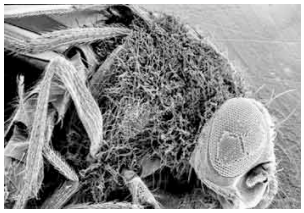
Interactions between innate immunity & adaptive immunity



1

What happens to T cells after they leave the thymus?

Naïve T cells exit the thymus and enter the bloodstream.

If they remain in the bloodstream, then they will pass through the spleen.

Naïve T cells can also enter lymph nodes by crossing high endothelial venules (HEVs).

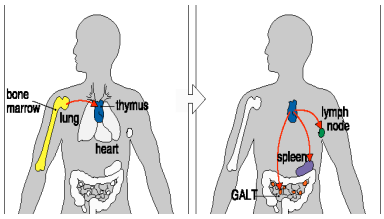
In these 2˚ lymphoid organs, naïve T cells encounter thousands of APCs.

If they are not activated, then they reenter the bloodstream and start all over.

2

T-cell activation

T cells migrate to secondary lymphoid tissues where they interact with antigen, antigen-presenting cells, and other lymphocytes:



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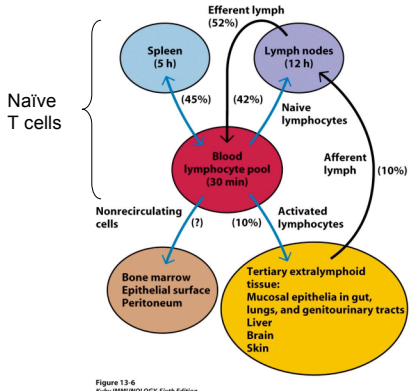

Figure 13-6
Kuby IMMUNOLOGY, Sixth Edition
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4

Lymphoid Tissues

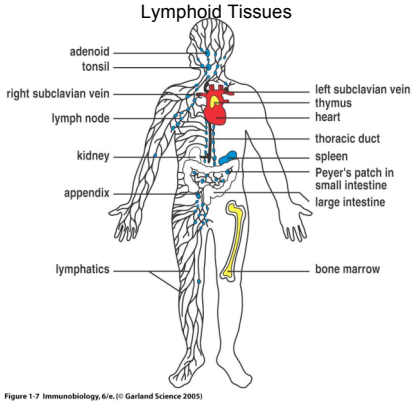


Figure 1-7 Immunobiology, 6/e. (© Garland Science 2005)

5

Naïve T cells enter lymph nodes via high endothelial venules (HEVs)

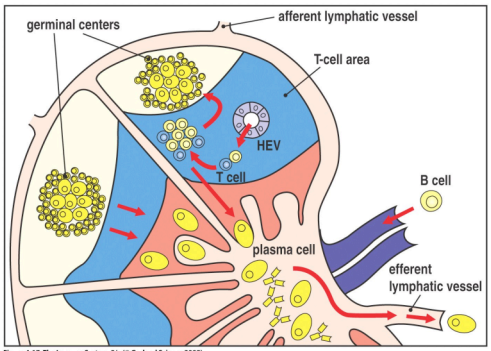


Figure 4-17 The Immune System, 2/e (© Garland Science 2005)

What happens when a naïve T cell is activated?

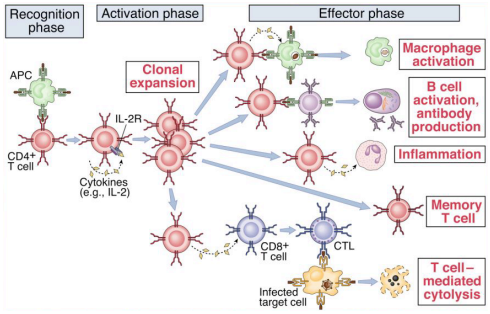
Proliferation
- Cell cycle entry and cell division
- Clonal expansion

Differentiation
- Secretion of cytokines (helper cells)
- Activation of killer functions (cytotoxic cells)
- Acquisition of effector function
- Memory

Death
- Important for down-regulation of immune response

7

Response of CD4 T cells



8

After activation, T cells remain in lymph nodes for 5-6 days

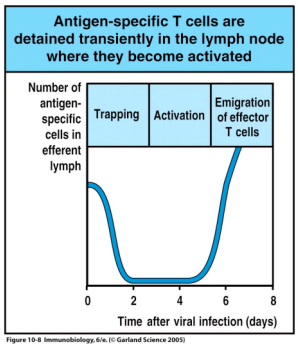


Figure 10-8 Immunobiology, 6/e. (© Garland Science 2005)

9

2˚ lymphoid organs bring cells of the immune system together

Facilitate the complex cellular and molecular interactions necessary to initiate the immune response

How do T cells and antigen meet in the lymph node?

10

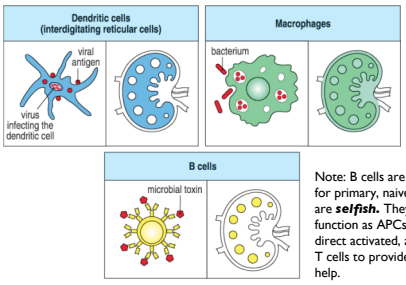
There are 3 types of professional antigen presenting cells

Tissue	MHC class I	MHC class II
Lymphoid tissues		
T cells	+ + +	+*
B cells	+ + +	+ + +
Macrophages	+ + +	+ +
Dendritic cells	+ + +	+ + +
Epithelial cells of the thymus	+	+ + +
Other nucleated cells		
Neutrophils	+ + +	–
Hepatocytes	+	–
Kidney	+	–
Brain	+	– †
Non-nucleated cells		
Red blood cells	–	–

Professional Antigen Presenting Cells

Figure 3-19 Immunobiology, 6/e. (© Garland Science 2005)

Dendritic cells are by far the strongest activators of naive T cells



Note: B cells are poor APCs for primary, naive T cells. They are ***selfish.*** They really only function as APCs in order to direct activated, antigen-specific T cells to provide them with help.

Fig 8.3 © 2001 Garland Science

Why?

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The Dendritic Cell Paradigm

- DC are present at all epithelial barriers, where they are sentinels for infection
- DC ingest invading pathogens (discriminate between self vs non-self)
- Upon this stimulus, DC migrate to local lymph nodes, where they present pathogen antigens to T cells, which initiate specific immunity
- Thus DC are critical for crosstalk between innate and adaptive immunity

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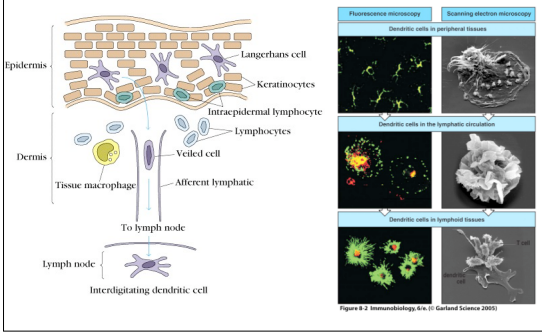
Dendritic cells pick up antigen at sites of infection and bring it to the regional lymph nodes or spleen



Immature dendritic cells

DCs in this state of maturation play a predominant role in antigen capture from within their local environments.

Immature DCs in the steady-state are thought to mature spontaneously and acquire the capacity to induce T cell tolerance.

15

Immature dendritic cells

- low expressors of MHC and costimulatory molecules
- weak stimulators of T cells.
- highly endocytic:
  - take up particles and microbes by phagocytosis.
  - form large pinocytic vesicles in which extracellular fluid and solutes are sampled, a process called macropinocytosis.
  - express receptors that mediate adsorptive endocytosis, including Complement receptor, C-type lectin receptors, as well as Fc receptors

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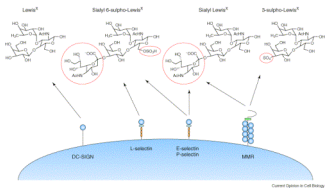
C-type lectins

Produced as transmembrane or secreted proteins.
Highly conserved Carbohydrate Recognition Domain (CRD)
Binds sugars in a calcium dependent manner
–Mannosylation of peptide result in 100 to 10,000 fold enhanced potency for T-cell stimulation



17

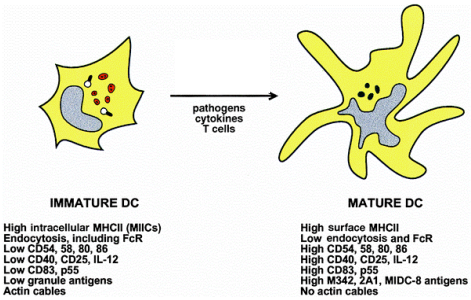
DC maturation

DC activators signals include proinflammatory cytokines (IL-1, GM-CSF and TNF-α) and bacterial or viral products such as LPS, CpG motifs, and double-stranded RNA through TLR.

Activated DCs can be distinguished from resting, mature DCs by expression of higher levels of MHC and costimulatory molecules or by production of cytokines such as interleukin (IL)-12 and interferon α

18

Dendritic cells undergo "maturation" to become potent antigen-presenting cells in lymphoid tissue



Poor T cell activators

Great T cell activators

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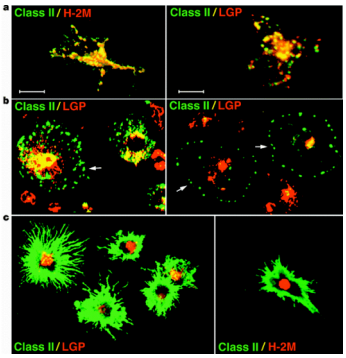
Dendritic cells undergo "maturation"



Immature phenotype: DCs from bone marrow cultures

Intermediate phenotype: 6 d culture with GM-CSF

Mature DCs: Day 8 of culture with GM-CSF

I. Mellman, P. Pierre, and S. Turley. *Nature* 1997, 388:787-792.

20

DC maturation is associated with migration to the lymph node

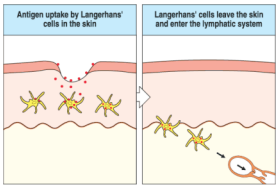


Fig 8.15 part 1 of 2 © 2001 Garland Science

Immature DC

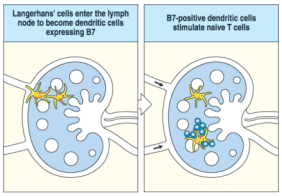


Fig 8.15 part 2 of 2 © 2001 Garland Science

Mature DC

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Why are DCs such good APCs?

1. The express both MHC class I and MHC class II
2. They start in the right place (tissues)
3. They end up in the right place (lymph node)
4. Immature DCs are highly efficient at taking up antigens in the tissues.
5. After maturation and migration, mature DCs present these antigens to T cells in the lymph node.
6. They express costimulatory molecules

22

Mature dendritic cells express key costimulatory molecules

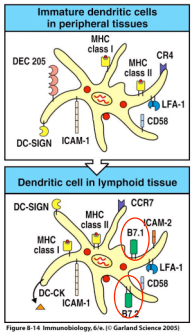

Figure 8-14 Immunobiology, 6/e, (© Garland Science 2005)

B7.1 (CD80) and B7.2 (CD86) are structurally related glycoproteins expressed on mature DCs.

Collectively called B7 molecules, they bind CD28 on T cells

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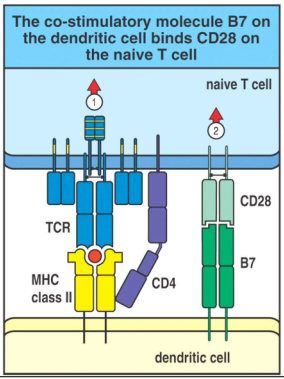
B7 molecules are ligands for CD28 on T cells



24

Naive T cells require two signals to become activated: TCR/MHC and costimulation

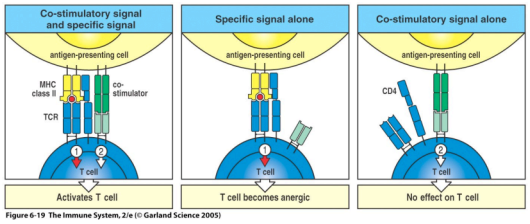
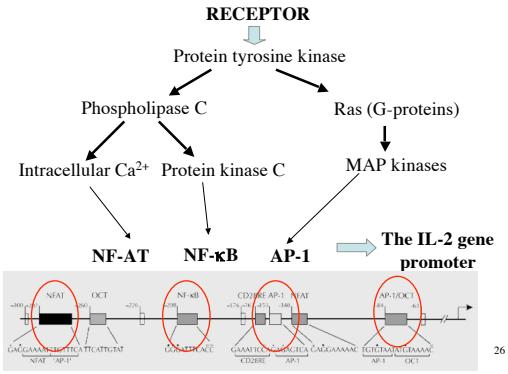


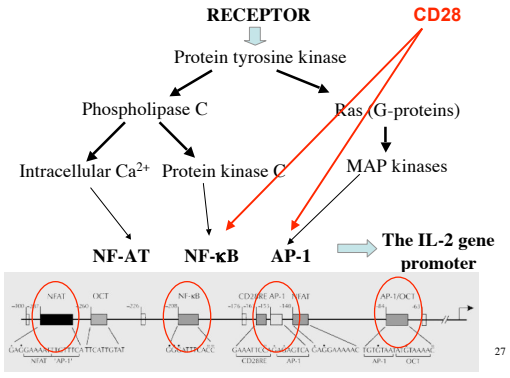
Figure 6-19 The Immune System, 2/e (© Garland Science 2005)

How does CD28 signaling enhance T cell activation?

Remember how TCR signaling leads to IL-2 transcription



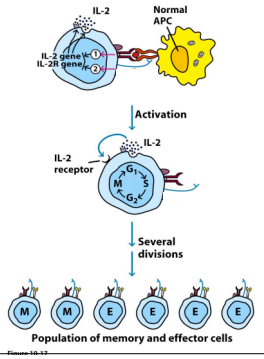
CD28 signaling activates AP-1 and NF-κB



CD28 signaling stabilizes IL-2 mRNA

- Most cytokine mRNAs are very short-lived because of "instability" sequences in their 3' untranslated regions.
- Instability is important for tight regulation.
- CD28 signaling stabilizes IL-2 mRNA leading to a 20-30 fold increase in protein secretion.
- Stabilization combined with the activation of NF-κB and AP-1 increases IL-2 secretion 100-fold.

The costimulatory signal is necessary for the synthesis and secretion of IL2



Summary I

- Naïve T cells leave the thymus and home to secondary lymphoid organs.
- Dendritic cells are the most potent activators of naïve T cells.
- DCs carry antigen from the periphery to the draining lymph nodes.
- This migration is associated with "maturation".
- Mature DCs express high levels of MHC as well as B7 costimulatory molecules.
- B7 molecules bind CD28 on T cells. CD28 signaling provides "Signal 2" to T cells and is necessary for T cell activation.
- CD28 costimulation is mediated, in part, by increasing IL-2 secretion.

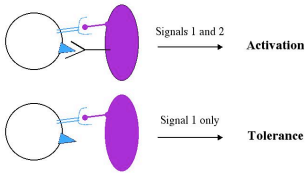
Why is costimulation necessary?

- Negative selection is not perfect.
- Some T cells will leave the thymus with specificities for self antigens not expressed in the thymus.
- The requirement for costimulation ensures that T cells will not be activated by cells expressing these antigens.

31

Lack of signal 2 leads to peripheral tolerance

Bretscher & Cohn 2-signal model for tolerance induction



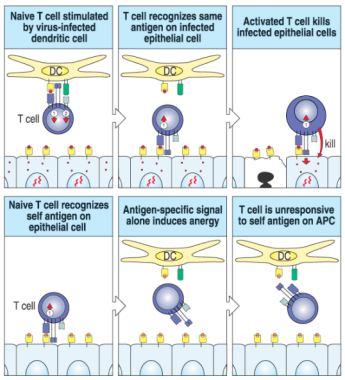
Peptides presented in the absence of costimulation will not activate T cells

32

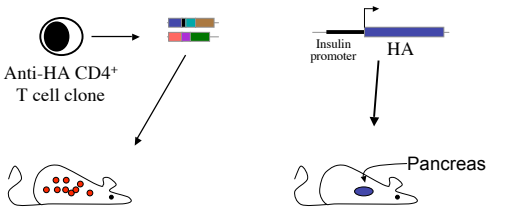
Signal 1 without Signal 2 leads to "Anergy"

T cell Activation

T cell Anergy (unresponsive state)



Experimental evidence for signal 2



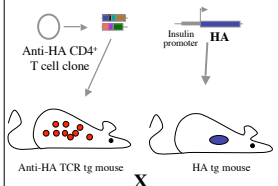
Anti-HA TCR tg mouse
(HA = hemaglutinin, an influenza virus protein)

HA tg mouse
(Expresses HA in beta cells of the pancreas)

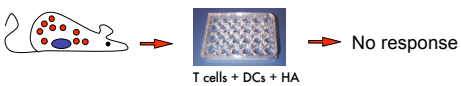
34

Experimental evidence for signal 2

Double-transgenic mouse



- Large numbers of anti-HA CD4+ T cells
- HA expressed in the pancreas.
- But no pancreatic infiltrate or diabetes!

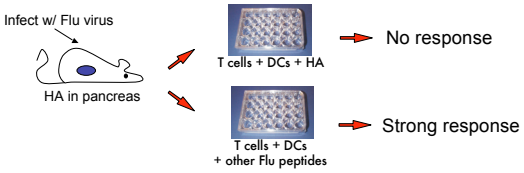


Potentially self-reactive cells are ANERGIC

35

Potentially self-reactive cells are Anergic

Influenza infection: normal immune response except no anti-HA T cells produced



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Summary II

Naïve T cells require 2 signals to be activated:
1 - TCR
2 - CD28 (costimulation)

Signal 1 in the absence of signal 2 leads to anergy

Anergic T cells are unresponsive to further stimulation, even in the presence of costimulation.

Anergy is thought to be a mechanism of ensuring tolerance to peripheral antigens not expressed in the thymus.

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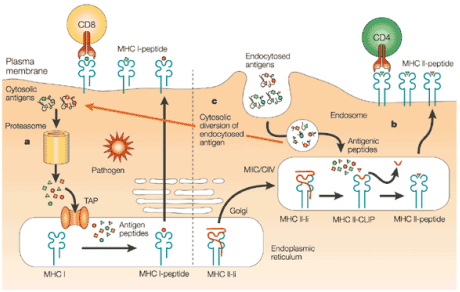
Cross-priming

To generate cytotoxic killer cells, which have the capacity to eliminate infected cells and attack transplants and tumour cells, DCs have to present antigenic peptides complexed to MHC class I molecules to CD8-expressing T cells.

- Straightforward if the DC is infected itself (ie. influenza virus).

- How DCs could process and present antigens that have no access to the cytosol in an MHC class-I-restricted manner (for instance transplant- and tumour-derived antigens, or antigens from viruses that cannot infect DCs)?

38



Nature Reviews | Immunology

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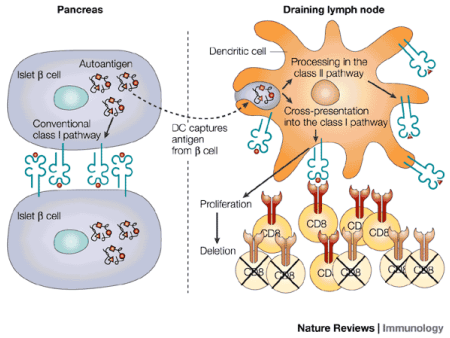
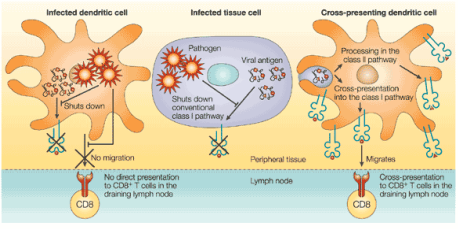


Nature Reviews | Immunology

40



Nature Reviews | Immunology

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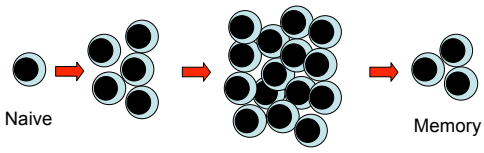
Once the T cell response is started, how is it turned off?



T cell activation is linked to signals that eventually limit activation and remove most activated cells.
CTLA-4 blocks costimulation
Fas induces apoptosis

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CD28 and CTLA-4

- CTLA-4, also expressed by T cells, is a CD28 homologue
- CD28 sends a positive co-stimulatory signal, enhancing T cell activation
- CTLA-4 sends an inhibitory signal
- TCR + CD28 signaling leads to upregulation of CTLA-4
- CTLA-4 has a much higher affinity for B7 than does CD28, so it outcompetes CD28 for B7 binding.

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T-cell activation through TCR and CD28 leads to surface expression of CTLA4

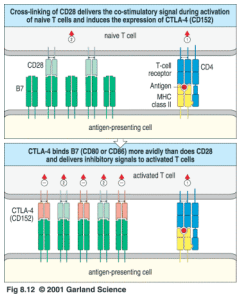

Fig 8.12 © 2001 Garland Science

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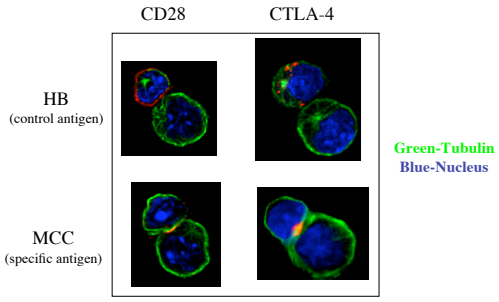
Antigen Induced Protein Translocation in 5C.C7 T Cells



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Manipulating signal 2

CTLA-4 null mouse. Dies of massive lymphadenopathy at several weeks of age. Idea is that there is nothing to counteract T cell activation
CD28 null mouse. Hyporesponsive immune system, but normal T cell development.
Transfect B7 into non-APC and convert it into effective APC. Consider implications for vaccine production
CTLA-4-Ig can inhibit immune responses (consider mechanism and potential uses).
Anti-CTLA-4-antibody can contribute to a more vigorous immune response.

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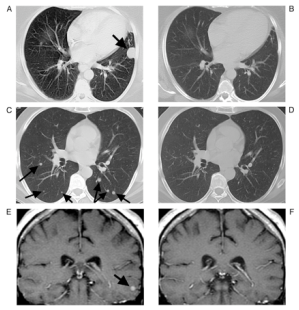
Cancer regression and autoimmunity induced by CTLA4 blockade in patients with metastatic melanoma



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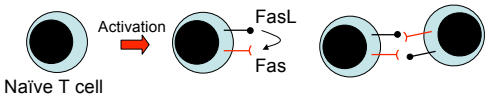
Activated T cells also upregulate Fas



Both Fas and Fas ligand (FasL) are upregulated on activated T cells

Signaling through Fas leads to activation of caspases and programmed cell death (apoptosis).

Mutations in Fas or FasL lead to lympoproliferative and autoimmune disorders

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Fas signaling leads to programmed cell death

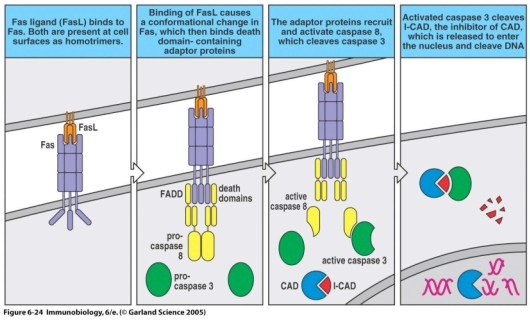

Figure 6-24 Immunobiology, 6/e. (© Garland Science 2005)

One the T cell response is started, how is it turned off?

CTLA-4 - puts on the brakes
- by competing for B7 molecules and sending inhibitory signals instead of activating

Fas - gets rid of unwanted T cells
- by inducing apoptosis in most of the activated T cells (after they do their job).

Both of these signals are induced by the TCR.

50

Summary III

Naive T cells leave the thymus and enter secondary lymphoid organs.

In secondary lymphoid organs, naïve T cells are activated by mature dendritic cells.

T cell activation requires 2 signals: TCR and costimulation.

Lack of costimulation during T cell activation leads to anergy.

T cell responses are downregulated by CTLA-4 and Fas
- CTLA4 competes for B7 binding
- Fas induces apoptosis

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