From : diann e@aol.co m
Date : Tue, 23 Aug 2005 09 :56 :17 EDT
Sub ject: CA PRI and NF2
To: smg8@colum bia.e du

Dear Dr. Greenberg,

As I read more and more about NF2, and Rac, Ras and Cdc42, it would seem CAPRI would have some role in NF2. What are your thoughts about that? Could you email me your article on CAPRI? I was only able to access the abstract.

My son has NF2. He is half blind and half deaf and is fighting many more brain and spinal tumors, which are progressing.

I've been following Pak inhibitors for NF2.

Please help me save my son.

Dianne

NF2: Neurofibromatosis 2, an inherited autosomal dominant disease characterized by multiple CNS tumors (schwannomas, meningiomas, and ependymomas) resulting from mutations in *merlin*

Merlin: An actin binding protein that inhibits Ras-induced foci-formation and associated AP-1 activity. Merlin blocks Ras-induced Rb phosphorylation, and inhibits the increase in cyclin D1 levels.

Rac, Ras, Cdc42: GTPases involved in cell signaling. Ras activates the MAP kinase pathway. Rac and Cdc42 activate cytoskeletal alterations and activation of the protein kinase, PAK

PAK: A protein serine/threonine kinase

CAPRI: An adaptor protein for Rac

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The *NF2* Tumor Suppressor Gene Product, Merlin, Inhibits Cell Proliferation and Cell Cycle Progression by Repressing Cyclin D1 Expression

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Inactivation of the *NF2* tumor suppressor gene has been observed in certain benign and malignant tumors. Recent studies have demonstrated that merlin, the product of the *NF2* gene, is regulated by Rac/PAK signaling. However, the mechanism by which merlin acts as a tumor suppressor has remained obscure. In this report, we show that adenovirus-mediated expression of merlin in *NF2*-deficient tumor cells inhibits cell proliferation and arrests cells at G_1 phase, concomitant with decreased expression of cyclin D1, inhibition of CDK4 activity, and dephosphorylation of pRB. The effect of merlin on cell cycle progression was partially overridden by ectopic expression of cyclin D1. RNA interference experiments showed that silencing of the endogenous *NF2* gene results in upregulation of cyclin D1 and S-phase entry. Furthermore, PAK1-stimulated cyclin D1 promoter activity was repressed by cotransfection of *NF2*, and PAK activity was inhibited by expression of merlin. Interestingly, the S518A mutant form of merlin, which is refractory to phosphorylation by PAK, was more efficient than the wild-type protein in inhibiting cell cycle progression and in repressing cyclin D1 promoter activity. Collectively, our data indicate that merlin exerts its antiproliferative effect, at least in part, via repression of PAK-induced cyclin D1 expression, suggesting a unifying mechanism by which merlin inactivation might contribute to the overgrowth seen in both noninvasive and malignant tumors.

Neurofibromatosis type 2 (NF2) is an autosomal dominant disorder that affects 1 in 33,000 to 40,000 people, with about one-half of cases representing new mutations. Germ line mutations of the *NF2* gene predispose individuals to the development of multiple benign tumors of the nervous system, the most common of which is bilateral vestibular schwannoma. Other tumor types include schwannomas of other cranial, spinal, and cutaneous nerves and cranial and spinal meningiomas, as well as ependymomas and gliomas of the central nervous system (9). The *NF2* gene is also implicated in the development of sporadic schwannomas and meningiomas, as well as in tumor types seemingly unrelated to the NF2 disorder, particularly malignant mesotheliomas, which are mesodermally derived, primarily pleural tumors (5–7, 29). Mutations of the *NF2* gene are present in about 50% of malignant mesotheliomas, and loss of heterozygosity at the *NF2* locus resulting in biallelic inactivation was reported in all cases of mesothelioma with *NF2* mutations (6). Heterozygous *Nf2* knockout mice develop a variety of metastatic tumors, mostly osteosarcomas (24), whereas conditional homozygous *Nf2* knockout mice, targeting Schwann cells, develop Schwann cell hyperplasia and schwannomas (10). How *NF2* inactivation plays a role in both benign and malignant tumors has remained obscure.

Consistent with its role as a tumor suppressor, ectopic expression of merlin in a variety of cell types has demonstrated that merlin plays an important role in regulating cell proliferation. Experiments with NIH 3T3 fibroblasts revealed that merlin can reverse Ras-induced anchorage-independent growth (33) and inhibit cell proliferation (22). Reexpression of merlin in schwannoma cells suppressed cell growth, which was accompanied by cell cycle arrest at G_0/G_1 (25, 28, 31). In *Drosophila melanogaster* mosaic tissue, *Merlin* mutant cells proliferate more rapidly than their wild-type neighboring cells (19). Despite these observations, the mechanism by which merlin regulates cell proliferation is not well understood.

Several lines of evidence have implicated merlin in the regulation of Rac/PAK signaling. Rac plays an important role in the regulation of cytoskeletal organization and intracellular pathways involved in cellular proliferation, transformation, and transcriptional activation. Merlin localizes at membrane ruffles, which can be induced by Rac activity. Extensive membrane ruffling observed in schwannoma cells can be reversed by inhibiting Rac1 (27) or by TAT-mediated merlin protein transfer (4), suggesting that Rac activity is deregulated in schwannoma cells and that merlin plays a role in Rac signaling. We and others have demonstrated a link between merlin and Rho GTPase signaling (17, 30, 35). In response to active Rac or Cdc42, but not active Rho, merlin is phosphorylated on serine 518 (30), and this phosphorylation is mediated by the PAK family of serine/threonine kinases that are immediate downstream effectors of both Rac and Cdc42 (17, 35). Recently, it has been shown that merlin expression inhibits Rac/PAK activation, which may be attributed to merlin's tumor suppressor function (12, 18).

Here we report that adenovirus-mediated expression of merlin inhibits cell proliferation and blocks cell cycle progression

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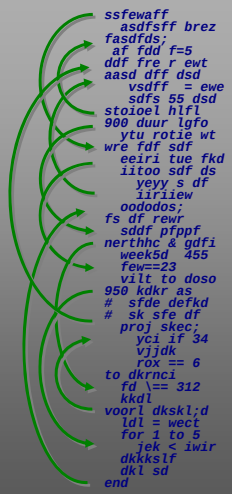
The Problem of Biological Complexity and "Information Overload"

Analogy: Structural Programming

In 1968 computer scientist Edsger Dijkstra wrote a now-classic short note entitled: "*GOTO Considered Harmful*."
(Dijkstra (1968) *Comm. ACM* **11**(3):147-148).

In it he criticized programming constructs that allowed undisciplined jumps in flow of control leading to so-called 'spaghetti code,' which made larger programs unwieldy



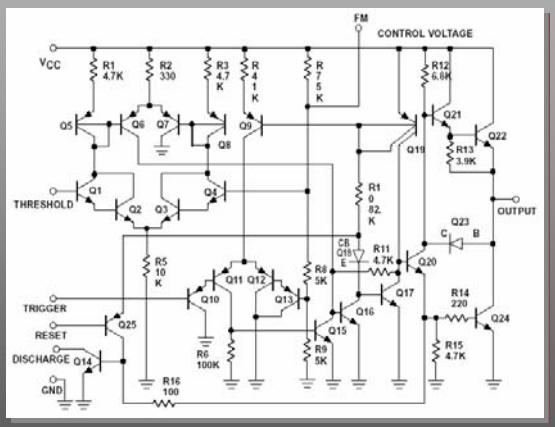
Courtesy: David Searls, Glaxo-Smith-Kline

Analogy: Structural Programming

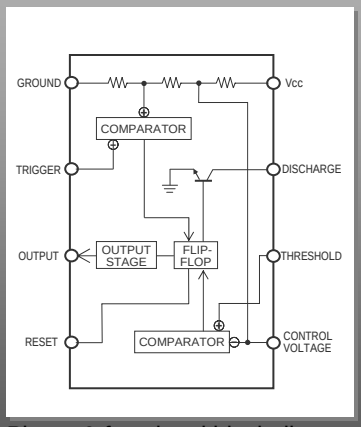
He helped to launch the structured programming movement, which enforced a strictly nested modularity for more manageable growth, debugging, modification, etc.

Courtesy: David Searls, Glaxo-Smith-Kline

Analogy: Modular Design of Integrated Circuits



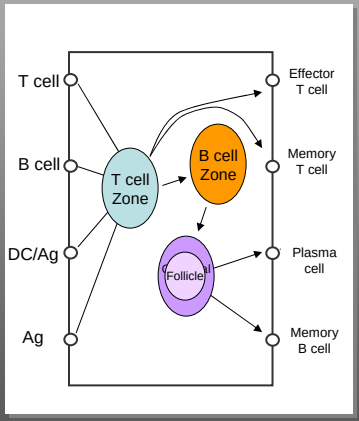
LM555 Timer



Pinout & functional block diagram

Courtesy: David Searls, Glaxo-Smith-Kline

The "Circuitry" of the Primary Immune Response



The "electronic lymph node"

Immunology-- The Whirlwind Tour

Ontogeny of the Acquired Immune System

Step 1. Lymphocytes develop in the bone marrow and thymus

Step 2. Naïve lymphocytes circulate in the blood and lymph

Step 3. The primary immune response occurs in the lymph nodes and spleen

Step 4. Lymphocytes exit the lymph nodes and spleen and become effector lymphocytes--they produce antibody (B cell-derived plasma cells) and become competent to produce cytokines, particularly CD4+ T cells, and kill (CD8+ T cells)

Stages in the Development of a Primary Immune Response

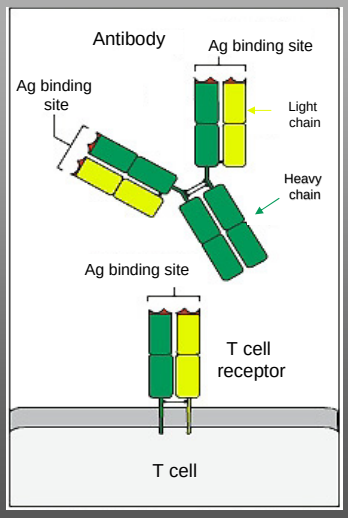
Step 1. Lymphocytes develop in the bone marrow and thymus

The immune repertoire develops

Lymphocytes develop early in life in the 1° lymphoid organs (bone marrow and thymus). They are competent to respond to a broad array of antigens. Diversity in antigen recognition is accomplished by random rearrangements of the **immunoglobulin (Ig) gene** in B cells and the **antigen receptor gene** in T cells (**TCR**).

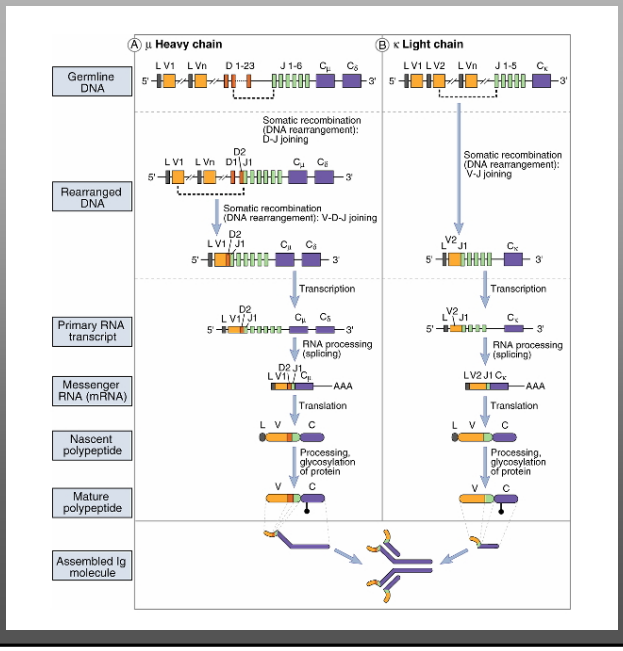
Those lymphocytes that survive do so through **positive** selection. Unproductive or inefficient interactions between lymphocyte and antigen results in death by **negative selection**.

Antibody (Ig) and TCR are the Only Genes that Undergo Somatic Cell Recombination



How is Diversity in Antigen Recognition Achieved?

Ig Maturation



What Happens in the Thymus?

Ordered TCR gene rearrangement and TCR expression

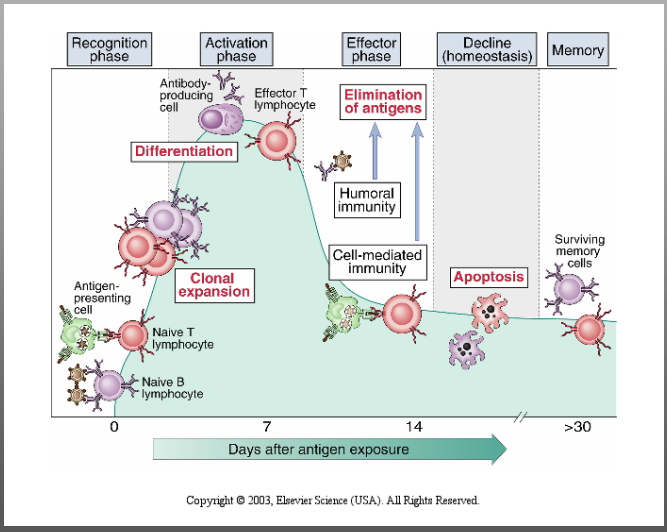
Ordered expression of surface molecules, including the TCR, CD4 and CD8

Thymocyte Education: Selection of the T cell repertoire through negative selection and positive selection

The Primary Immune Response

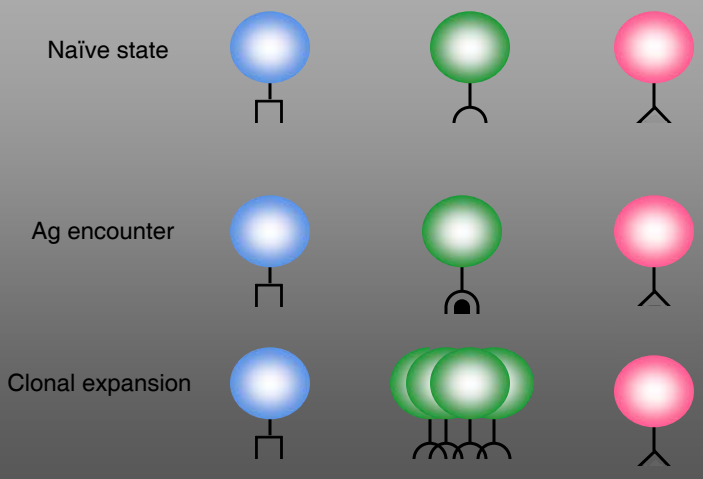
Input: Ag-loaded APCs and naïve lymphocytes
Output: Effector and memory lymphocytes

Phases of the Primary Immune Response



How Do the Relevant Lymphocytes "Know" to be Activated?

The Clonal Selection Theory



Three Types of APCs

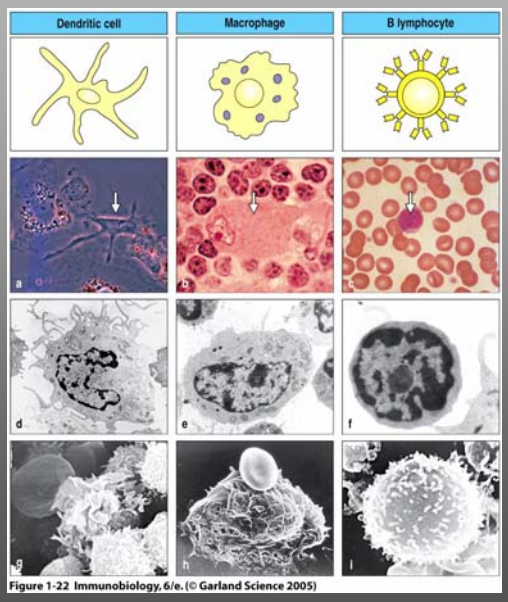


Figure 1-22 Immunobiology, 6/e. (© Garland Science 2005)

The Itinerant Dendritic Cell

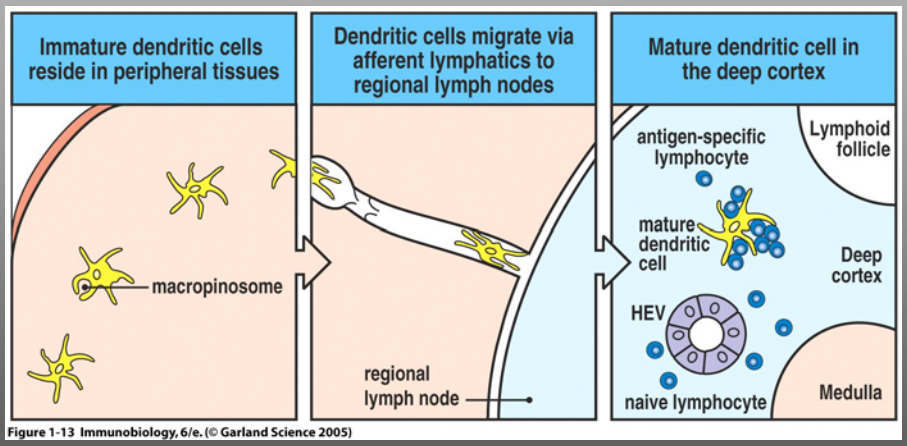
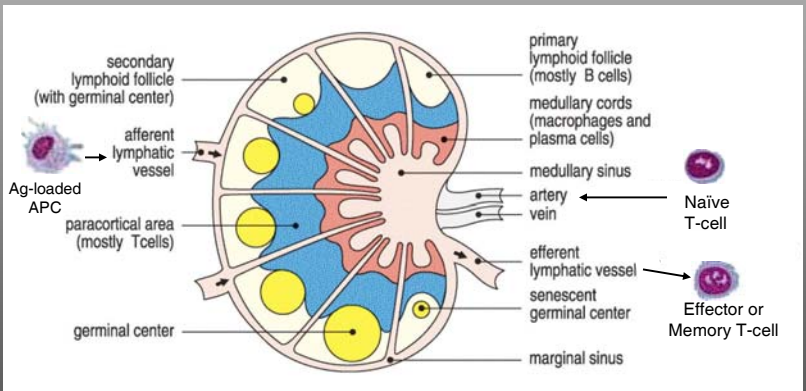


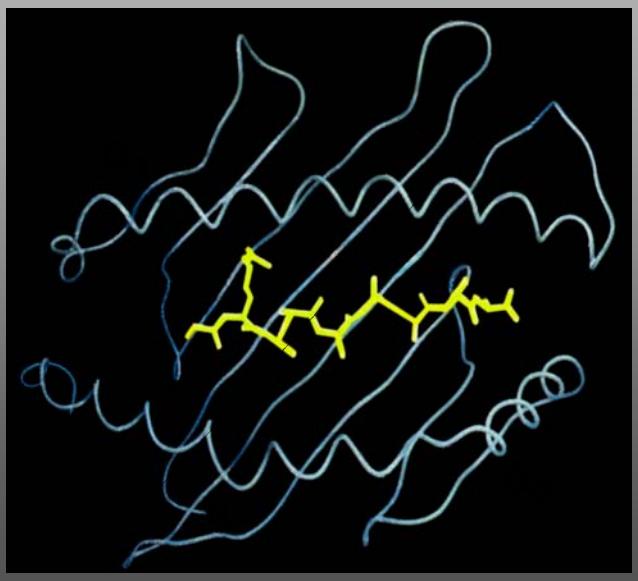
Figure 1-13 Immunobiology, 6/e. (© Garland Science 2005)

Functional Anatomy of a Lymph Node

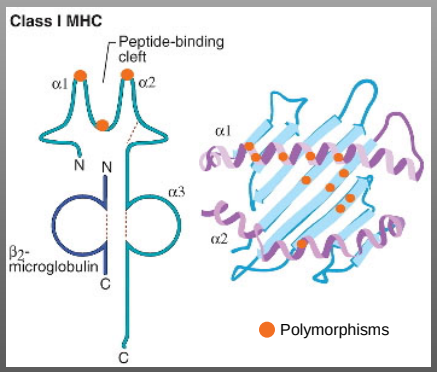


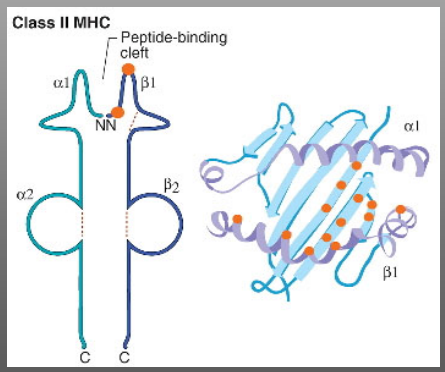
The Antigen "Vetting" Process: Who Decides Which Antigens are Presented

Structure of Peptide-binding Class I MHC Domains



Contact Between the TCR and MHC/peptide: Not All MHC Molecules are Created Equal

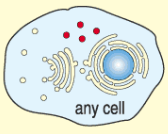
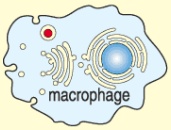
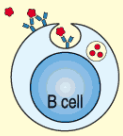




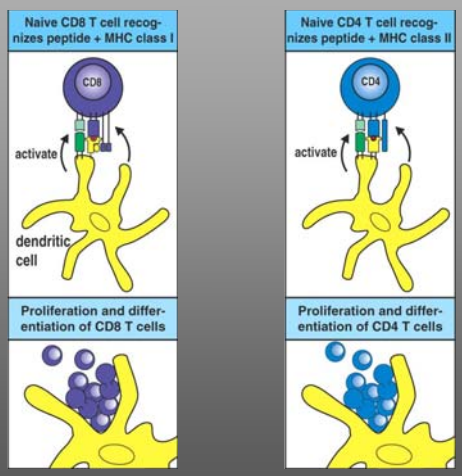
The "Fit" Between MHC Molecules and Peptide Defines MHC Restriction

Polymorphisms (allelic differences within a population) of the MHC loci account for the variability of the immune response between individuals

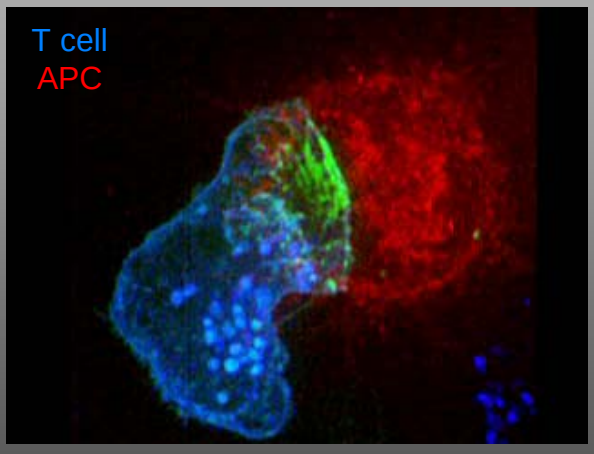
Functions of MHC I and II

	Cytosolic pathogens	Intravesicular pathogens	Extracellular pathogens and toxins
			
Degraded in	Cytosol	Endocytic vesicles (low pH)	Endocytic vesicles (low pH)
Peptides bind to	MHC class I	MHC class II	MHC class II
Presented to	CD8 T cells	CD4 T cells	CD4 T cells
Effect on presenting cell	Cell death	Activation to kill intravesicular bacteria and parasites	Activation of B cells to secrete Ig to eliminate extracellular bacteria/toxins

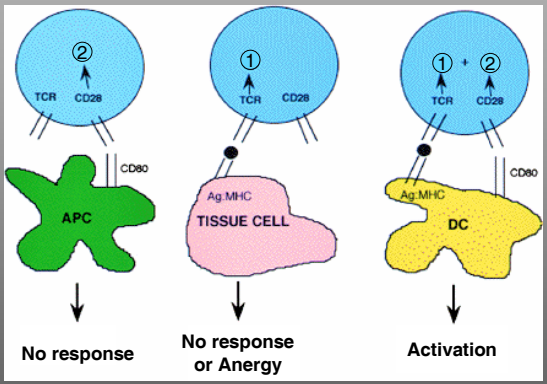
Antigen Presentation at the Cellular Level



The "Immunologic Synapse"



The Two-Signal Theory of T-cell Activation



APC = Antigen-presenting cells
TCR = T cell receptor for antigen
DC = Dendritic cell
CD80 = Co-stimulatory receptor

CD4+ T Cells Activate Macrophages and B cells

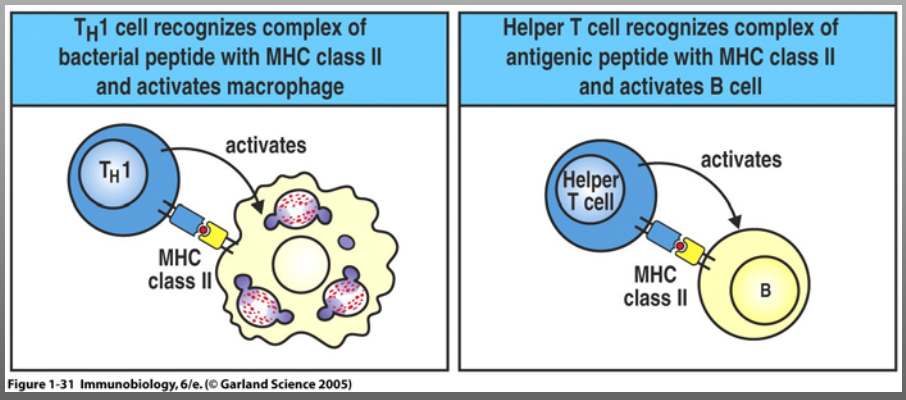
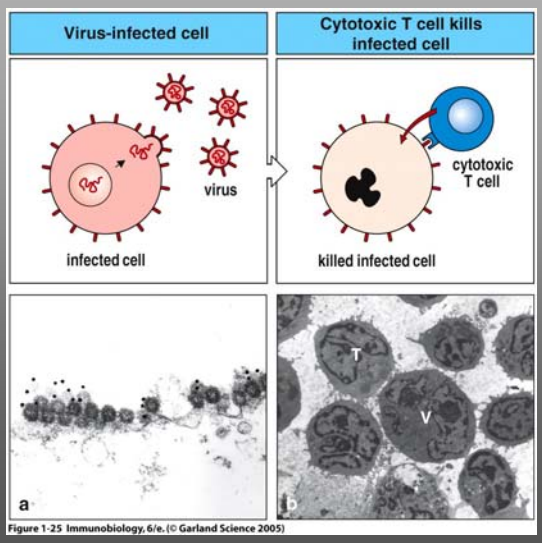


Figure 1-31 Immunobiology, 6/e. (© Garland Science 2005)

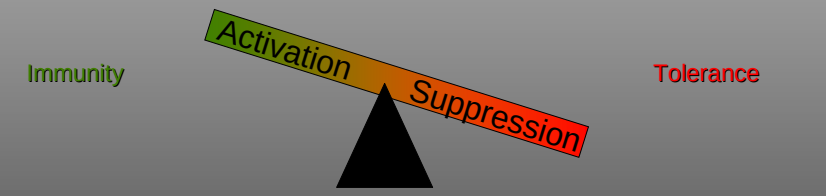
CD8+ CTLs Kill Viral-infected Cells



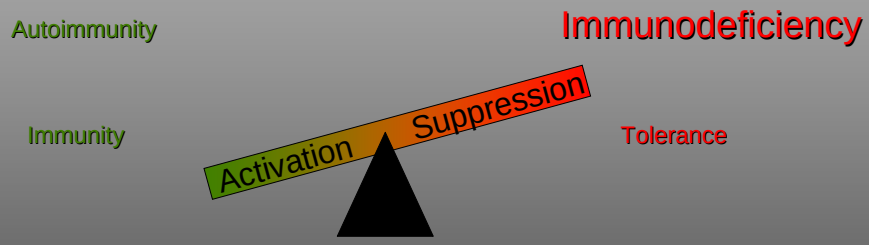
Regulation of the Immune Response: a Conceptual View

Immunity Activation Suppression Tolerance

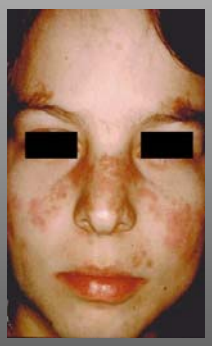
Regulation of the Immune Response: a Conceptual View

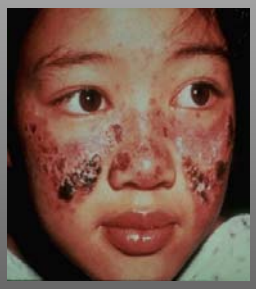


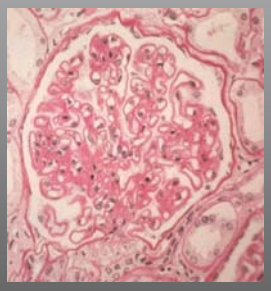
Regulation of the Immune Response: a Conceptual View



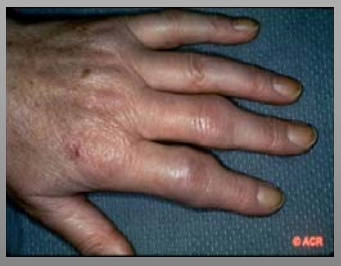
Systemic Lupus Erythematosis (SLE): An Autoimmune Disease

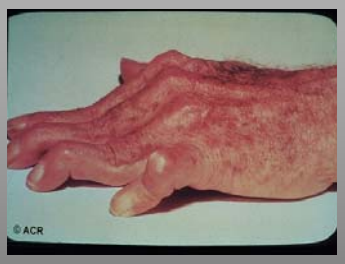


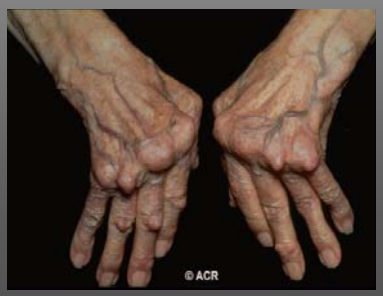




Clinical Manifestations of Rheumatoid Arthritis







Summary

1. The immune system is complex. Try to understand it in terms of specific functional modules.

2. Diversity in antigen recognition is accomplished, in part, by rearrangements in the Ig and TCR loci. This occurs in the bone marrow and thymus, respectively.

3. The T and B cell repertoire determines the spectrum of antigens that can be recognized in an individual's lifetime. The nature of this repertoire is determined by the Major Histocompatibility Complex (MHC), which binds peptide antigen.

4. In a primary immune response, antigen presenting cells (APCs) present antigen bound to MHC molecules to T cells in the lymph nodes and spleen. T cells "help" B cells to develop further and clonally expand in germinal centers of these organs.

5. Lymphocytes exit these organs to become effector or memory cells. Effector cells secrete Ab (plasma cells) or cytokines (CD4+ T cells) and kill virally-infected cells (CD8+ T cells). Memory cells re-circulate until they encounter Ag again.

6. The immune system is tightly regulated. It exists in a delicate balance of immunity and tolerance. A lack of tolerance to self antigen coupled to excessive immune activation (or inadequate immune suppression) can lead to autoimmunity.