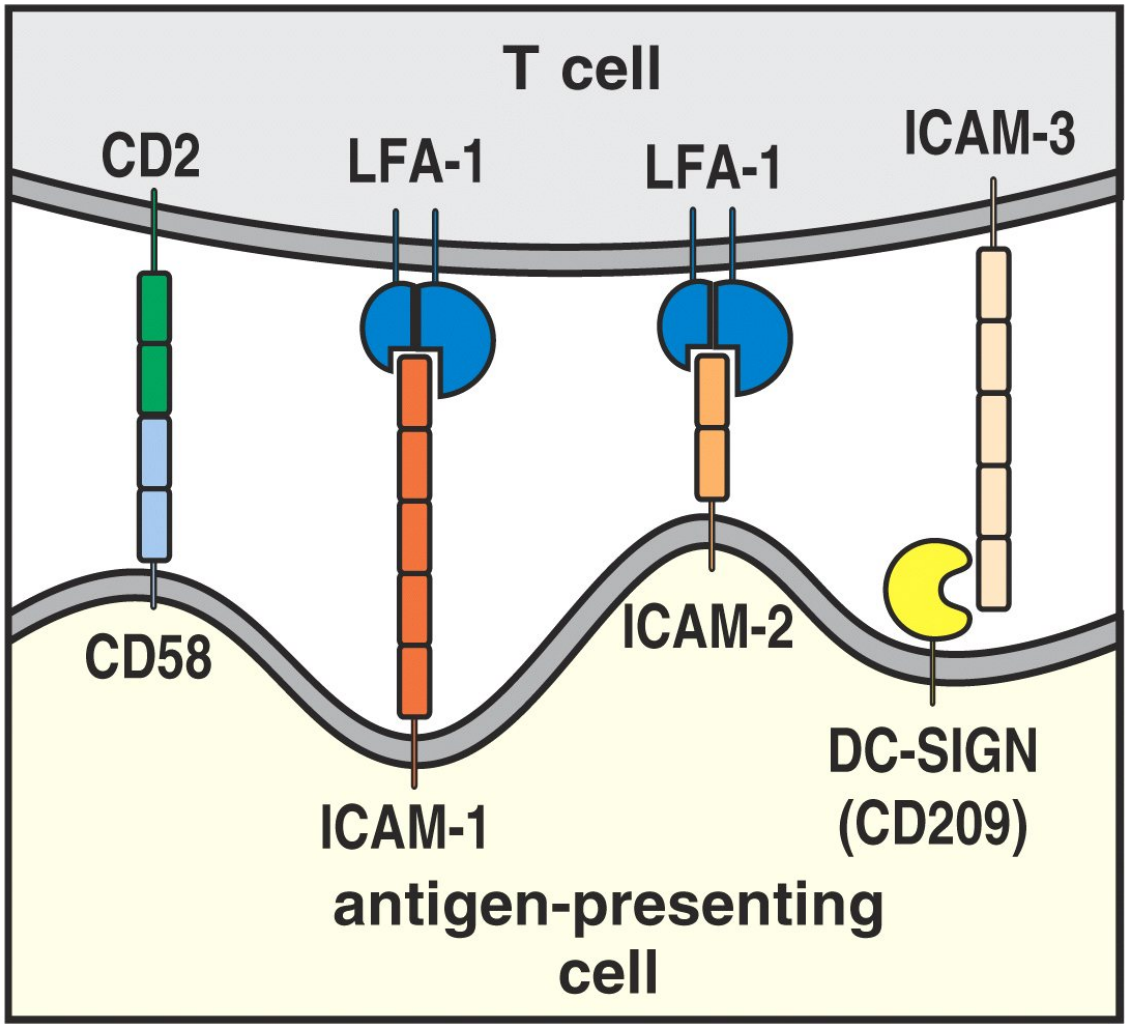
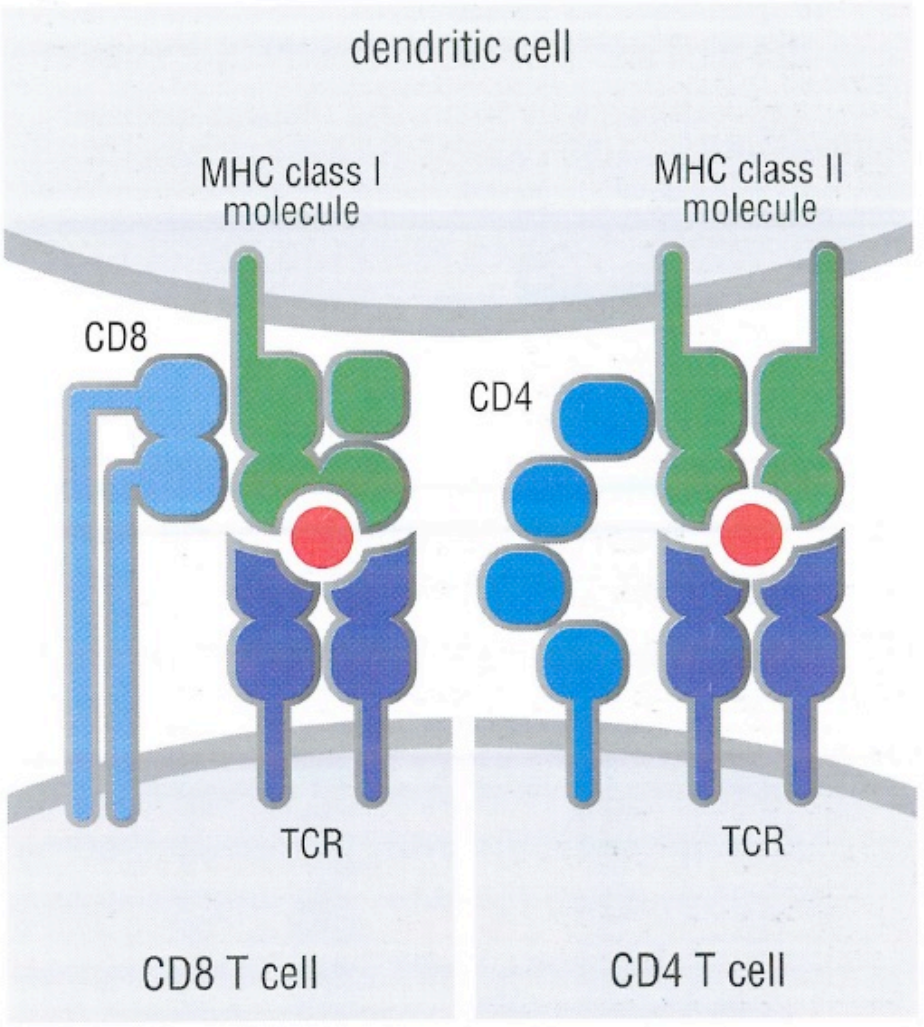
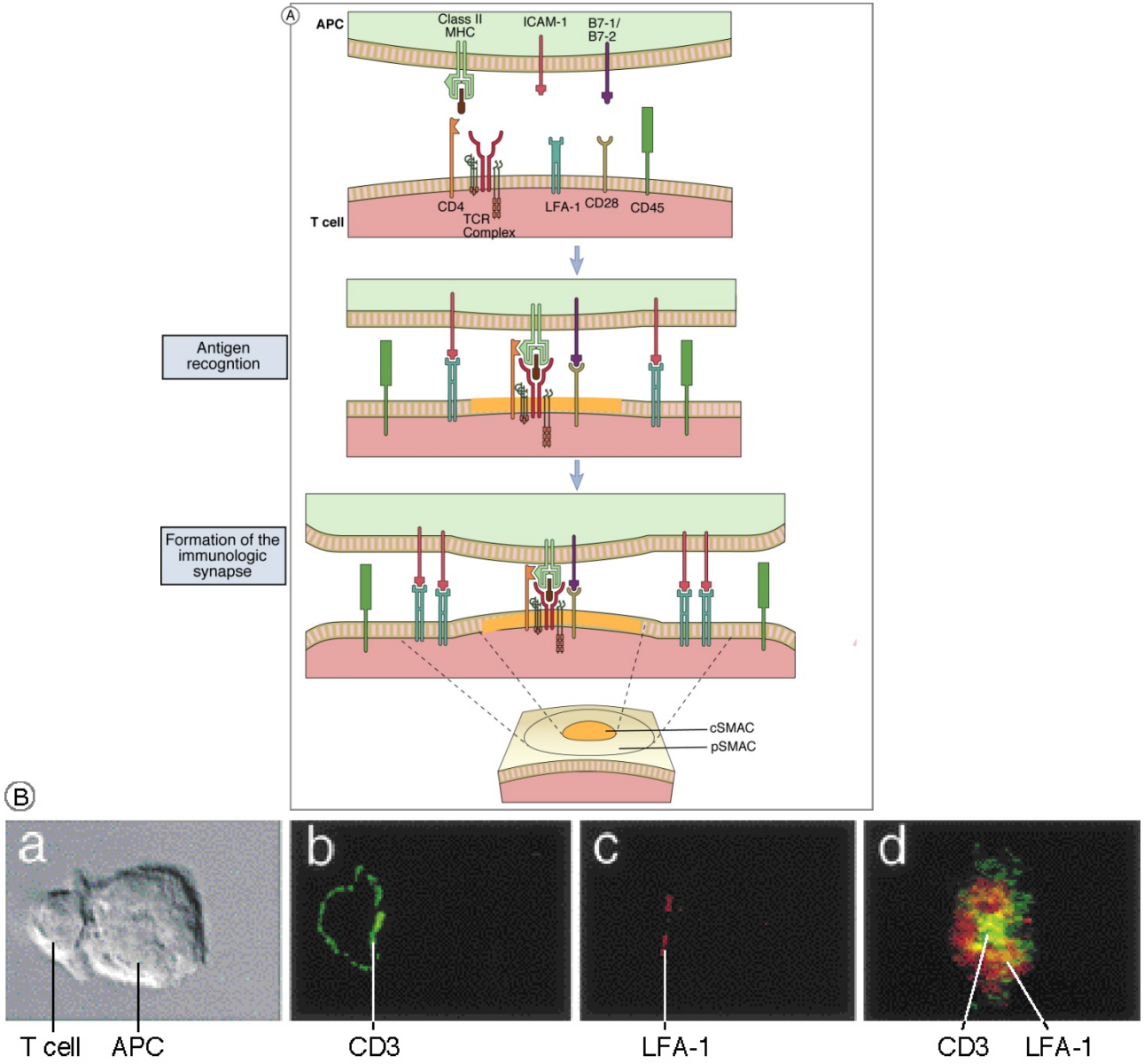



Figure 8-8 Immunobiology, 6/e. (© Garland Science 2005)

# T cells use co-receptors for antigen recognition



# Formation of the immunological synapse

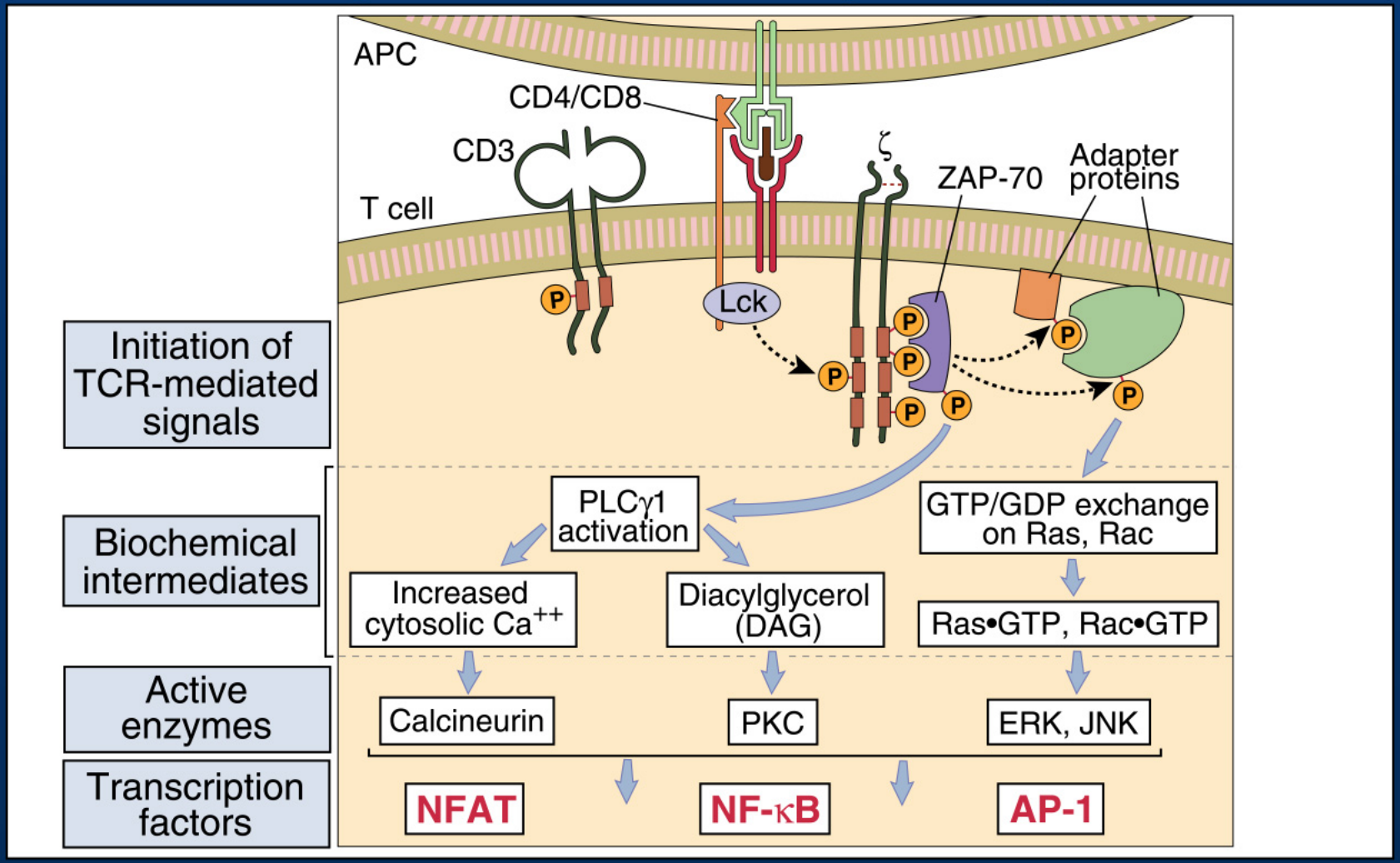



*Regulated way of bringing together key signaling molecules*

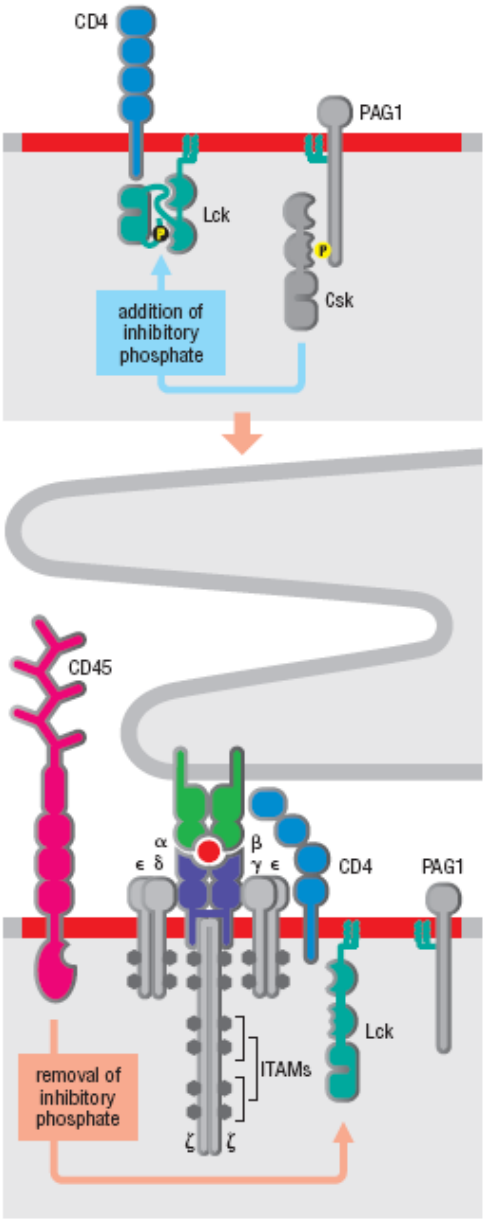
# Functions of the immune synapse

- Promote signaling
- Terminate signaling: recruitment of phosphatases, ubiquitin ligases, inhibitory receptors to the site of the TCR complex
- Direct effector molecules to the relevant target: cytokines, CD40L, perforin, etc

# T cell receptor-induced signal transduction pathways



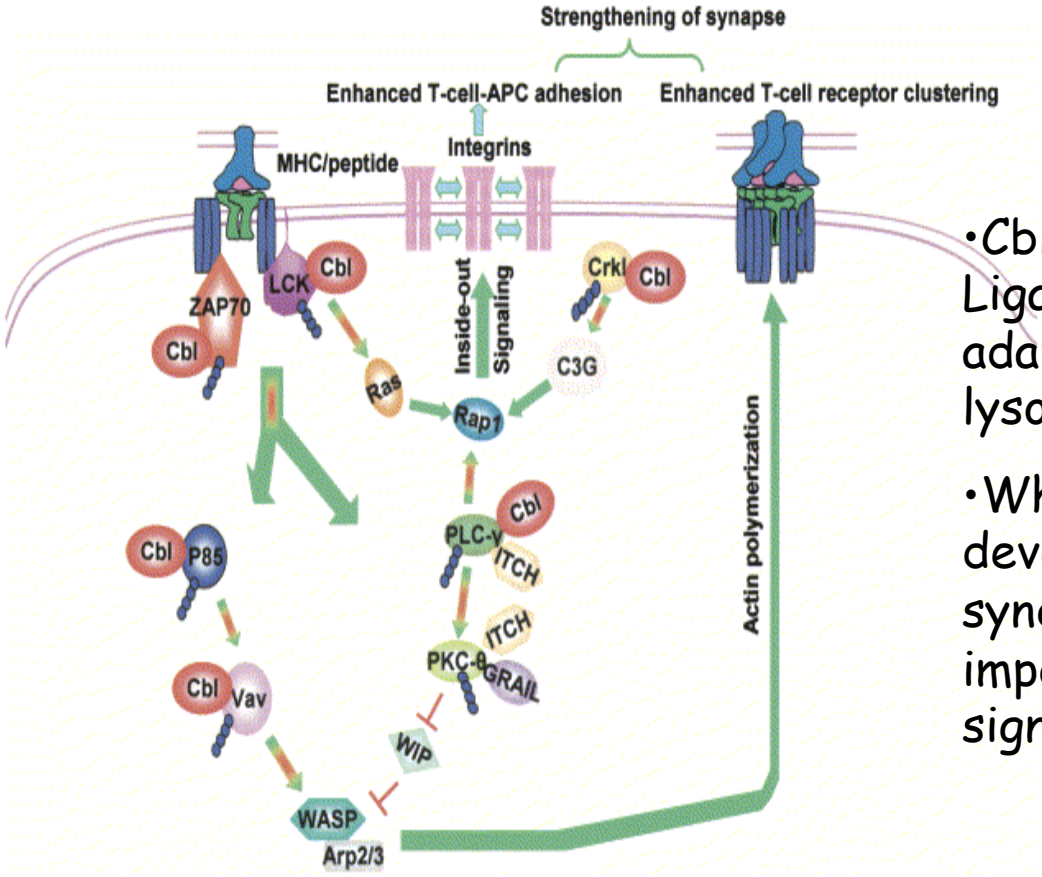

From Abbas, Lichtman, & Pober: Cellular and Molecular Immunology. W.B. Saunders, 1999, Fig. 8-8




# TCR signalling is dynamically regulated

- Csk and CD45 are continually phosphorylating and dephosphorylating Lck
- Phosphorylation of Lck inhibits its activation acitivity
- When TCR stimulation occurs PAG1 is dephosphorylated and Csk is released thus removing the inhibitory phosphorylation of Lck

# TCR signalling is dynamically regulated (cont).




- Cbl family proteins are Ubiquitin Ligases that tag phosphorylated adaptors for destruction in the lysosome
- When Cbl-b is knocked out, mice develop a severe autoimmune syndrome highlighting the importance of the termination of signaling

# Initial responses to activation

- #1 rule- key cytokine the T cell needs to make is IL-2
- Proliferation. Mostly dependent on IL-2 through an autocrine pathway.
- Other cytokines, cytokine receptors will also get produced and lead to effector T cell development (lecture upcoming…)

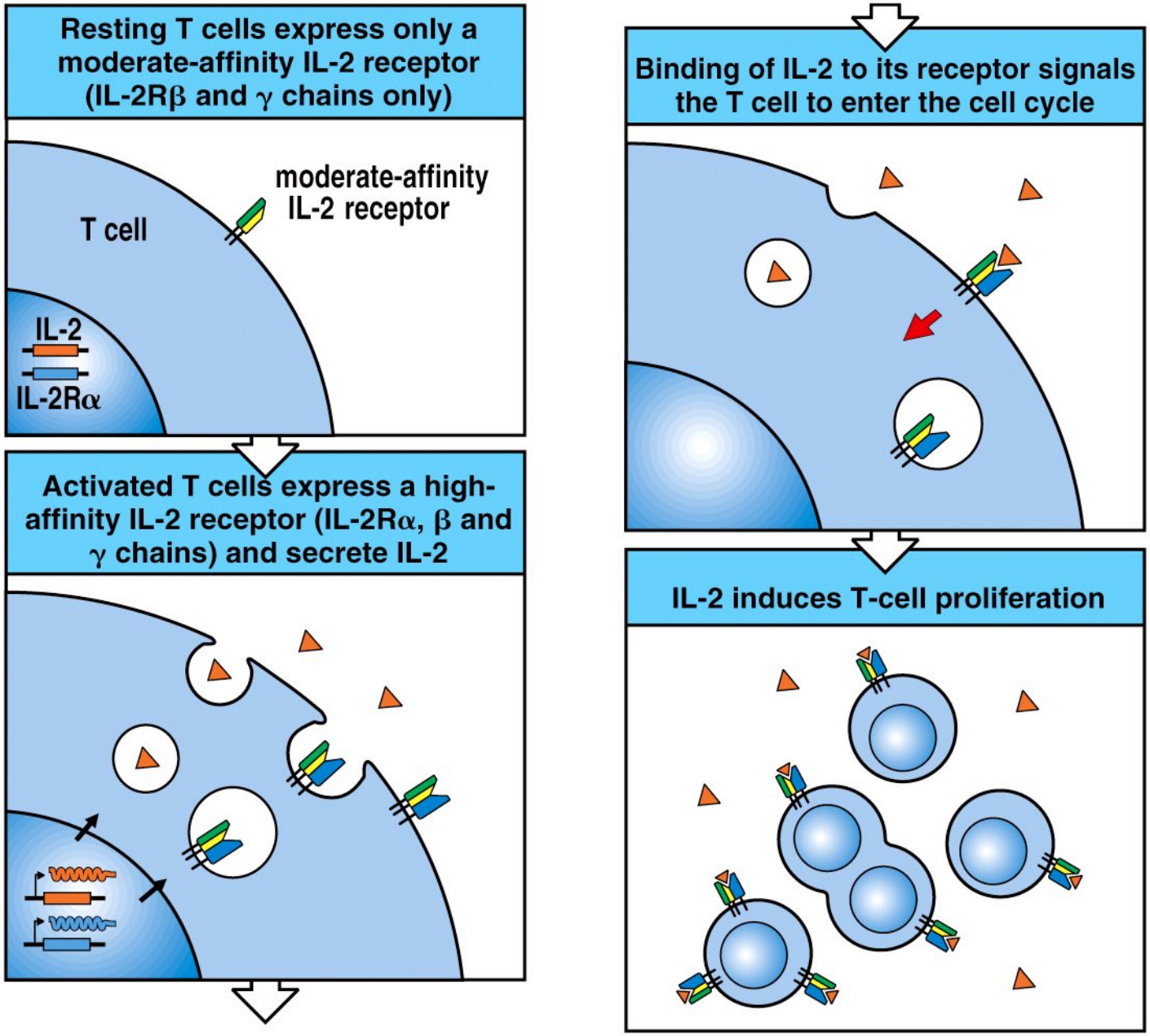
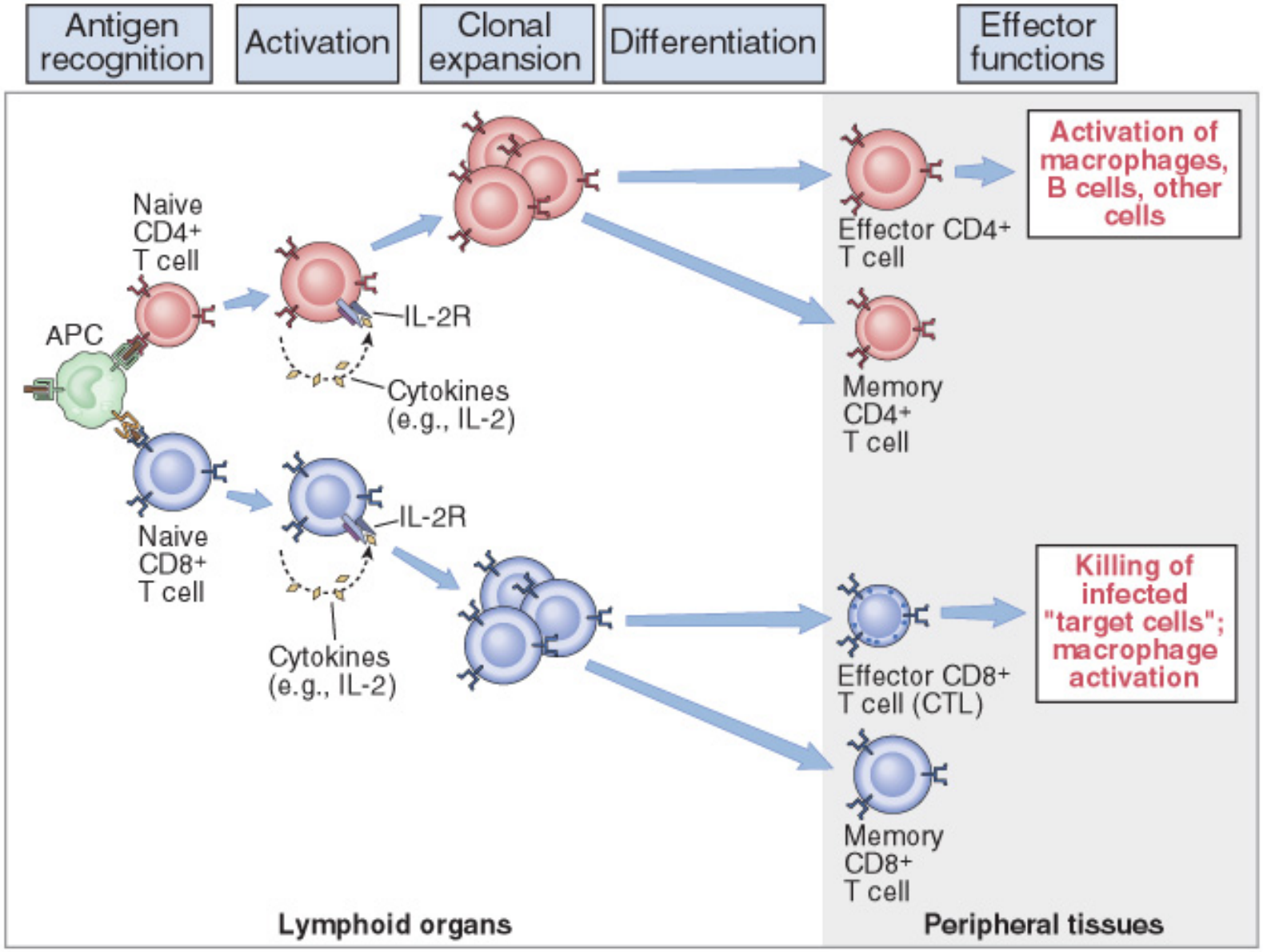
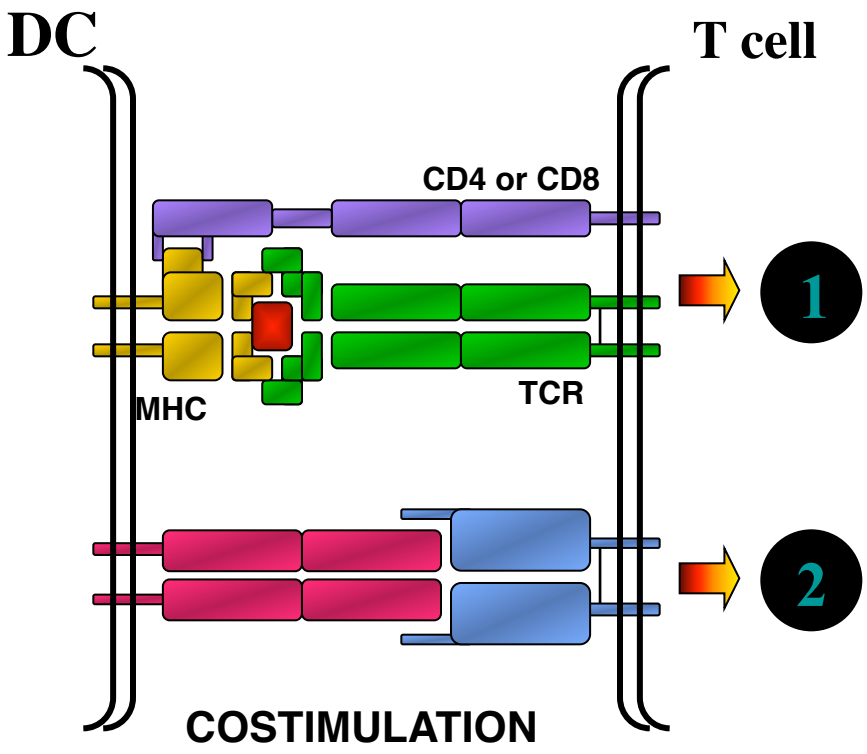



Figure 8-20 Immunobiology, 6/e. (© Garland Science 2005)

**Steps in the activation of T lymphocytes.**



# The Two-Signal Model of T-cell Activation




**1+2=Full activation**

# Two signal requirement for lymphocyte activation

- Naïve lymphocytes need two signals to initiate responses

- Signal 1: antigen recognition
  - Ensures that the response is antigen-specific

- Signal 2: microbes or substances produced during innate immune responses to microbes
  - Ensures that the immune system responds to microbes and not to harmless antigenic substances
  - Second signals for T cells are "costimulators" on APCs and cytokines produced by APCs

# The Experimental Evidence of Co-stimulation

**Marc Jenkins and Ronald Schwartz in the late 80's :**
**The first definitive experimental demonstration that TCR engagement alone was insufficient for T cell activation.**

**Proliferative response of T cell clones**
**(pigeon cytochrome c peptide 81-104 presented by I-$E^k$)**
**to normal or ECDI(chemical crosslinker)-fixed peptide-pulsed APCs**

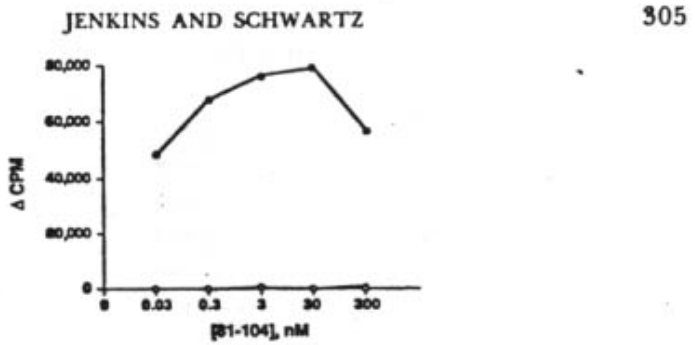
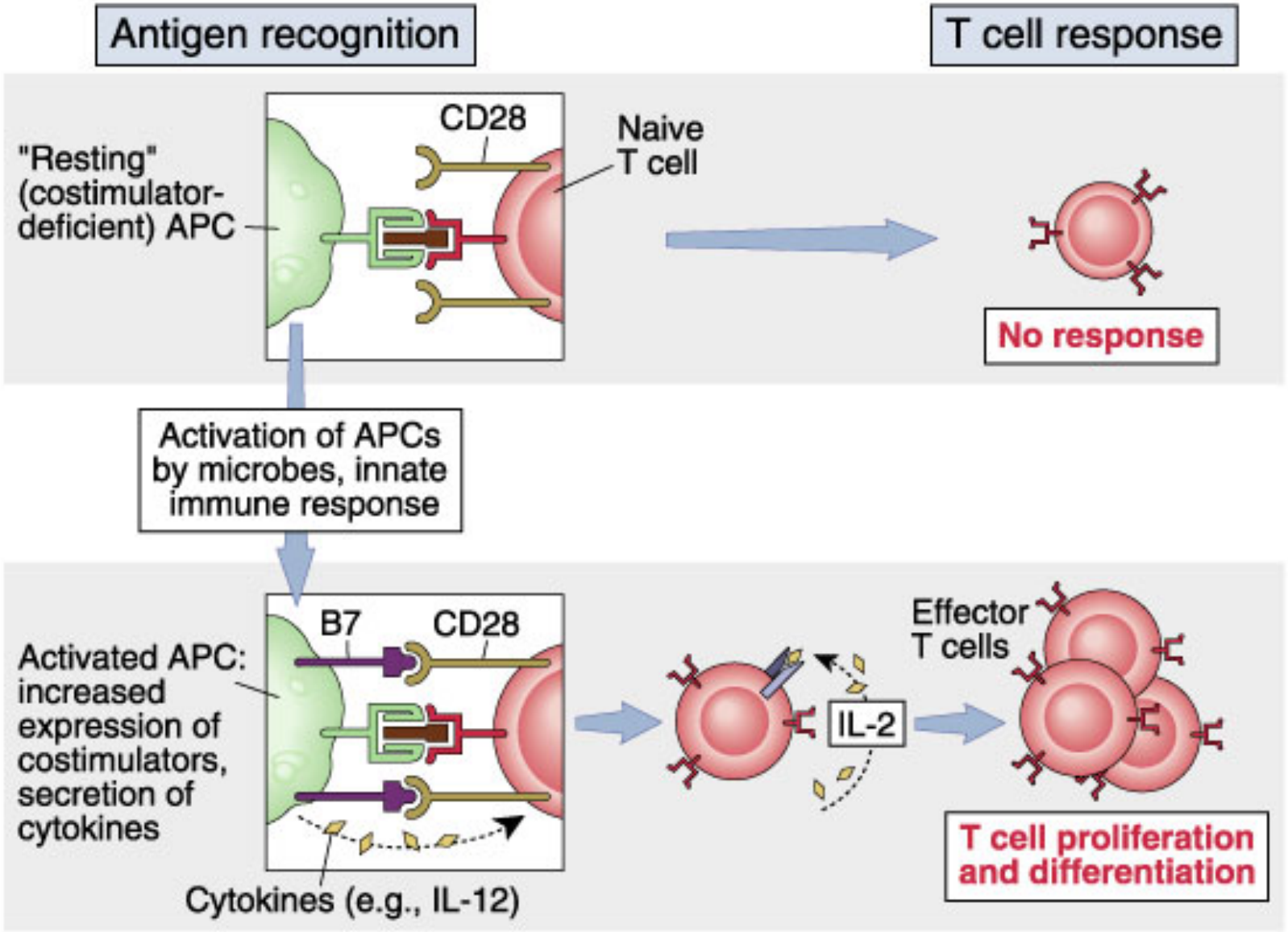



FIGURE 1. ECDI-treated splenocytes fail to stimulate proliferation by a normal T cell clone. $5 \times 10^5$ ECDI-treated (*open circles*) or 3,000 rad irradiated normal (*filled circles*) B10.A splenocytes were cultured with $2 \times 10^4$ F1.A.2 normal cloned T cells and the indicated doses of pigeon fragment 81-104. Proliferation by F1.A.2 was determined by [$^3$H]thymidine incorporation with the results expressed as Δ cpm.

*Jenkins M.K., and Schwartz R.H. J. Exp. Med. 165:302-319, 1987.*

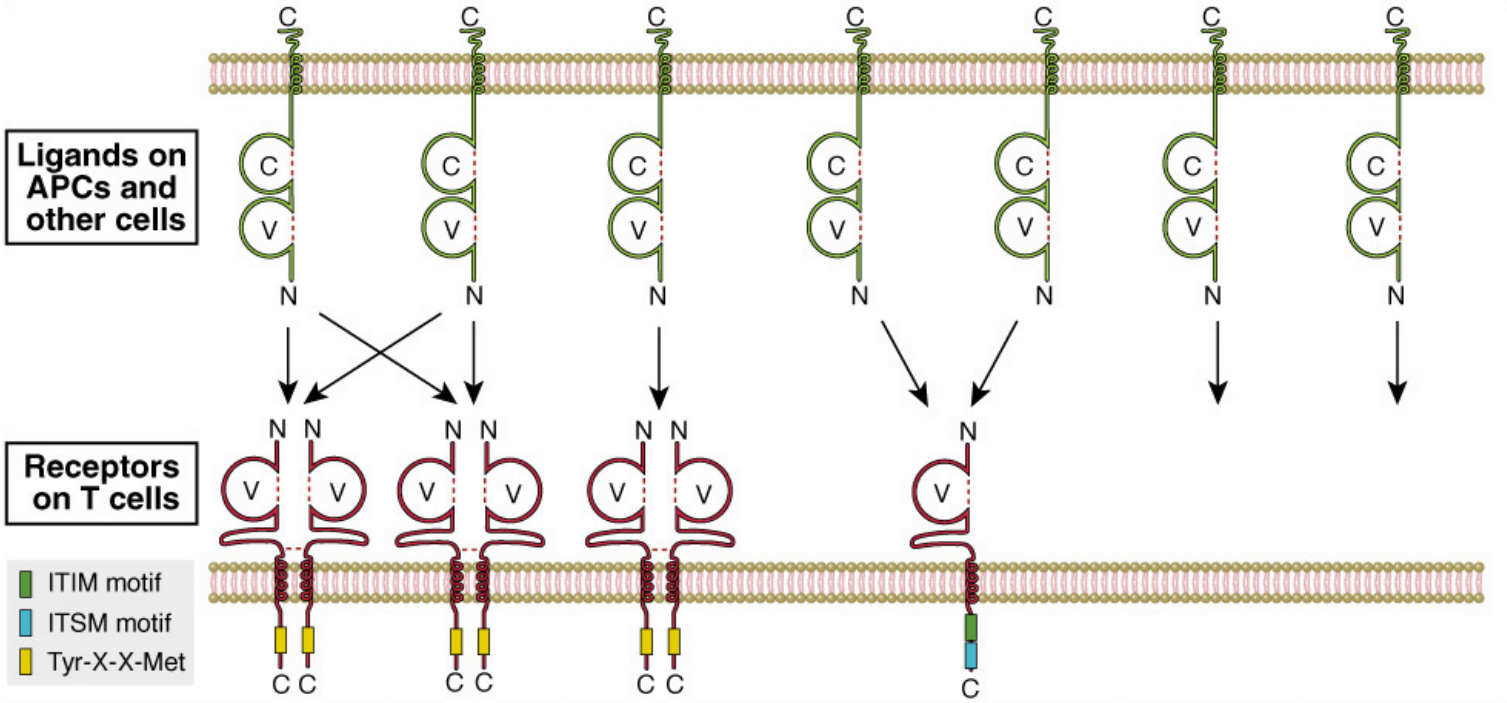
**ECDI-treated APCs fail to stimulate proliferation by normal T cell clones :**
**Not the result of extensive modification of the MHC class II molecule**
**ECDI treatment inactivated an accessory (costimulatory) function of the APC**

# The role of costimulation in T cell activation.



# The B7:CD28 families

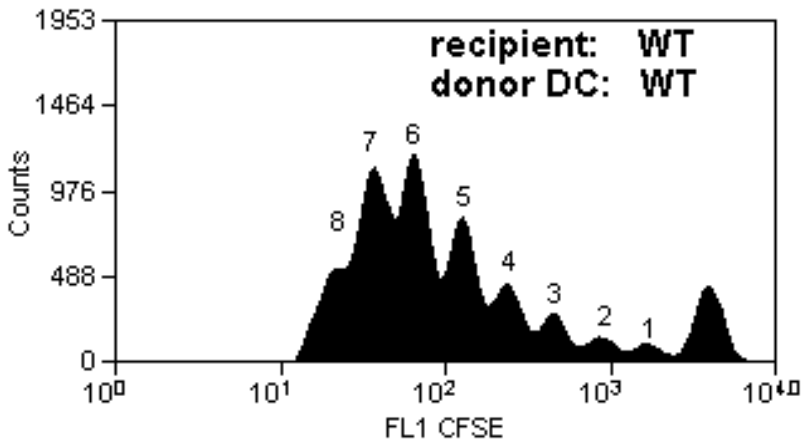
| Expression | DCs, macrophages, B cells | DCs, macrophages, B cells | DCs, macrophages, B cells, other cells | DCs, macrophages, B cells, other cells | | DCs, lymphoid and nonlymphoid cells | DCs, lymphoid and nonlymphoid cells |
|---|---|---|---|---|---|---|---|
| **Name** | **B7-1 (CD80)** | **B7-2 (CD86)** | **ICOS-L** | **PD-L1 (B7-H1)** | **PD-L2 (B7-DC)** | **B7-H3** | **B7-H4** |

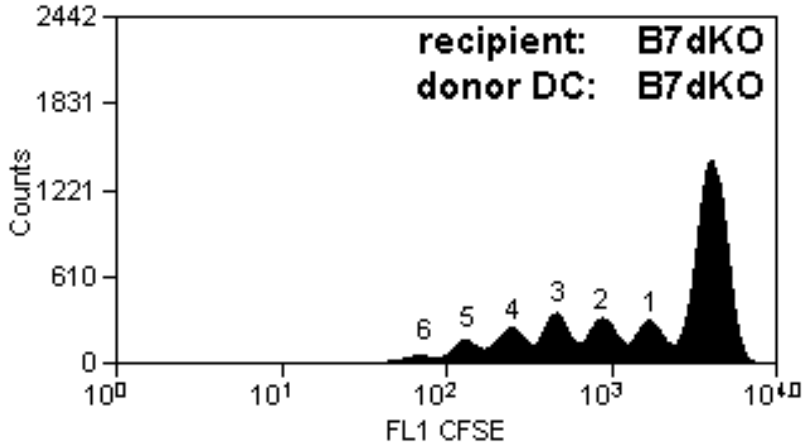



| Name | CD28 | CTLA-4 | ICOS | PD-1 | ? | ? |
|---|---|---|---|---|---|---|
| **Expression** | T cells; constitutive | T cells; inducible | T cells; inducible | T cells, B cells, Myeloid cells; inducible | | |
| **Major function** | Costimulation of naive T cells; generation of regulatory T cells | Negative regulation of immune responses; self-tolerance | Costimulation of effector T cells | Negative regulation of T cells | Costimulation or negative regulation of T cells | Negative regulation of T cells |

From Abbas, Lichtman and Pillai. Cellular and Molecular Immunology 6th edition, Elsevier, 2007

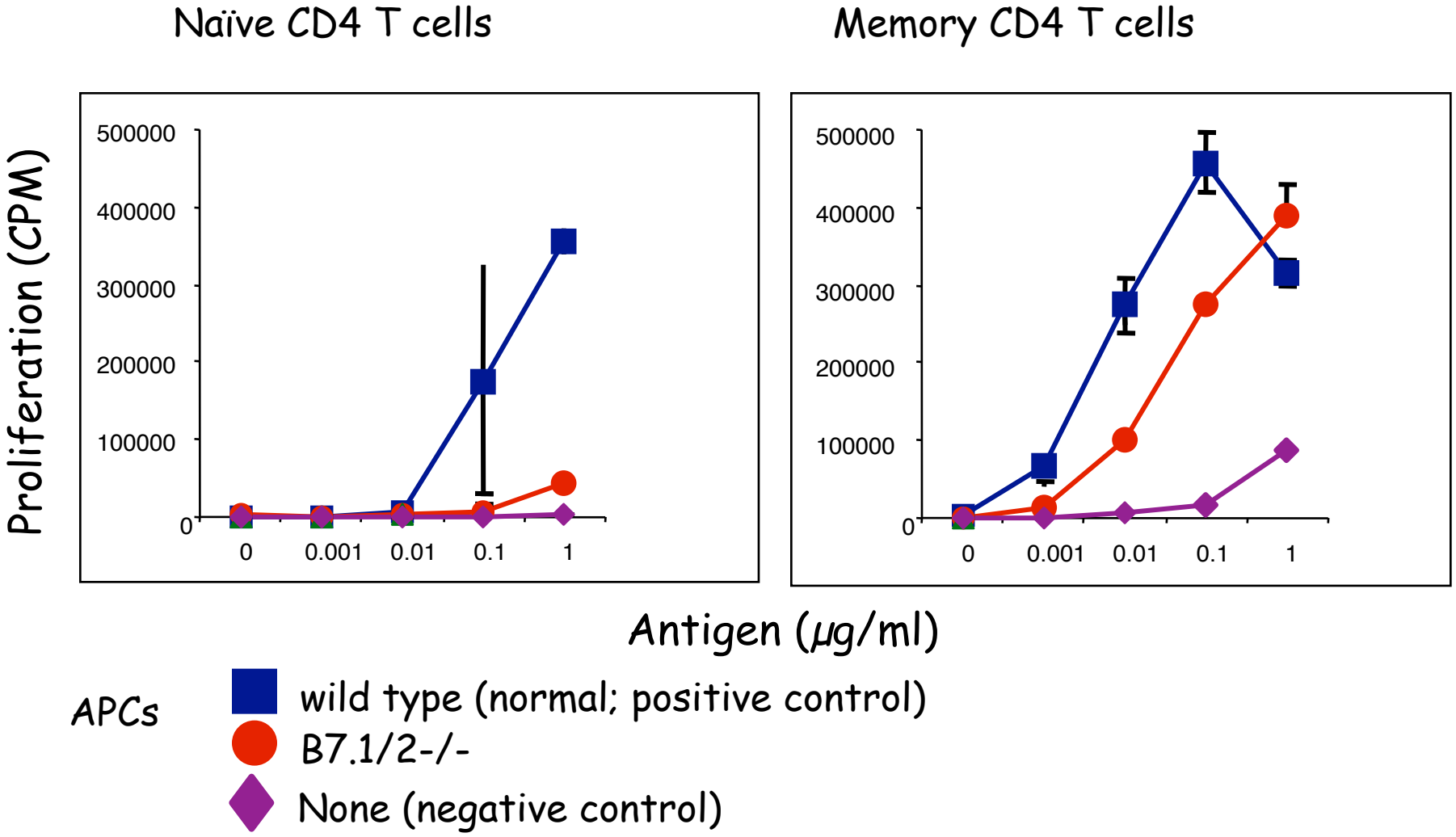
# Activation of T cells by peptide-pulsed DCs in vivo: requirement for B7




*DO11 T cells (Ova-specific TCR transgenic) labeled with CFSE and transferred into normal or B7-knockout recipients ----> immunized with Ova peptide-pulsed cultured dendritic cells from normal or B7-knockout recipients ---> response of DO11 cells assayed*

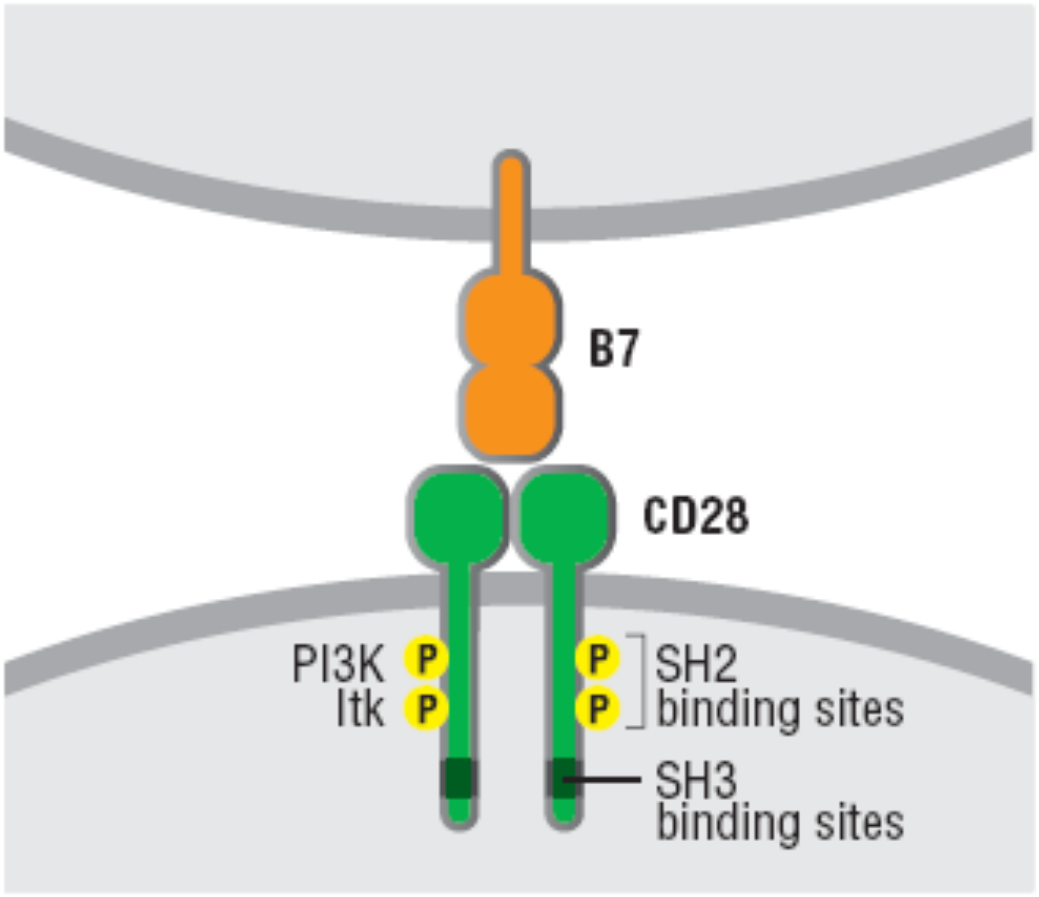
# Memory cells are less dependent on B7 costimulation than are naïve T cells



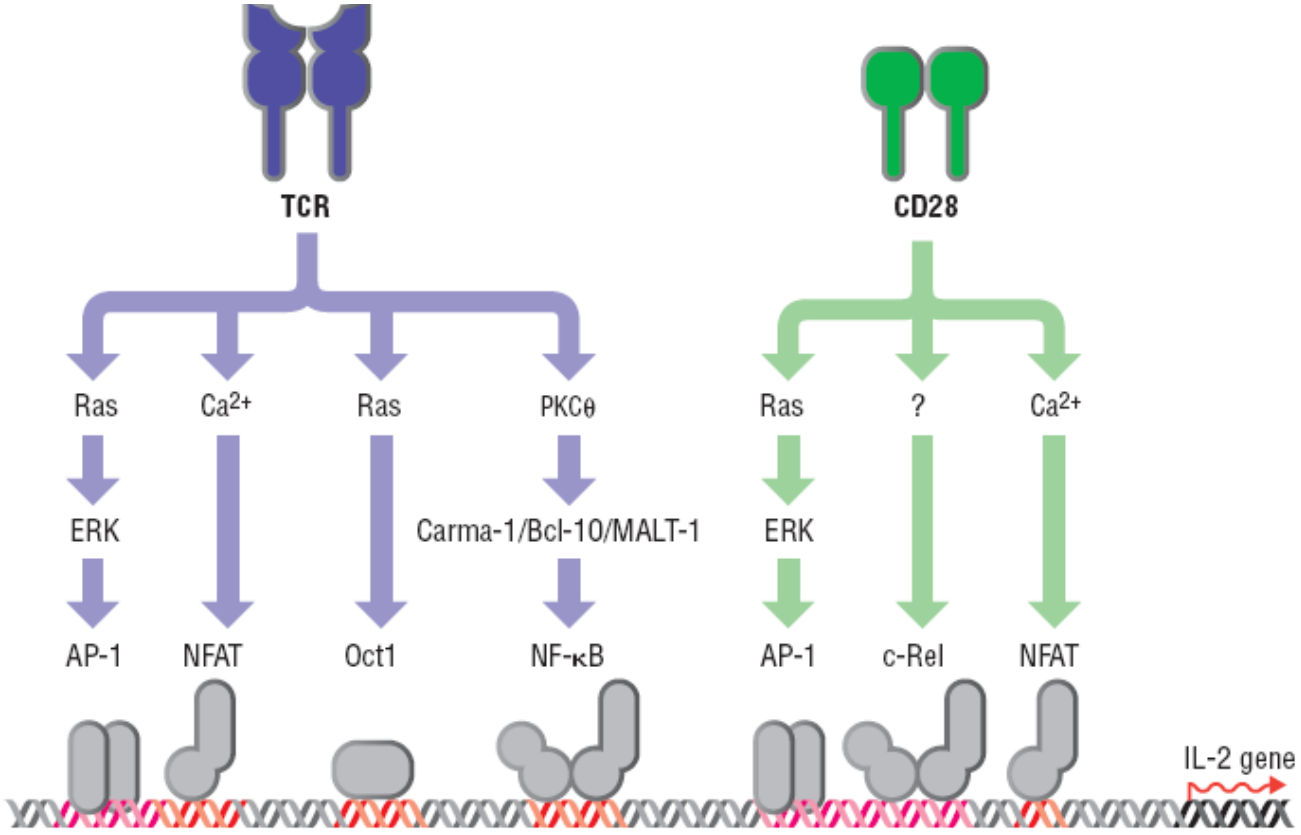
# B7:CD28 dependence of T cells

- Initiation of T cell responses requires B7:CD28
- Dependence on B7-CD28:
  - Naïve > Th1 > Th2 > memory
  - CD4 > CD8
  - Regulatory T cells

# CD28 Signals through its cytoplasmic tail SH2-binding sites. A major downstream signaling enzyme is PI3 kinase.



# TCR and CD28 Signaling cooperate to help promote IL-2 production



# Major effects of CD28-mediated costimulation in T cells

**Proliferation**
IL-2 (transcription, mRNA stabilization)
IL-2R up-regulation
↑G1 cell cycle kinases
↓Cell cycle inhibitor p27Kip

**Survival**
Bcl-xL

**Effector function**
↑ CD40-L, OX-40, 41BB, ICOS
↑ cytokines expression
↑ cytotoxic molecules

The major effects of CD28-mediated costimulation are to augment and sustain T cell responses initiated by antigen receptor signal by promoting T-cell survival and enabling cytokines to initiate T cell clonal expansion and differentiation.

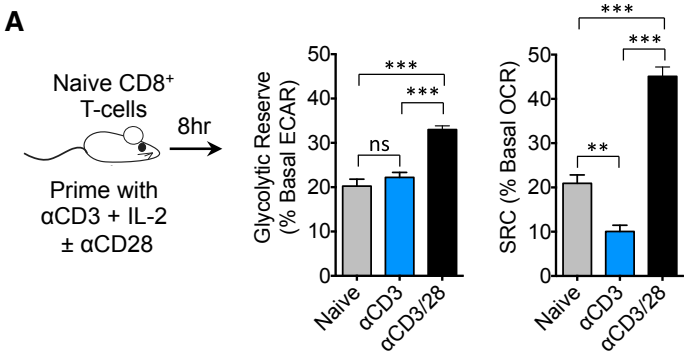
# Major effects of CD28-mediated costimulation in T cells

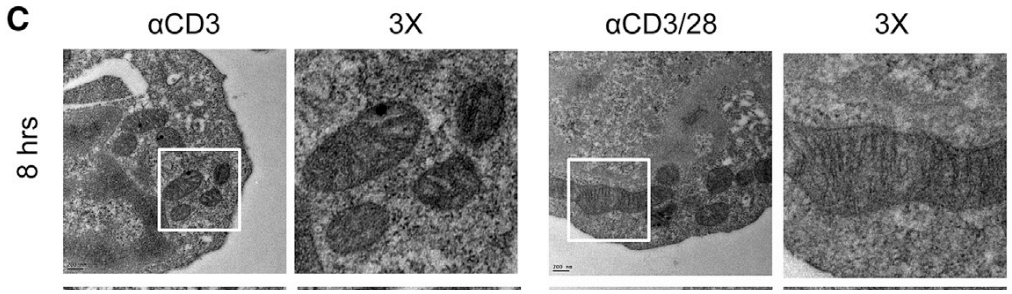
## Mitochondrial Priming by CD28

Cell *171*, 385–397, October 5, 2017 © 2017 Elsevier Inc.

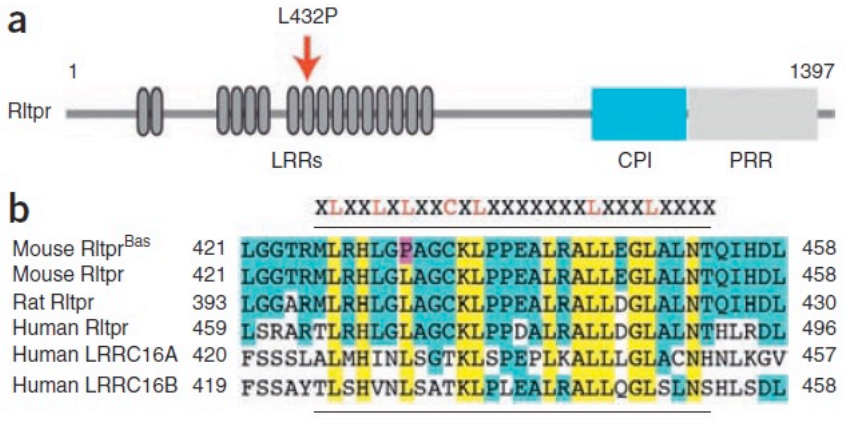
Ramon I. Klein Geltink,[1] David O'Sullivan,[1] Mauro Corrado,[1] Anna Bremser,[2,3] Michael D. Buck,[1] Joerg M. Buescher,[1] Elke Firat,[4] Xuekai Zhu,[5] Gabriele Niedermann,[4,6] George Caputa,[1] Beth Kelly,[1] Ursula Warthorst,[2] Anne Rensing-Ehl,[2] Ryan L. Kyle,[1] Lana Vandersarren,[7,8] Jonathan D. Curtis,[1] Annette E. Patterson,[1] Simon Lawless,[1] Katarzyna Grzes,[1] Jing Qiu,[1] David E. Sanin,[1] Oliver Kretz,[9,10] Tobias B. Huber,[10,11,12] Sophie Janssens,[7,8] Bart N. Lambrecht,[7,8] Angelika S. Rambold,[2,3] Edward J. Pearce,[1,13] and Erika L. Pearce[1,14,*]

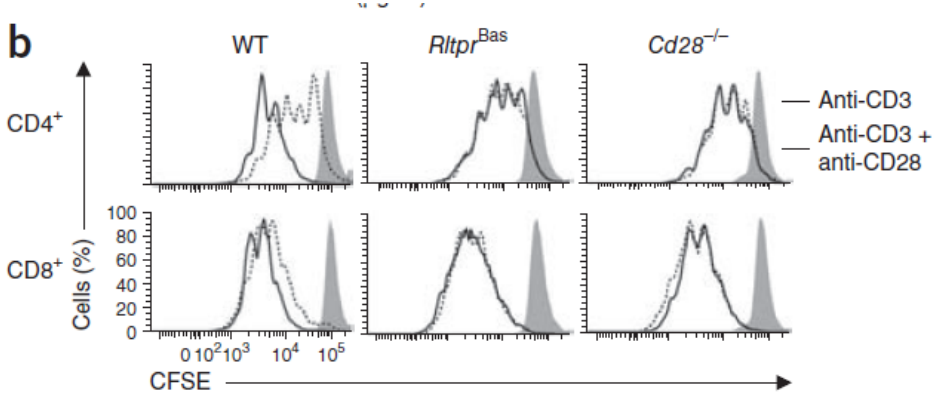
[1]Department of Immunometabolism, Max Planck Institute of Immunobiology and Epigenetics, 79108 Freiburg, Germany

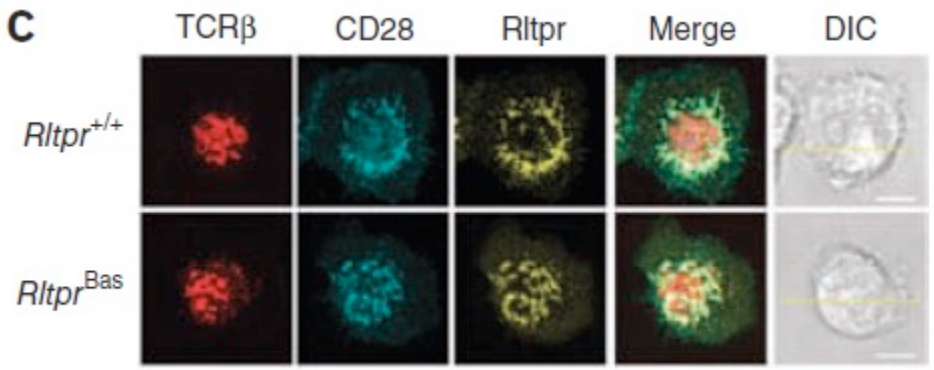


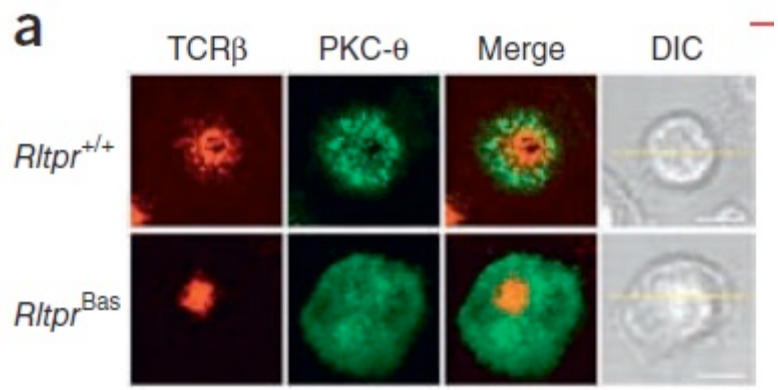



# Are there unique pathways for CD28 signaling?



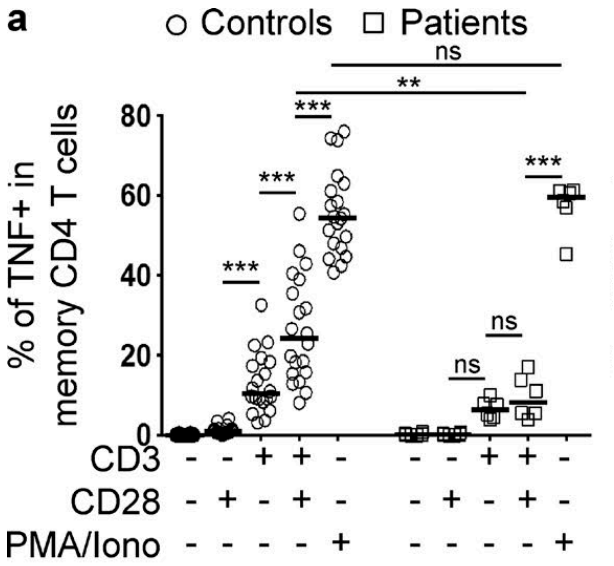

Liang et al. 2013, Nature Immunology

# Are there unique pathways for CD28 signaling?

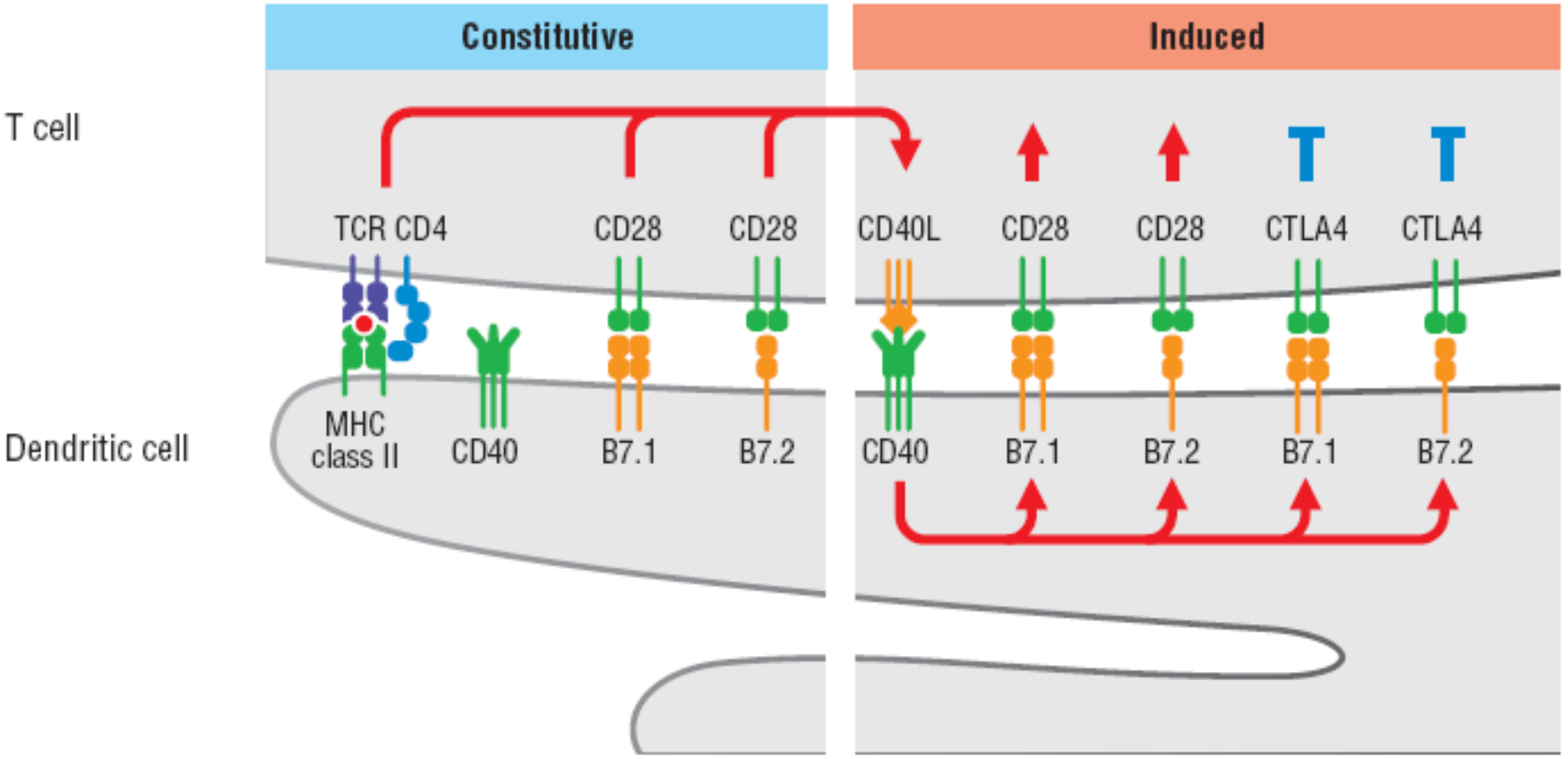
## Dual T cell– and B cell–intrinsic deficiency in humans with biallelic *RLTPR* mutations

Yi Wang,[1,3] Cindy S. Ma,[4,5*] Yun Ling,[1,3*] Aziz Bousfiha,[6*] Yildiz Camcioglu,[7*] Serge Jacquot,[8,9*] Kathryn Payne,[4] Elena Crestani,[10] Romain Roncagalli,[12] Aziz Belkadi,[1,3] Gaspard Kerner,[1,3] Lazaro Lorenzo,[1,3] Caroline Deswarte,[1,3] Maya Chrabieh,[1,3] Etienne Patin,[13,14] Quentin B. Vincent,[1,3] Ingrid Müller-Fleckenstein,[15] Bernhard Fleckenstein,[15] Fatima Ailal,[6] Lluis Quintana-Murci,[13,14] Sylvie Fraitag,[16] Marie-Alexandra Alyanakian,[17] Marianne Leruez-Ville,[18] Capucine Picard,[1,3,20] Anne Puel,[1,3] Jacinta Bustamante,[1,3,20] Stéphanie Boisson-Dupuis,[1,3,21] Marie Malissen,[12**] Bernard Malissen,[12**] Laurent Abel,[1,3**] Alain Hovnanian,[2,3**] Luigi D. Notarangelo,[10,11**] Emmanuelle Jouanguy,[1,3,21***] Stuart G. Tangye,[4,5***] Vivien Béziat,[1,3***] and Jean-Laurent Casanova[1,3,19,21,22***]

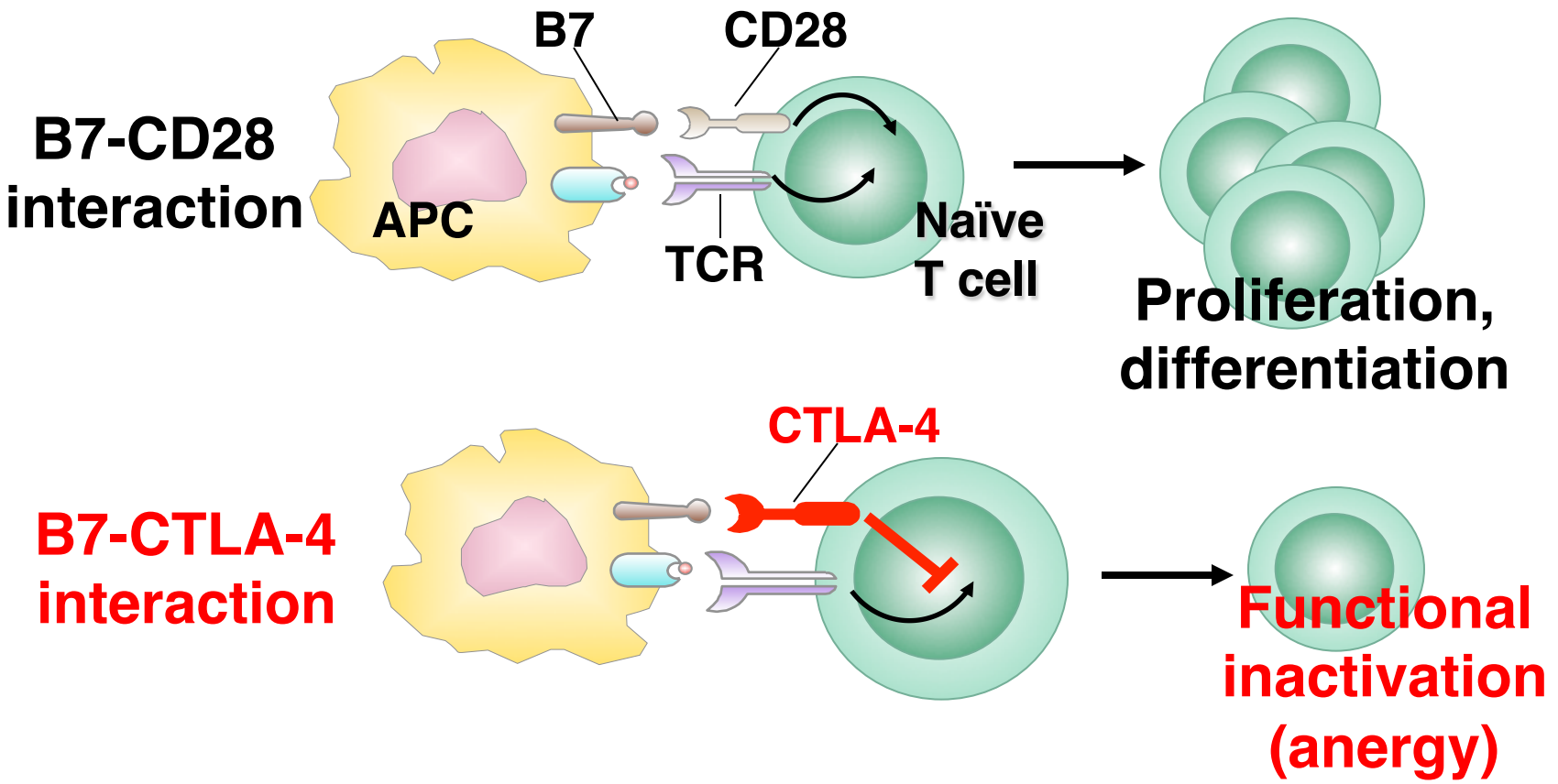
J Exp Med 2016



# CD40L is upregulated on T cells after initial priming. This causes APC's to further upregulate B7 ligands.

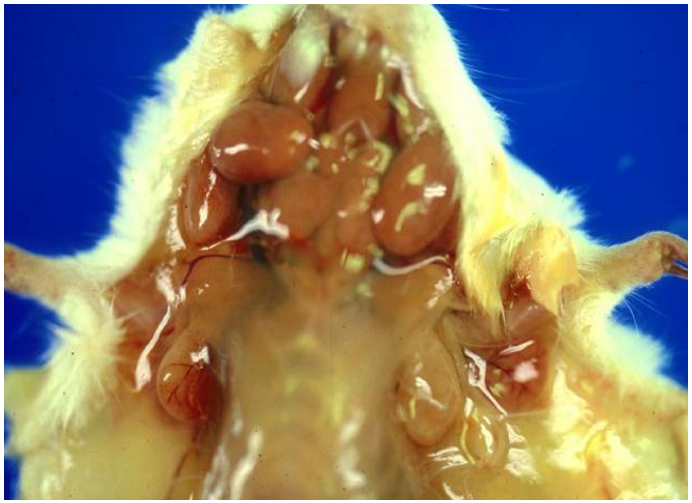


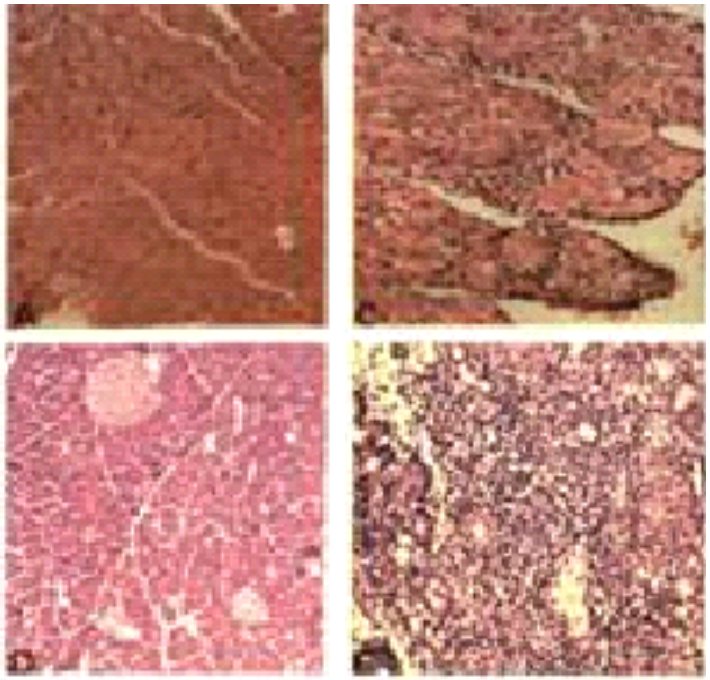
# The opposing functions of CD28 and CTLA-4

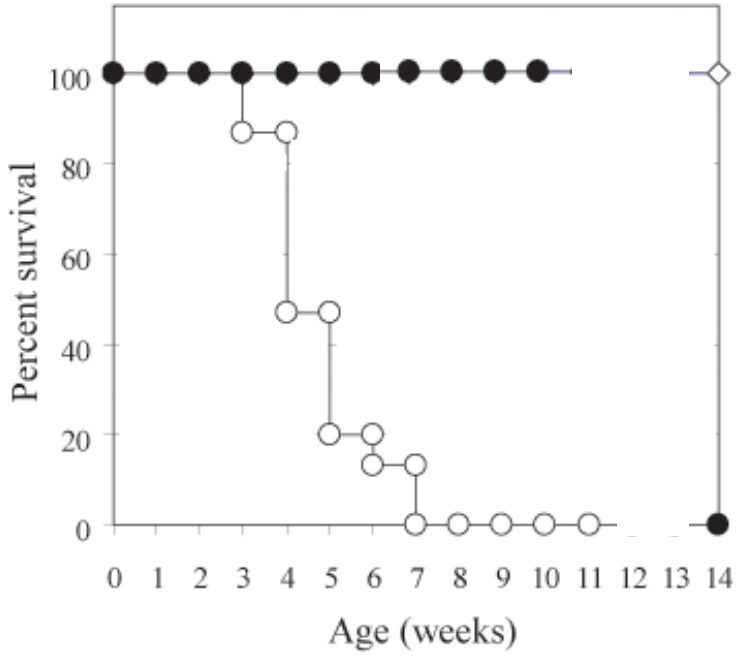



- *Knockout of CTLA-4 results in autoimmune disease and loss of normal homeostasis:*
  - *multi-organ lymphocytic infiltrate, lethal by 3-4 weeks*
  - *lymphadenopathy, splenomegaly*

# CTLA-4 – Master regulator of T cell activation



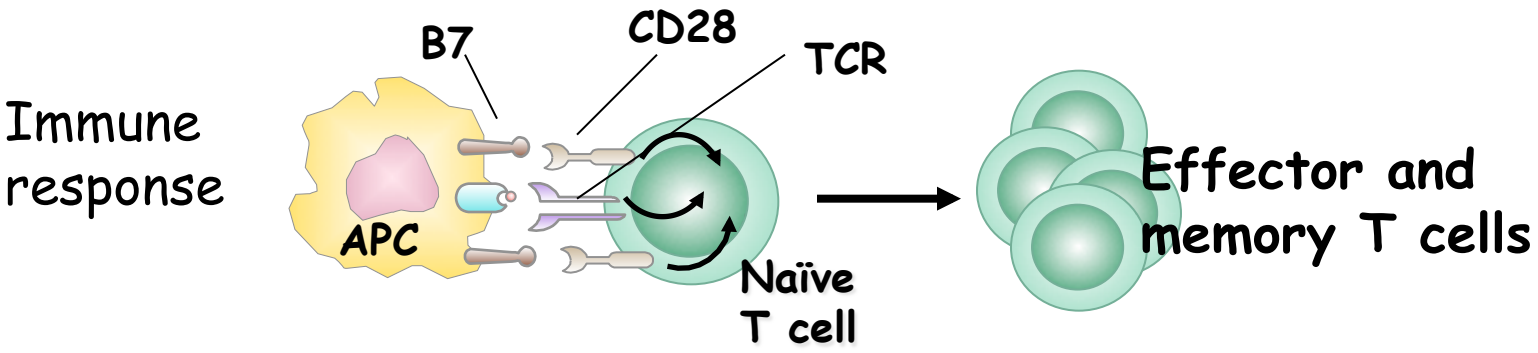


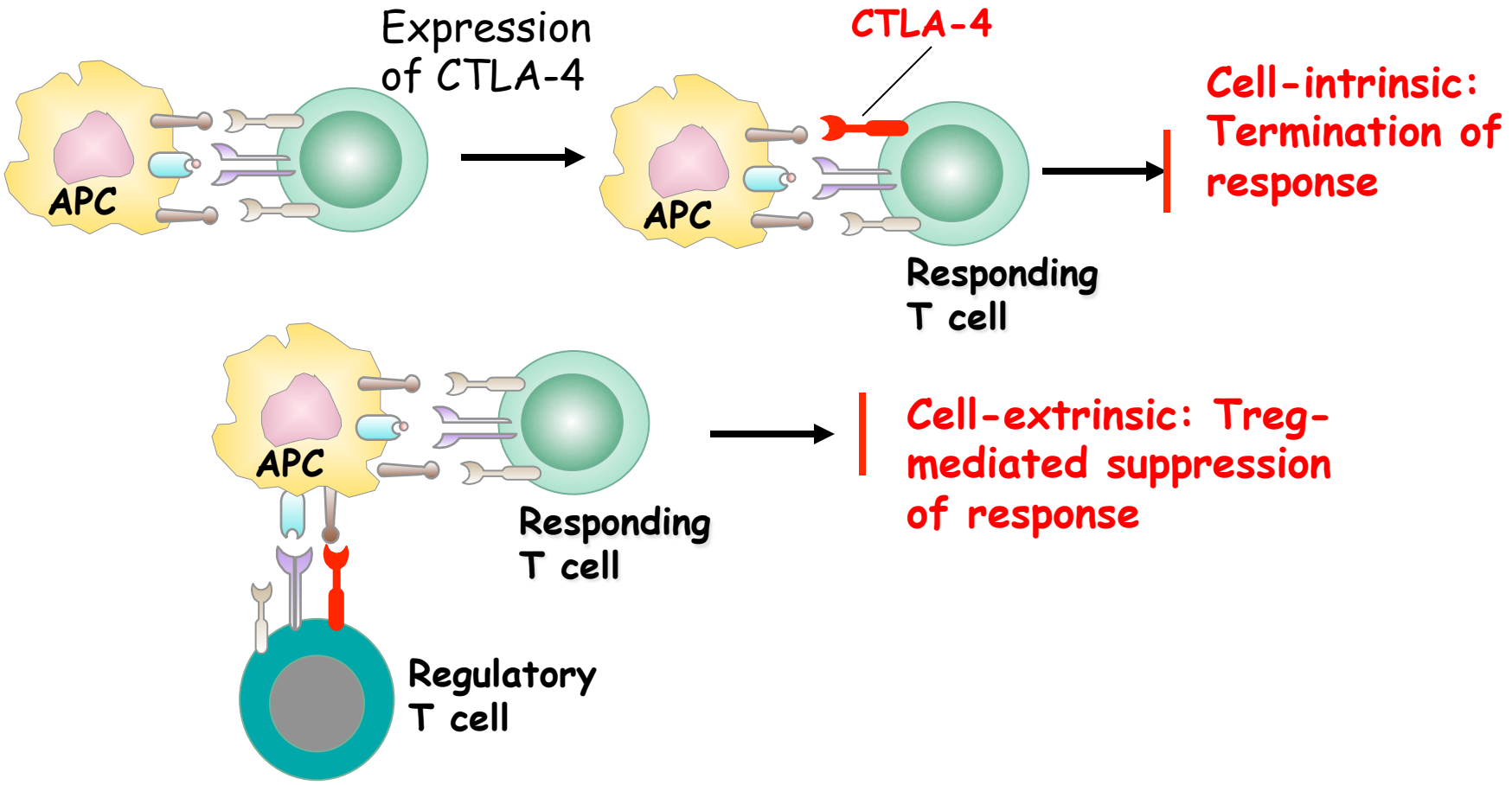


# The inhibitory functions of CTLA-4

- **Role in self-tolerance:**
  - Autoimmunity and lymphoproliferation in knockout mice
  - Polymorphism associated with autoimmune diseases in humans
  - Blockade or deletion makes T cells resistant to tolerance, exacerbates autoimmune diseases (EAE, type 1 diabetes)

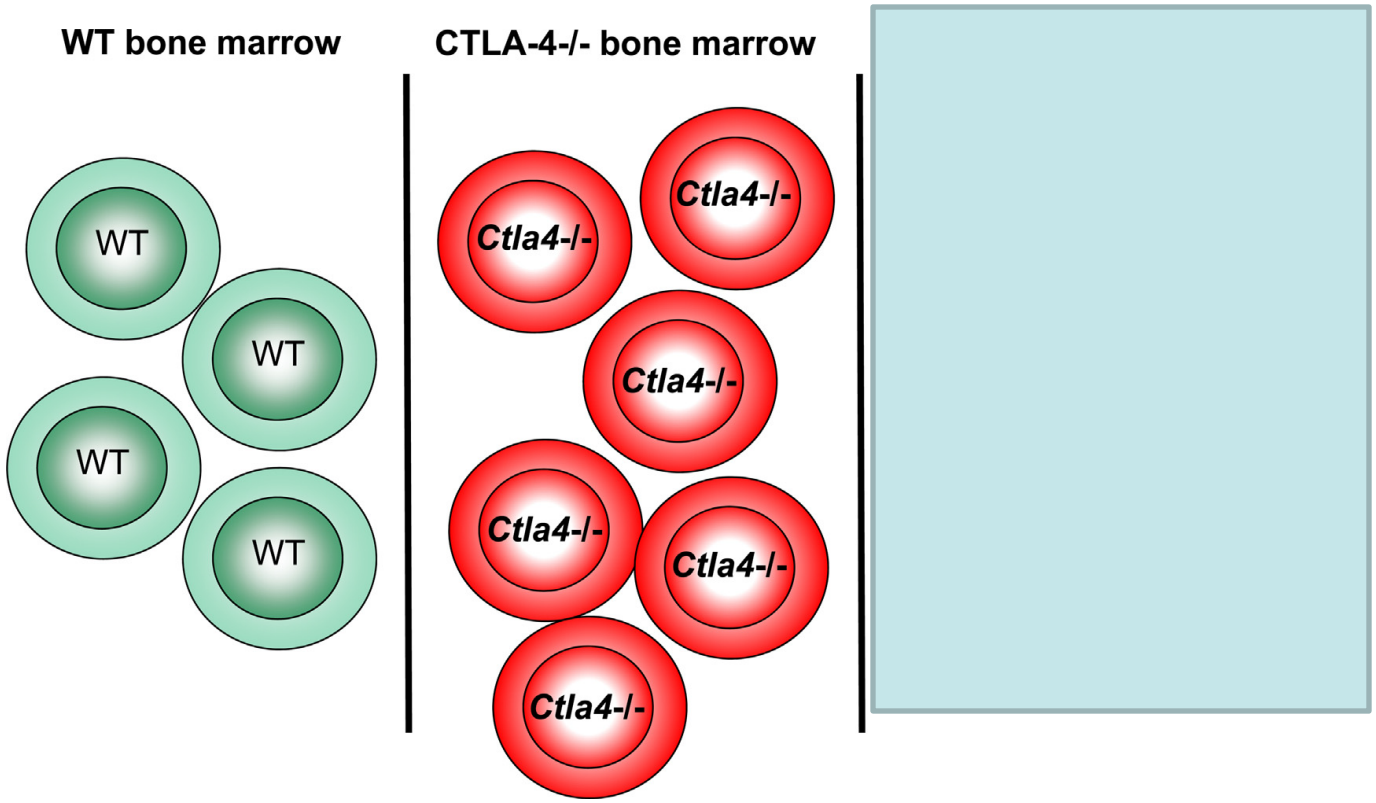
# Actions of CTLA-4



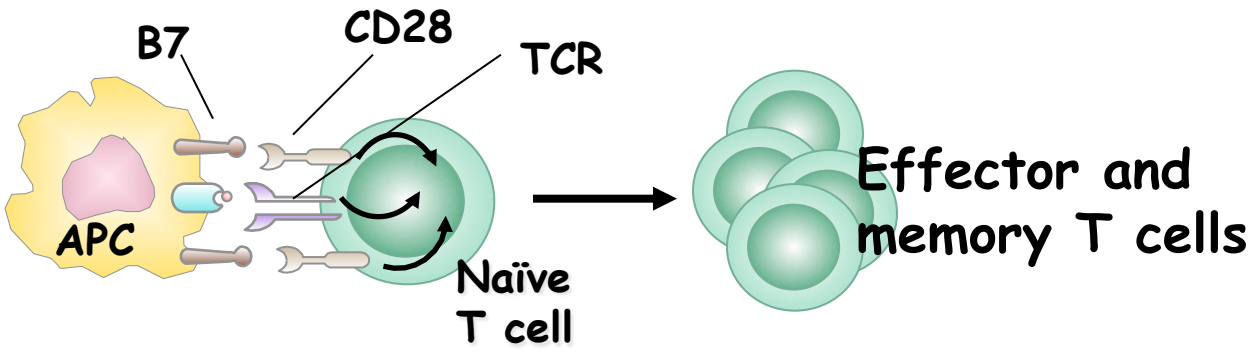
# How does CTLA-4 regulate T cell function

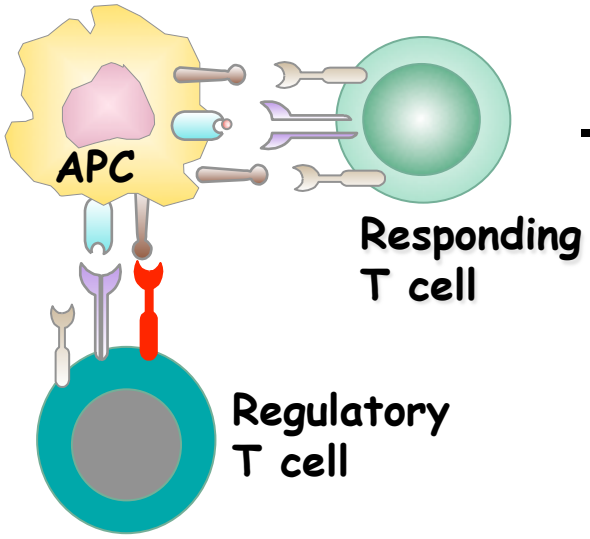
L.S.K. Walker / Immunology Letters 184 (2017) 43–50



# Actions of CTLA-4

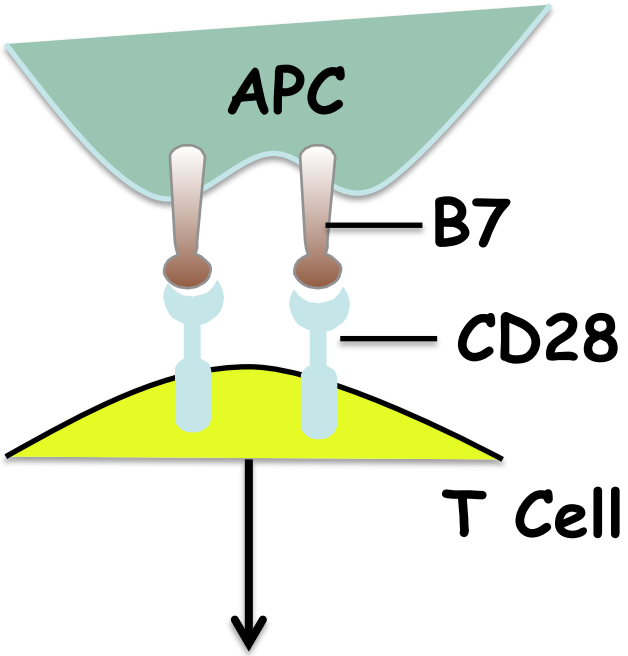
Immune response



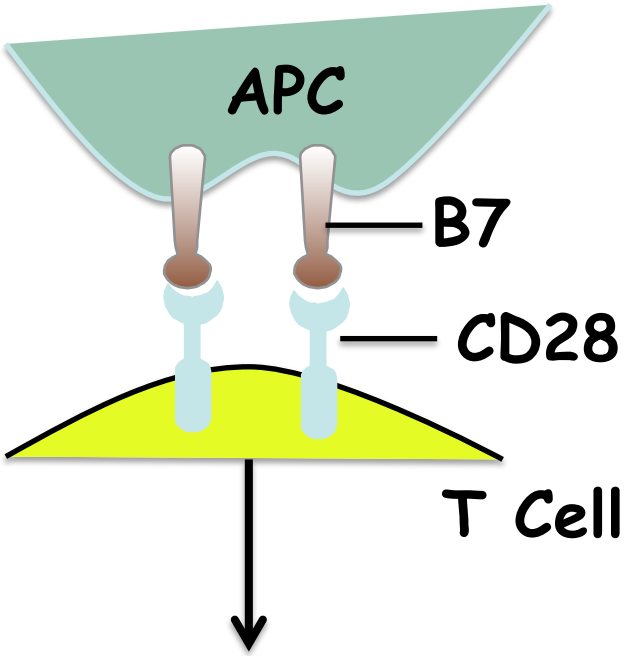

Cell-extrinsic: Treg-mediated suppression of response

# CTLA-4 competitively inhibits B7-CD28 engagement

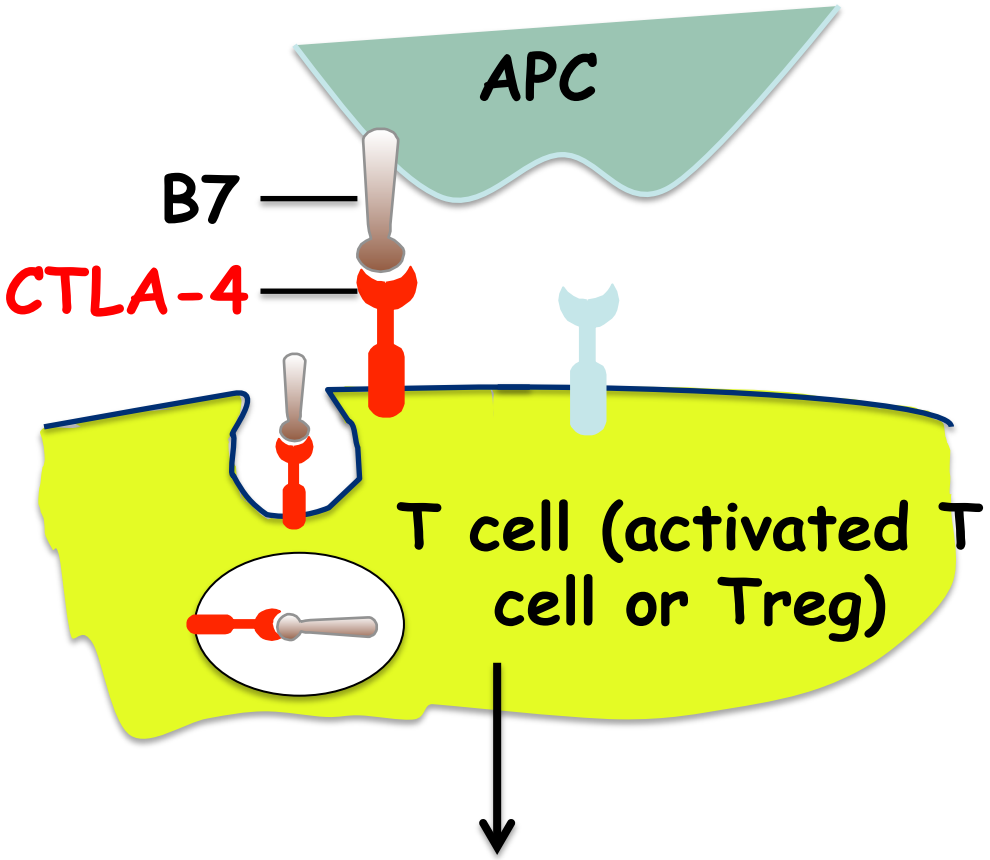



Costimulation →
T cell activation

# CTLA-4 competitively inhibits B7-CD28 engagement

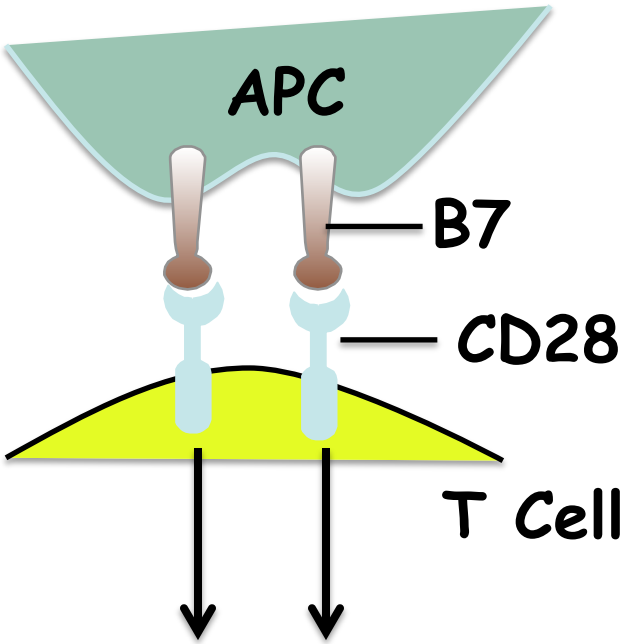



Costimulation → T cell activation




CTLA-4 blocks and removes B7 → lack of costimulation → T cell inhibition

# Consequence of mutations in the CTLA-4 pathway




Unopposed costimulation →
Excessive T cell activation

Therapy?

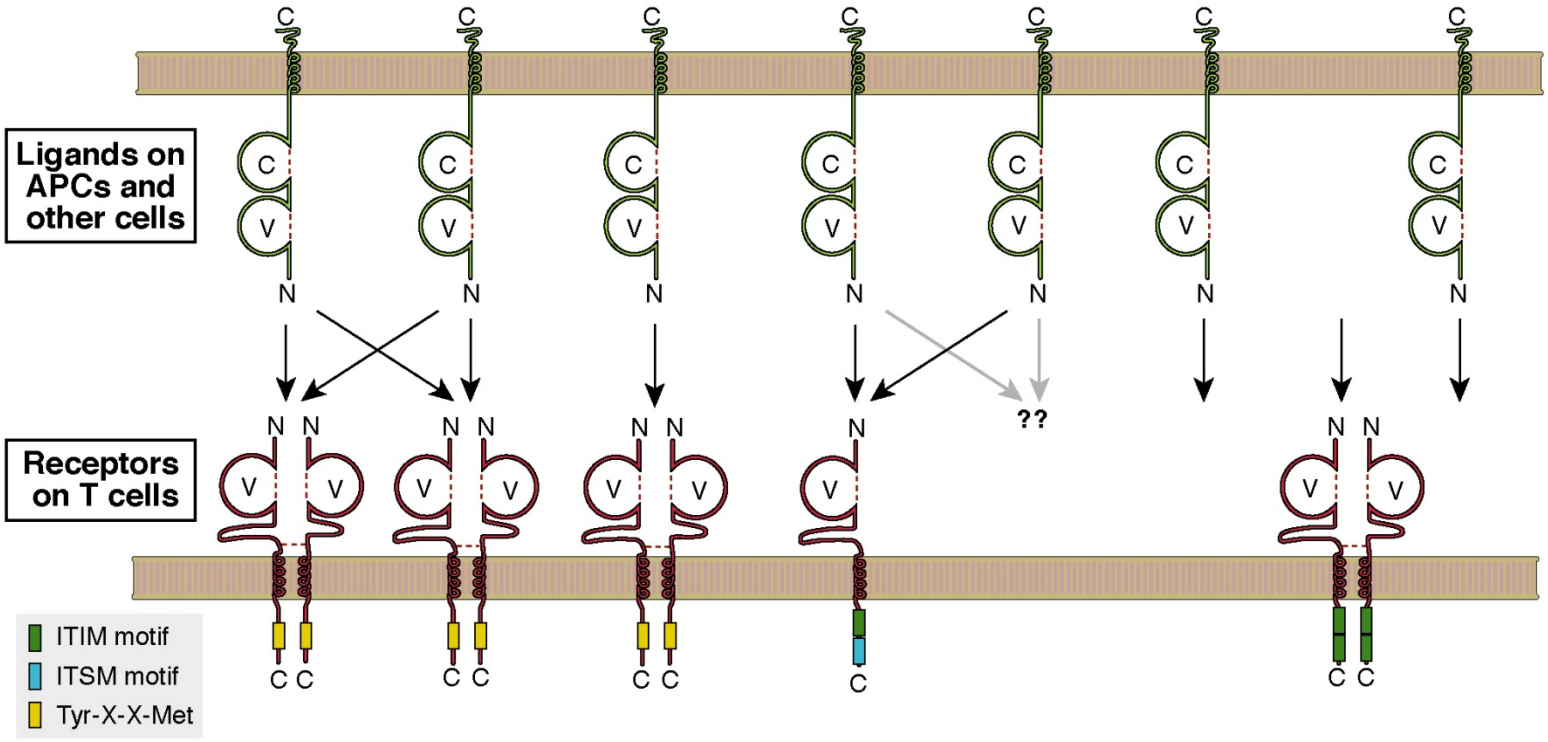
# The opposing actions of CD28 and CTLA-4

## CD28 and CTLA-4 both recognize B7-1, 2; yet CD28 stimulates and CTLA-4 inhibits

- Kinetics: CD28 is expressed constitutively and initiates responses; CTLA-4 appears later and terminates responses
- Affinity: CD28 binds to B7 only when B7 levels are high (microbes?), CTLA-4 (high affinity) binds when B7 is low (self antigens?)
- Preferential ligands: CD28-->B7-2 (constitutive); CTLA-4-->B7-1 (inducible)

# The B7:CD28 families

| Expression | APCs; inducible | APCs; inducible | APCs, B cells, other tissues; inducible | APCs, B cells, other tissues; inducible | | APCs, B cells, other tissues; inducible | | APCs, B cells, other tissues; inducible |
|---|---|---|---|---|---|---|---|---|
| **Name** | **B7-1 (CD80)** | **B7-2 (CD86)** | **ICOS-L** | **B7-H1 (PD-L1)** | **B7-DC (PD-L2)** | **B7-H3** | **?** | **B7-H4** |




| Name | CD28 | CTLA-4 | ICOS | PD-1 | ? | ? | BTLA | ? |
|---|---|---|---|---|---|---|---|---|
| **Expression** | T cells; constitutive | T cells; inducible | T cells; inducible | T cells, B cells, Myleoid cells; inducible | | | T cells, B cells; inducible | |
| **Major function** | Costimulation of naive T cells; generation of regulatory T cells | Negative regulation of immune responses; self-tolerance | Costimulation of effector T cells | Negative regulation of T cells in peripheral tissues | Costimulation or negative regulation of T cells | Costimulation or negative regulation of T cells | Negative regulation of T cells | |

# B7h/ICOS costimulatory pathway

A new molecule with structural characteristic similar to the B7 molecules was identify in 1999, and was named **B7h** (B7-related protein 1; also GL-50 or B7RP-1 or ICOS-L).

B7h does not bind to CD28 or CTLA-4, but bind to **ICOS** (inducible costimulatory molecule). ICOS shares 30-40% sequence similarity with CD28 and CTLA-4.

**ICOS expression:**
ICOS is not constitutively expressed on naïve T cells but is induced on CD4+ and CD8+ T cells following stimulation through the TCR and is further enhanced by CD28-mediated costimulation.

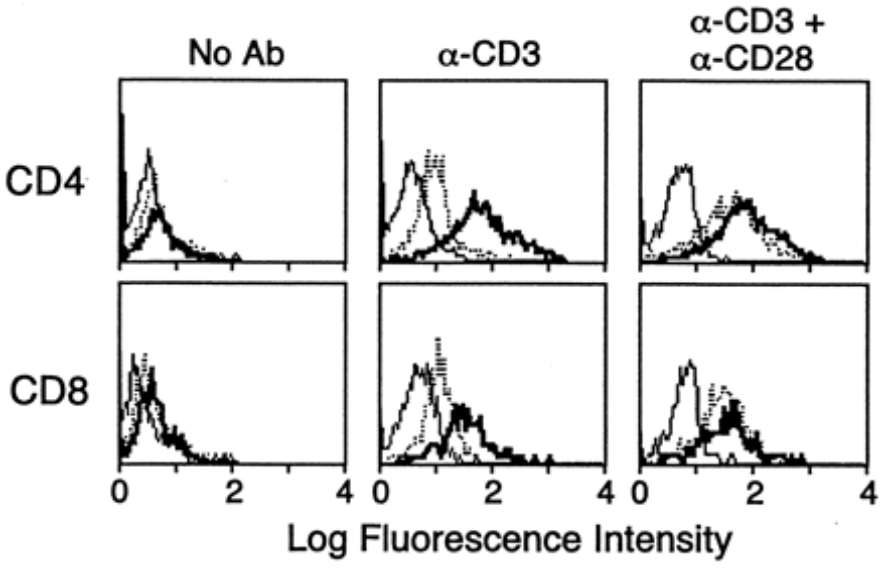
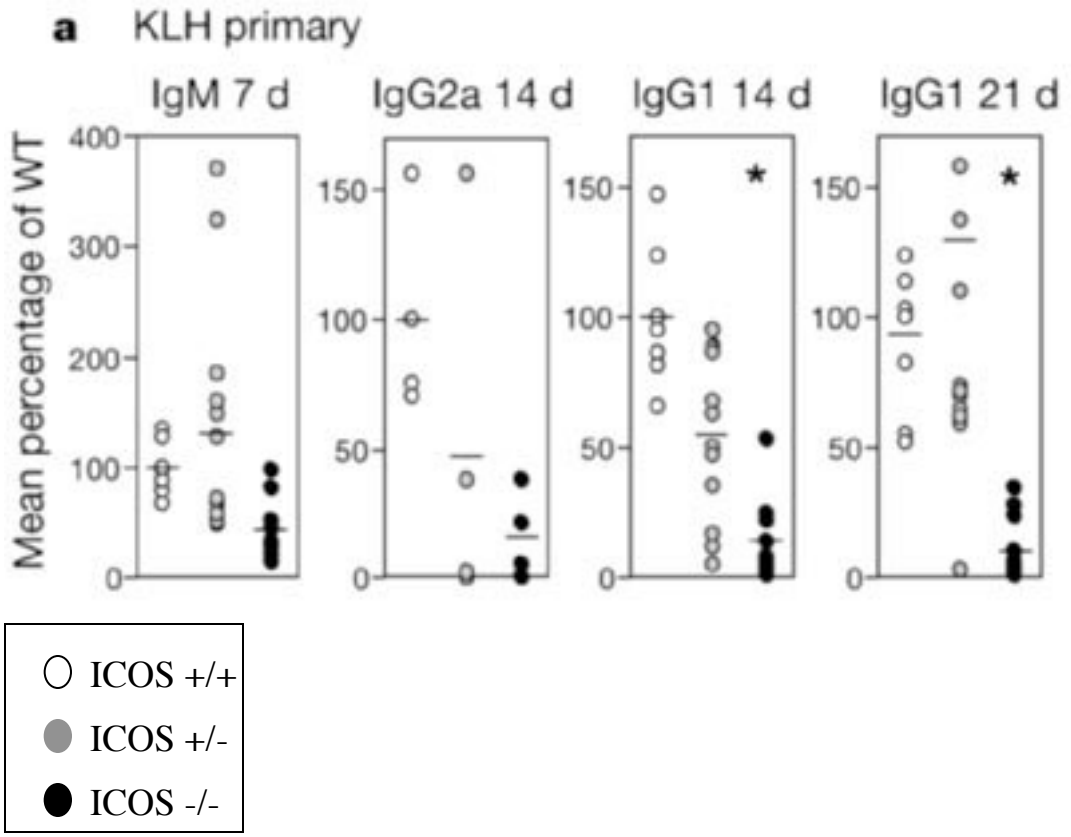
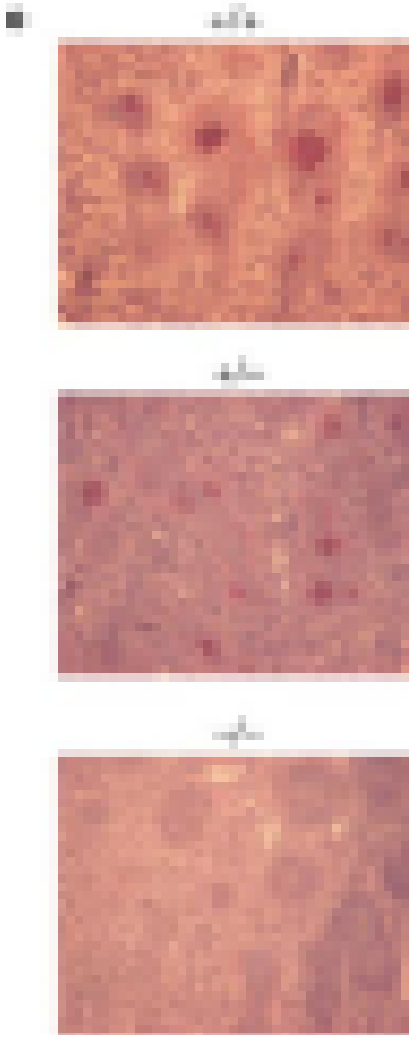



**FIGURE 2.** Expression of ICOS on activated T cells. Dissociated splenocytes from wild-type or B7-1/2$^{-/-}$ 129/SvS4Jae mice were incubated with anti-CD3, anti-CD3 and CD28, or no Ab. The thick line shows ICOS expression on T cells from wild-type splenocyte cultures, the dotted line shows ICOS expression on T cells from B7-1/2$^{-/-}$ splenocyte cultures, and the thin line represents a negative staining control (rat IgG-FITC).

*McAdam A.J. et al. J. Immunol. 165:5035, 2000.*

# Antibody response and germinal center formation in ICOS -/- mice



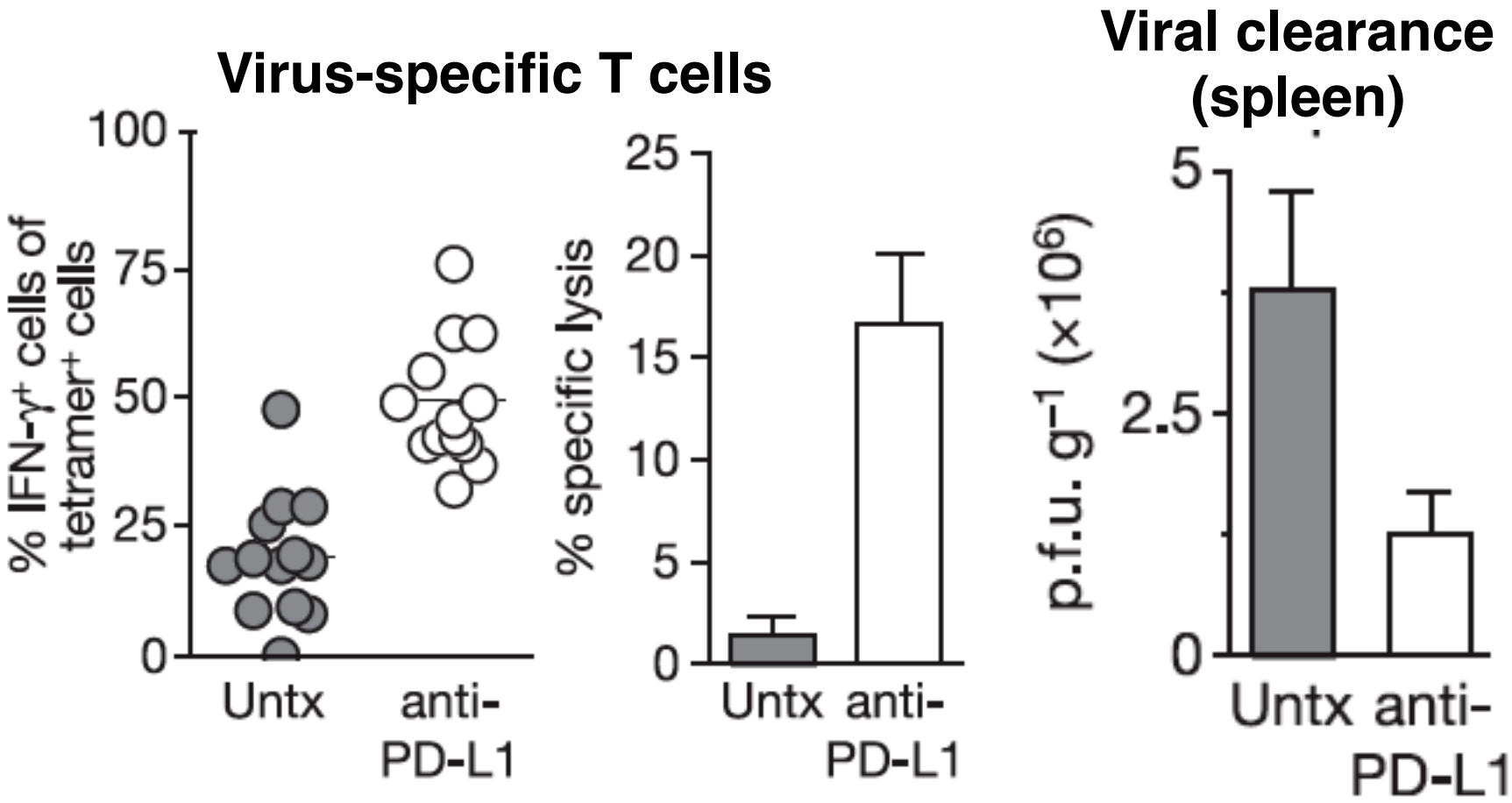
*Tafuri A. et al (2001). Nature, 409: 105-109.*

**ICOS is required for antibody responses and GC formation.**

# The PD-1 inhibitory pathway

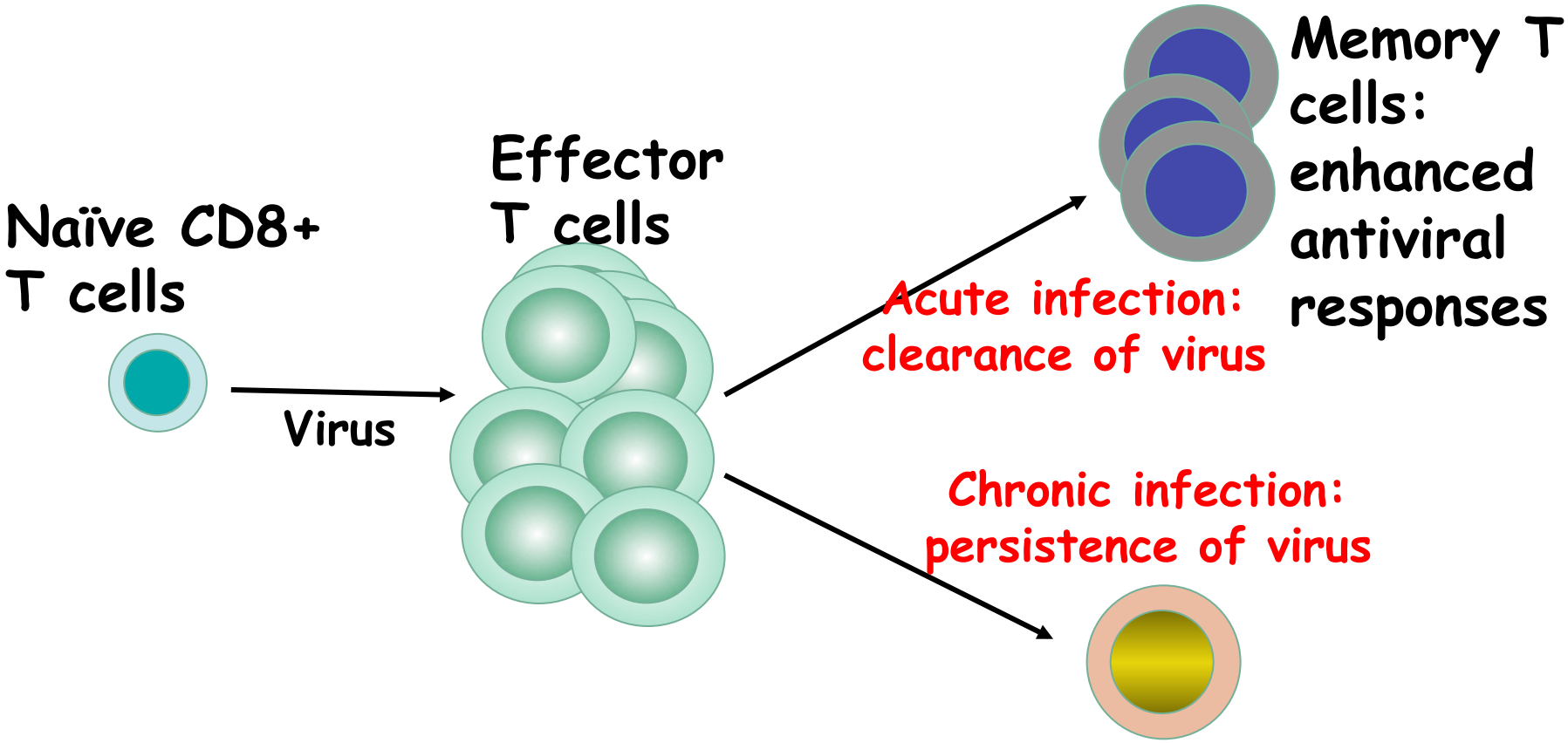
- PD-1 recognizes two ligands (PD-L1, PD-L2)
- Upregulated on T cells after activation
- Knockout of PD-1 leads to autoimmune disease (different manifestations in different strains)
- Role of PD-1 in T cell suppression in chronic infections?

# Inhibitory role of PD-1 in a chronic infection



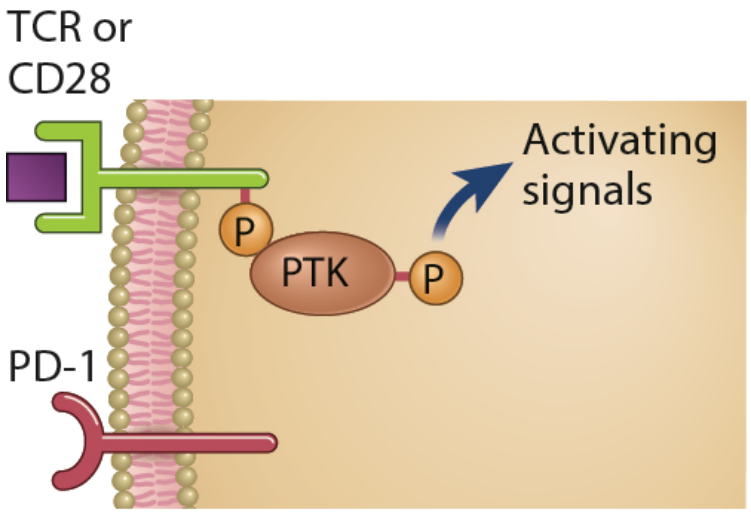

*In chronic LCMV infection in mice, virus-specific T cells become paralyzed; express high levels of PD-1; function restored by blocking the PD-1 pathway. Barber et al (Ahmed lab) Nature 2006*

# T cell "exhaustion" in chronic viral infections

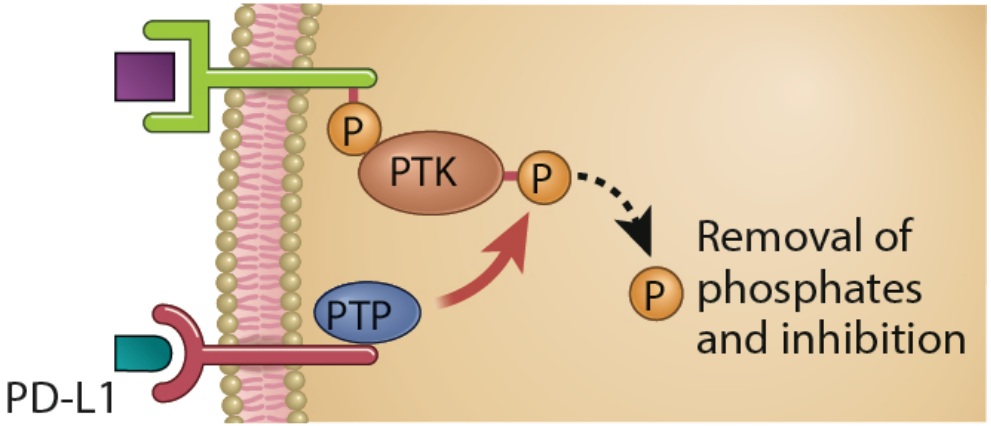


# Action of PD-1

Normal response




PD-1 engagement



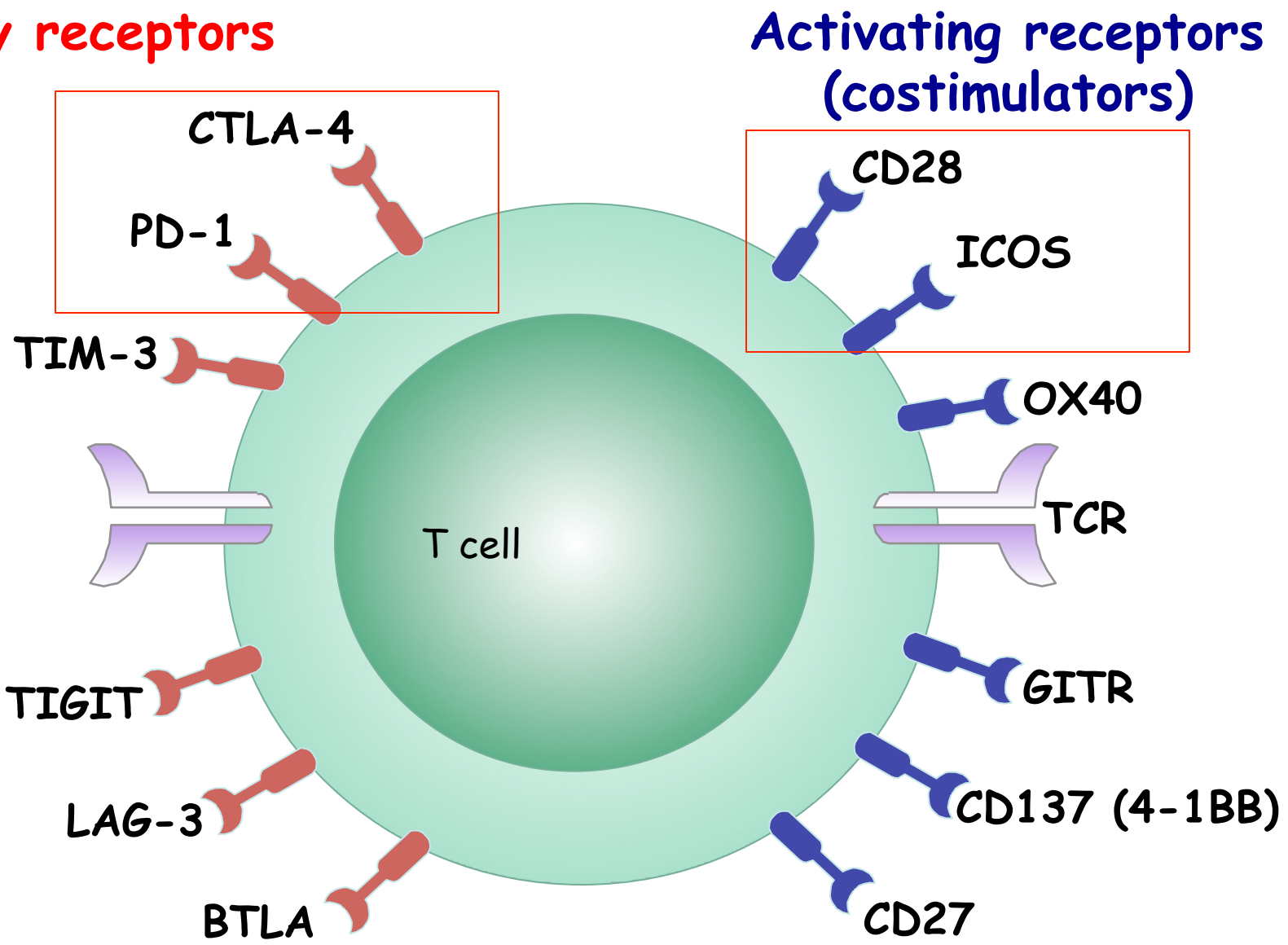
# Roles of inhibitory receptors

- Maintenance of self-tolerance
- Immunosuppression in chronic infections (HCV, HIV?)
- Termination of normal immune responses?
- Why so many inhibitory pathways?

# Functions of CTLA-4 and PD-1

| | CTLA-4 | PD-1 |
|---|---|---|
| Major site of action | Lymphoid organs | Peripheral tissues |
| Stage of immune response suppressed | Induction | Effector phase |
| Mechanism of action | Competitive inhibitor of CD28 | Signaling inhibitor of CD28 and TCR |
| Cell type suppressed | CD4+ and CD8+ | CD8+ > CD4+ |

# T cell activating and inhibitory receptors



# Putting it back together

# Context matters: APC's upregulate B7 upon recognition of microbes

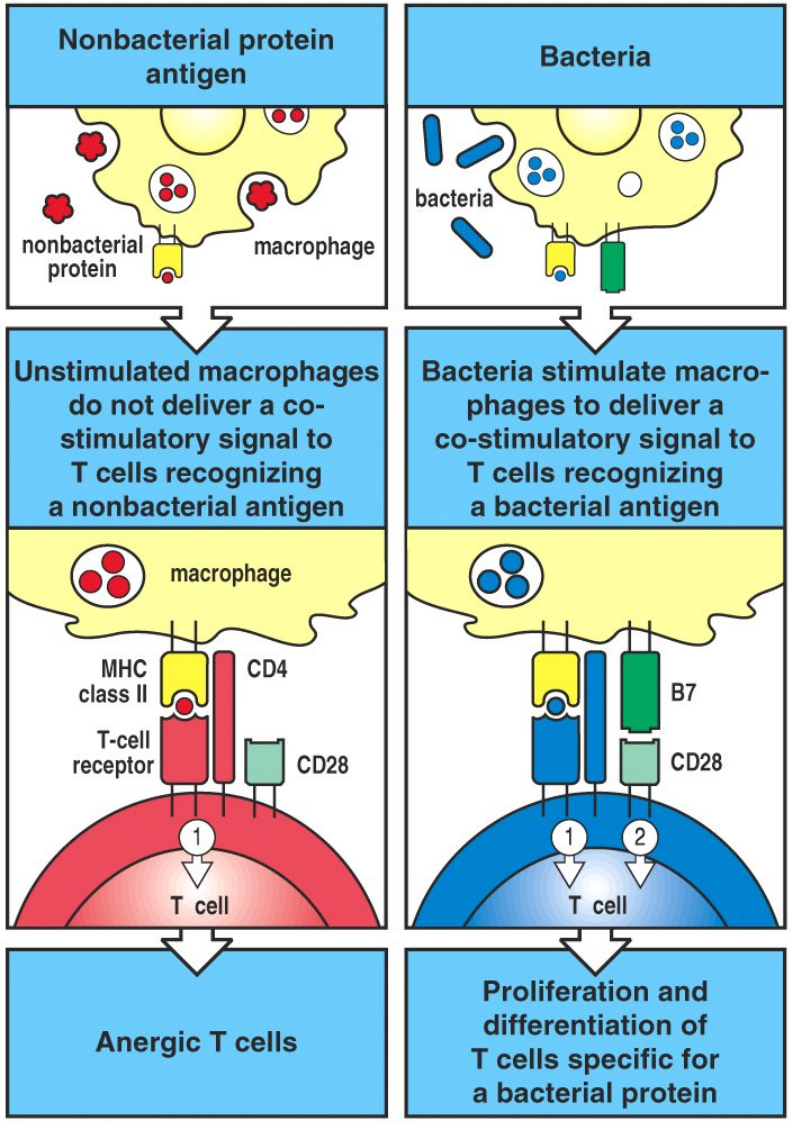
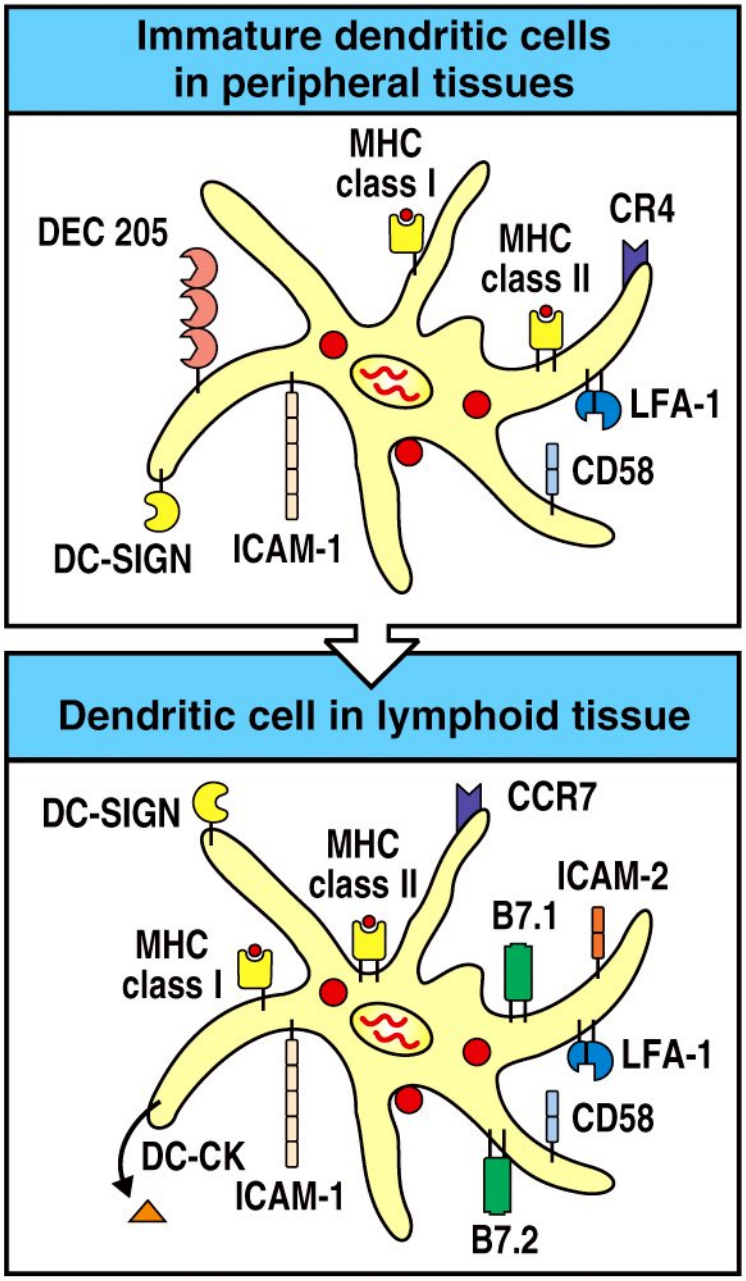



Figure 8-16 Immunobiology, 6/e. (© Garland Science 2005)



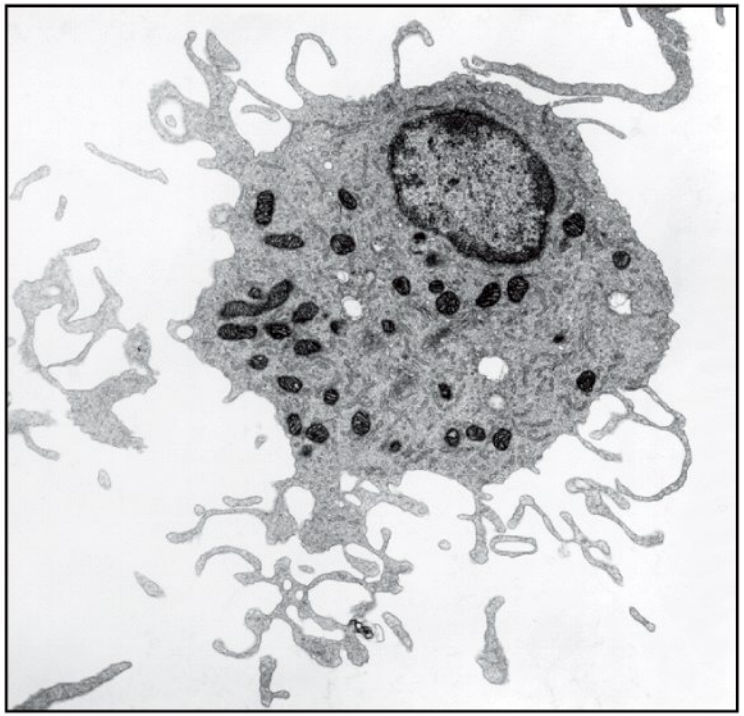



Figure 8-14 Immunobiology, 6/e. (© Garland Science 2005)

# Anatomy of naïve T cell priming-APC's

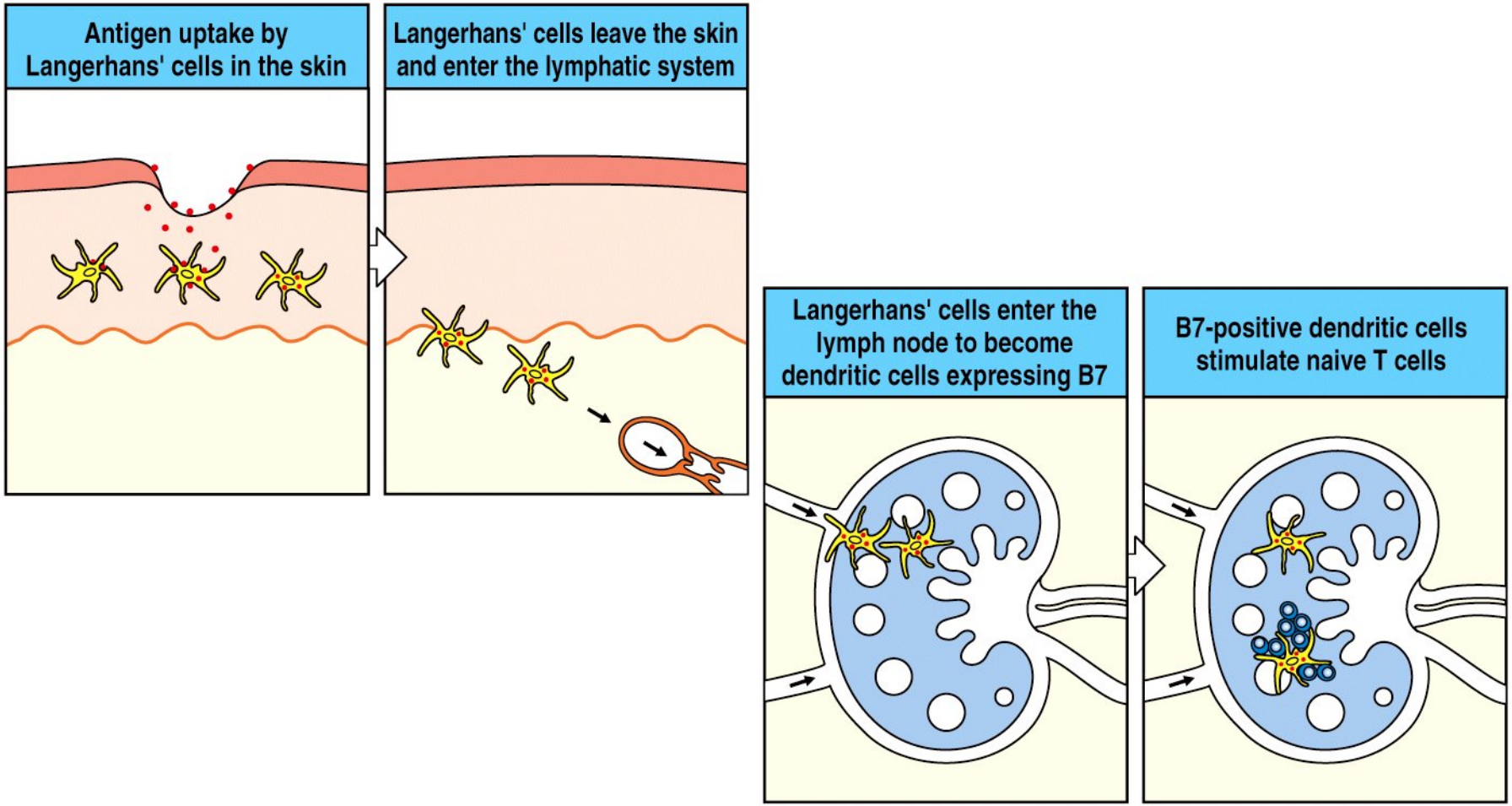



Figure 8-15 Immunobiology, 6/e. (© Garland Science 2005)

# Anatomy of naïve T cell priming (cont.)

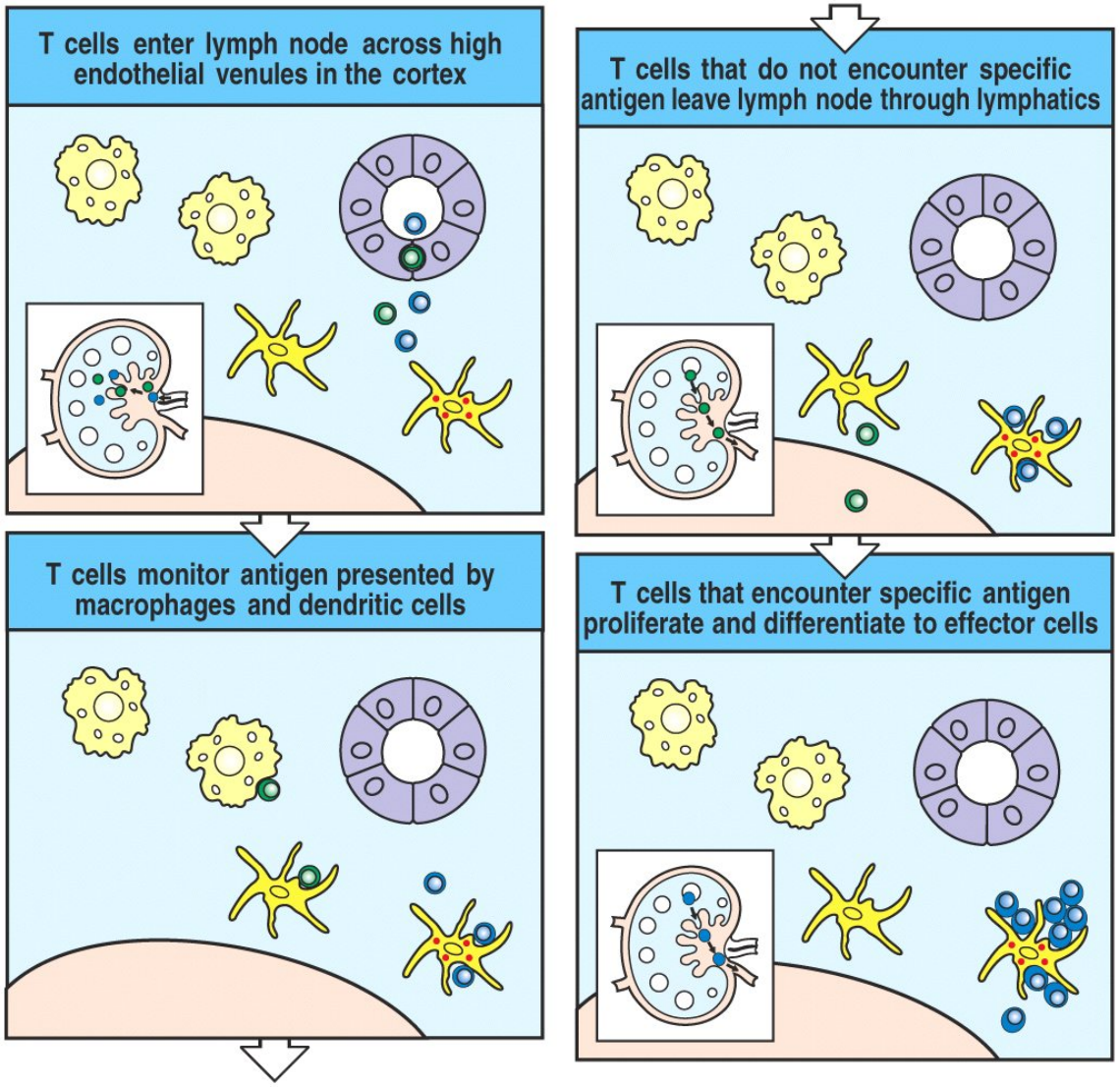
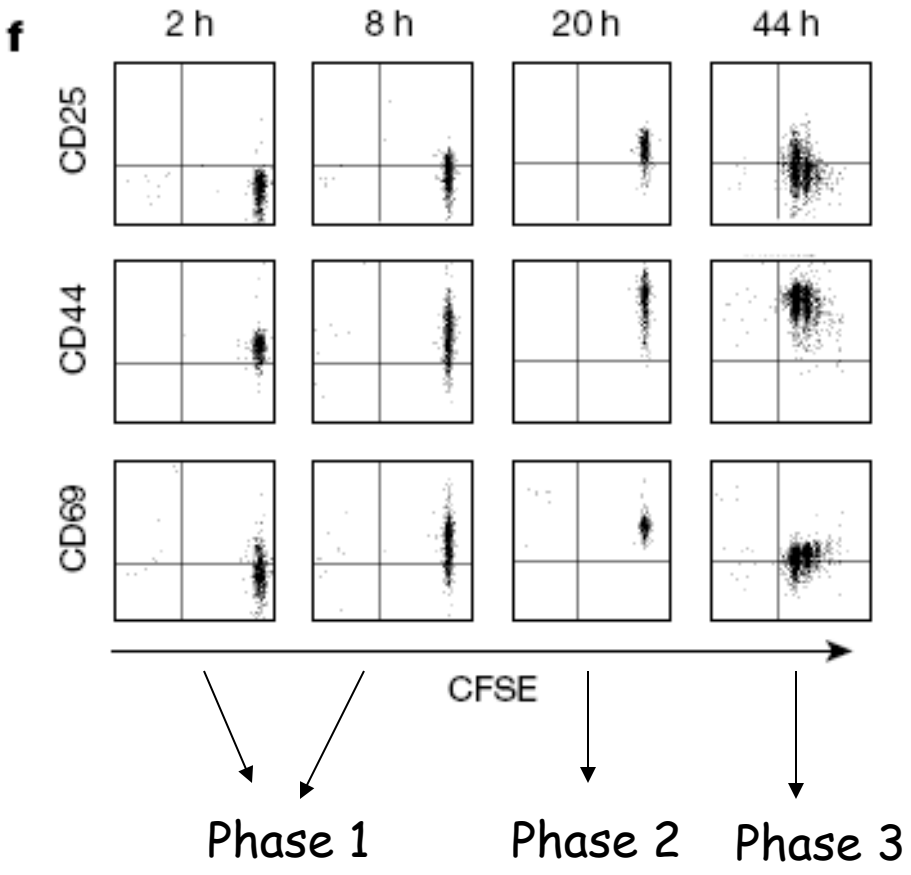



Figure 8-4 Immunobiology, 6/e. (© Garland Science 2005)

# In Vivo T cell activation

## Mempel et al. Nature 2004




In vivo imaging of T cells adoptively transferred into mice with antigen loaded DC's

DC's are red and T cells are green

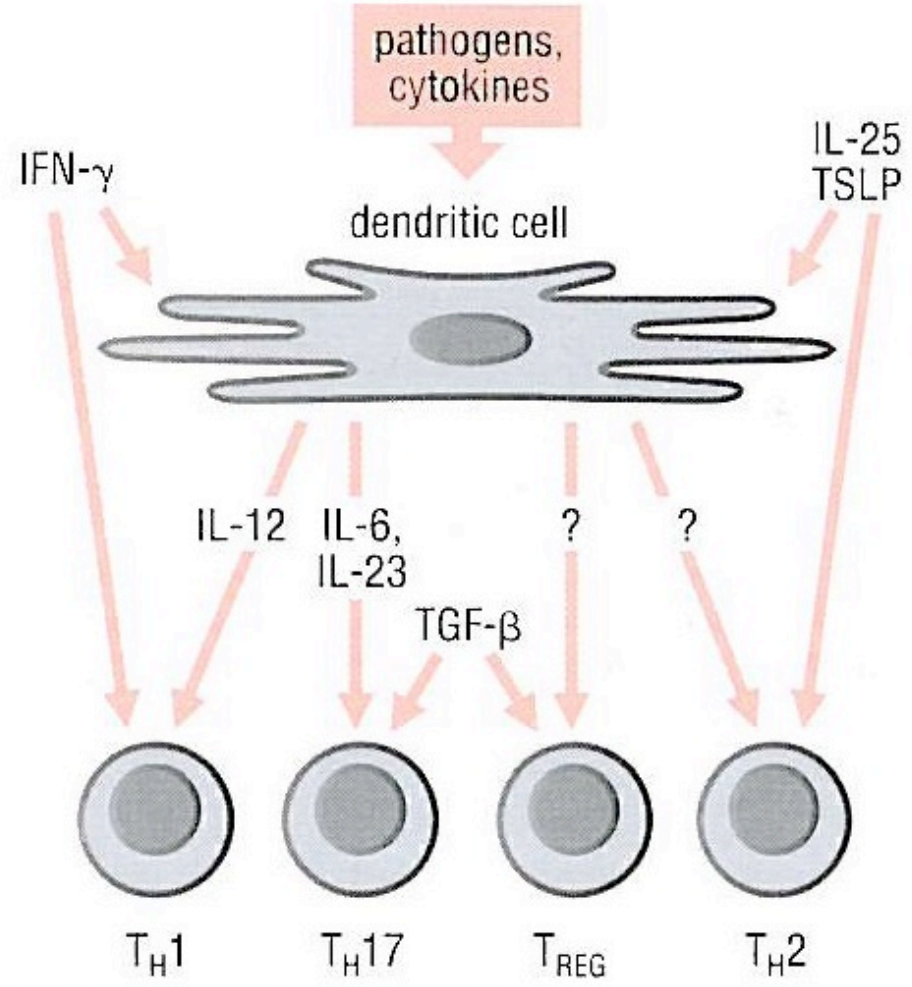
Observed three phases of T cell behavior:

Phase 1: multiple short encounters with DC's

Phase 2: long-lasting stable contacts with DC's

Phase 3: resumed short contacts and rapid migration

# After T cell activation, differentiation into other subsets



# Summary

- TCR-MHC/peptide interaction is low affinity. T cells use multiple mechanisms to overcome this (anatomy, adhesion, synapse, etc.)
- Context of MHC-antigen is critical to outcome
- Balance of positive and negative signals determine the magnitude and nature of T cell responses
- Challenges:
  - Which signals are dominant in vivo under different conditions?
  - How do we use this knowledge to design therapeutic strategies?