LEARNING GOAL
Understanding in vitro assessment of immunologic responses that have contemporary clinical relevance

BACKGROUND READING
Janeway: 8th edition: P 749-756; 310. Optional article of interest is posted on the Web Forum

DEVELOPED BY
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IV. Immunologic Assays used for detection and assessment of antibody responses

A. One of the first methods used (and still is) in patients suspected to have hemolytic anemia (also known as antibody mediated hemolytic anemia) is the Coombs test. The fundamental question the clinician is asking is: is this patient making an antibody that binds to her own red cells and causing them to break up or be phagocytized and destroyed?

1. The question can be answered by incubating the patient’s erythrocytes with antibodies that react with the antibody isotype that is suspected of causing the hemolysis. For example, if IgG is thought to be the culprit, the laboratory would use anti-human IgG. If the patient had her own IgG bound to her own red cells, they would agglutinate, can then be seen by the naked eye, and the test would be positive.

![Direct Coombs Test](image)

2. The above figure depicts the classic Coombs test.
   a. Describe how you would set up appropriate controls for the Coombs test.

   b. How would the test be set up if you were not sure the hemolysis causing antibody was IgG?
c. How would the test be set up if you had only the patient’s serum but did not have her red blood cells?

**B. Immunofluorescence (fluorescent antibody techniques)** - the principle underlining this method exploits the ability of certain chemical compounds to emit light of restricted wavelength during ultraviolet excitation.

1. **Direct fluorescence.** If these light emitting compounds (tags) are linked to antibodies that **directly bind** to antigens (for example in tissue biopsies) or **indirectly bind** to a patient’s immunoglobulin that has **previously bound** to antigens in tissue (or test substrates), the specific binding can be documented by direct microscopic visualization during UV excitation.

2. **Indirect immunofluorescence.** The indirect fluorescent method is useful for detecting and quantitating by titer, patient antibodies to various antigens. One widely used assay is for the detection of anti-nuclear antibodies in systemic lupus. Patient serum containing possible antibodies to nuclei is layered on a substrate rich with nuclei, incubated, washed and then anti-human Ig-fluorochrome is added. If there were specific anti-nuclear antibodies in the patient serum they would bind to the nuclear antigens. This complex, in turn, would then react with antihuman Ig - fluorochrome, and UV light passing through the sample then emits green fluorescence at the site of binding which can be detected by fluorescence microscopy.
3. You only need to understand the concept of this method at this time-specific ways to use it will become evident in a later Small Group.

V. Techniques that can be used to identify and quantify immune cell populations.

A. FLOW CYTOMETRY is a technique that can provide information on the characteristic of single cells in a mixed cell population. This technique also depends on tagging cells with specific light emitting compounds. The cell population to be studied is shepherded into a thin stream of single cells that have been previously incubated with the desired fluorescent- labeled monoclonal (For production of the latter, see figure below) or a monospecific antibody probe. Be sure you understand how to use flow cytometry for diagnosis-it is always on the test.

1. Flow cytometry is heavily dependent upon availability of monoclonal antibodies. These reagents have high specificity for individual antigens. Their use is widespread in basic research, clinical testing and now have many clinical applications in cancer, inflammatory arthritis and many other diseases.

2. You need to understand how monoclonals are made.

From: immunobiology, 8th edn.
Author: Janeway, et al
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a. If you understand how a monoclonal is made, you should immediately be able to see how this technology could be applied to the bird flu case in the prior small group.

3. **Back to flow cytometry technique.** The single cell queue flows past a laser that emits a light wavelength unique to the characteristics of the fluorochrome. If a cell has bound the antibody-fluorochrome, the emitted wavelength is then photo multiplied and quantitated by computer software. A cartoon of the technique is shown below. A typical plot using two monoclonal antibodies, each with a different light emitting fluorochrome, is depicted below on the right.
4. **A clinical scenario where flow analysis can be used**

   a. 84 year male fainted during a church service. A complete blood count in the ER revealed a markedly reduced hemoglobin, a low platelet count and his white blood cell (leukocyte) count was **100,000/mm³** (normal 4-10,000).

   b. Set up the flow cytometry experiments to solve the following questions:

   1. **Lineage** of his white blood cells- does he have a leukemia- abnormal high # of white cells in his blood- and if so, what kind? For example, lymphocytic, myeloblastic, etc.

   2. **Phenotype** of the white blood cells

   3. **Clonality** of the white blood cells
      a. describe what diagnostic antibodies could be used to prove that the majority of his white blood cells were B cell descendants of a single B cell clone.
b. Describe how you could prove that this patient had a T cell leukemia (malignant proliferation of a T cell clone) by flow cytometry, not Southern Blot or PCR.

VI. Techniques that characterize clonality of serum immunoglobulins.

A. Serum protein electrophoresis. Electrophoresis separates proteins based on their size and charge. This simple, cheap technique can be used to confirm intense activation of B cells or production of a homogeneous immunoglobulin by an expanded clone of B cells. In human serum, albumin is negatively charged and migrates to the positive pole, while the immunoglobulin family does the opposite (and by coincidence, was named the gamma region). If the electrophoresis is carried out in a gel or on a paper medium, an optical reader will roughly quantify the separated components and produce a tracing (see below). One can then stain the component with specific antibodies to identify its composition. This second step is termed immunofixation and common reagents that are used are anti-human kappa, lambda, and heavy chain antibodies.

![Electrophoresis Tracing](image)
If a **single** clone of plasma cells is expanding, the immunoglobulin the clone produces will be homogeneous with respect to size and charge. All the **monoclonal** antibody that single clone makes will migrate to a single point in an electrophoretic field. However, if a normal individual is undergoing intense, persistent antigenic stimulation, a sequence of plasma cells with slightly different antigenic specificity to the inciting antigen will make antibodies. Each plasma cell clone will produce an antibody of slightly different charge/size that migrates to its own unique site in the electrical field. You will see in a later small group how increases in **polyclonal** antibody can be used diagnostically.

1. Just to be sure you understand the concept, explain how you could you **specifically** identify the following serum protein abnormality in the gamma region of the serum electrophoresis shown below:

![Electrophoresis Diagram]

2. What would a polyclonal increase in serum IgG look like on a serum protein electrophoresis?
T-CELL IMMUNITY 1 and 2

Date: Wednesday, April 3rd & Thursday, April 4th, 2013
Time: 9:00 AM & 10:30 AM, respectively

LEARNING GOALS
You will be able to understand the complex relationships between cytokines and immune effector cells and identify the differences between and unique aspects of T-cell mediated responses.

OBJECTIVES
You will be able to:
• Identify mechanisms that generate T-cell mediated macrophage immunity
• Identify mechanisms that generate lymphocyte cytotoxic responses
• Identify specific regulatory mechanisms that control immune responses
• Identify the T cell pathway to chronic inflammatory and auto-immune responses

BACKGROUND READING

As always, do NOT memorize any Table or Figure in background reading.
WARNING! USE THE SUPPLEMENTARY CYTOKINE TABLE WITH THE LECTURE NOTES, NOT THE ONE(S) IN THE TEXT

LECTURER
John A. Robinson, M.D.
Introduction: T Lymphocytes.

I. CD3+ 4+ T lymphocytes. T directed immune responses are heterogeneous and based upon the development of specific subsets of T cells identified by unique cytokine profiles and functions. The terminology here is unfortunate and needs to change. The CD3,4 T cell is conventionally designated a T helper (Th) cell because the original functions of these cells were ones that “helped” promote immune responses. They do many more things than “help”. At this point we are stuck with the term-Th cell.

A. The fundamental rules of T cell immunity.
   1. T cells are the orchestrators of adaptive immune responses
   2. T lymphocytes are required for the optimal development of both cell mediated immunity (CMI) and antibody responses by B cells (also called the humoral response or humoral immunity).
   3. T cell responses are regulated by specific cytokines and T regulator cells (Treg).
   4. In contrast to antibodies that recognize three dimensional conformations of antigens, all T-cells recognize antigens as fragments of macromolecules presented to them in the context of self-MHC.
   5. Antigen presented in the context of Class I MHC is recognized by CD8 T-cells.
   6. If the antigen is processed and presented by Class II MHC of dendritic cells, macrophages (professional APCs) or B cells to a naïve CD4 T helper cell - one of 4 responses-Th1, Th2, Th17 or T regulator - will occur.
   7. Commitment to a subset depends upon host genetics, the type of infection and which type of TLR and cytokine profile dominates the early phase of T-cell activation.
   8. Each Th subset has specific functions and is associated with specific cytokine profiles. The Th1 subset enhances and amplifies cellular mediated immunity (CMI), mainly by activating macrophage defense mechanisms and promoting cytotoxic responses by CD8 lymphocytes. The Th2 helper subset promotes optimal antibody production. The Th17 subset promotes chronic inflammation and T regs modulate or suppress immune responses.

II. The type of threat and the genes that drive TLR and cytokine expression dictate the type of the immune response. This concept is the critical for understanding how a wide
spectrum of infectious and autoimmune diseases can occur in humans.

III. A CD3, 4 T helper cell can be directed 4 different ways (maybe 5 ways but will touch on that later!).

A. The “classic” Th1 reaction - also called T cell mediated macrophage immunity (TMMI).

1. Any scenario that requires uptake of a complex antigen, either alone or as part of an infecting organism, by a dendritic cell (DC), will eventuate in the presentation of processed antigens on MHC Class II and simultaneous production of specific cytokine profiles. If the dominant cytokine produced by the APC (usually a DC) is IL-12, T cell mediated macrophage immunity (TMMI) is the end result.

2. The recognition and uptake of an intracellular pathogen or its complex antigens by DC triggers maturation of the immature DC that is characterized by:
   a. Termination of phagocytosis
   b. Intracellular processing of the pathogen proteins to peptides
   c. Upregulation of MHC II on their cell surface
   d. Upregulation of co-stimulatory molecules on their cell surface
   e. Migration to lymphoid tissue
   f. Upregulated synthesis of IL-12 & 18.
   g. presentation of the processed antigen in their MHC-II

3. IL-12 is the obligatory initiation cytokine for a Th1 reaction. IL-18 is also a critical cytokine for initiating Th1 reactions and its presence strongly amplifies all IL-12 properties.

4. The 2 cytokines, IL-12 & 18, initiate the commitment of a Th0 (uncommitted helper T cell) cell to a Th1 subset and also have potent activation effects on NK cells. The committed Th1 cell can be identified by the induction of the transcription factor “master regulator” T-bet.

5. When antigen specific Th1 cells bind to antigen in the presence of these initiation cytokines, the Th1 cell upregulates CD 28 and CD154 (40L). CD28/B7 and CD40/40L interactions are critical co-stimulatory signals required during T and B cell mediated immune reactions.

6. The committed Th1 cell then produces IFN-γ, a cytokine with a central role in Th1 responses.

Concepts: Th1 Initiation steps
a. **IFN-γ** is produced *predominately by CD4 subset (Th1) lymphocytes and natural killer cells* but also by activated CD8 cells and γδ T cells

b. **IFN-γ receptors** can be found or induced on almost all cell types

7. The introduction of **INF-γ** into the sequence is the pivotal step for *amplifying T cell mediated macrophage activation.*

a. **IFN-γ is a powerful activator of macrophages and the signature cytokine of an ongoing Th1 reaction.**

b. **IFN-γ is also a potent inducer of endothelial adhesion and homing receptors that attract additional effector cell traffic to the area.**

c. Another critical role of **IFN-γ** is its ability to control the display of MHC-I and II determinants on APC such as DC, M/M and endothelium. Thus, IFN-γ upregulates an antigen specific response in exponential fashion by controlling MHC elements.

8. Propagation and maintenance of the TMMI response
   a. Th1 cells, after antigen receptor binding to antigen (Signal 1) and activation by IL-12 and IL-18 (Signal 2), rapidly up-regulate synthesis of **IL-2 and increase the display of IL-2 receptors on their cell surface.**

b. IL-2 is the critical growth cytokine in Th1 reactions that stimulates and supports the rapid proliferation of antigen stimulated T cells.

   i. It is produced by activated T-helper (Th) cells, acts in an **autocrine or paracrine** fashion, and also enhances NK and B-cell responses.

   ii. The high affinity IL-2 **receptor** consists of 3 polypeptide chains that are only expressed as a functional unit after specific antigen TCR interactions. One of the three chains is also expressed in several other cytokines providing important redundancy for T-cell growth requirements

   iii. Genetic defects in the assembly of the IL-2 receptor have already been shown to result in a severe immunodeficiency state (will discuss later in a small group).

9. **IFN-γ** continues to be synthesized and released by the expanding IL-2 stimulated Th1 cells.
10. IFN-γ is a potent down-regulator of the Th-2 and Th17 (more later) subsets. As long as IFN-γ is present in dominant concentrations, development of the Th2 and Th17 subsets are strongly inhibited.

![Th1 Propagation and Maintenance](image)

Figure by J. Robinson

11. Il-12 activated Th1 cells also produce IL-21 - a very potent activator of CD8 cytotoxic cells (explanation to follow shortly)

12. The end result of T cell mediated macrophage immunity (TMMI). (Please note: the initiation and activation steps of Th1 & Th2 reactions usually occur in a lymph node or spleen and the in situ details will be discussed in detail by Dr Clancy in a later lecture.)

   a. The operational purpose of Th1 activation is to provide an antigen specific, efficient and rapid way for the immune system to recruit activated killer cells to the site of the infection.

   i. A small number of antigen specific cells are able to recruit a large number of neutrophils and macrophages to resist the pathogen.
ii. The major activating cytokine is IFN-γ, the Th1 derived cytokine that stimulates killer cells to activate their hydrolytic and oxidizing systems and markedly increase the production of proinflammatory cytokines.

iii. The activation sequence occurs in lymphoid tissue, the activated cells then migrate back to site of antigen uptake to mediate the reaction.

B. PRO-INFLAMMATORY CYTOKINES-these cytokines have a major role in promoting inflammatory responses by activating immune effector cells and recruiting the final arbiters of inflammation, e.g. neutrophils and monocytes, to sites of inflammation. The classic tetrad of pro-inflammatory cytokines, IL-1, IL-6, IL-8 and TNF-α, is produced in vast amounts by activated macrophages.

1. IL-1 has an extraordinarily wide range of biological effects that are not restricted to the immune system. IL-1:
   a. can be viewed as a prototype primordial cytokine with broad spectrum effects that facilitate host reactions to stress and infection.
   b. is produced by a wide range of cell types, especially M/M and keratinocytes that respond to other cytokine stimuli, a wide diversity of microbial products, crystals such as silica, and even certain UV wavelengths.
   c. is a cardinal pro-inflammatory mediator that can, in concert with IL-6, elevate body temperature by its effects on the hypothalamus, mobilize neutrophils from the bone marrow and induce marrow colony stimulating growth factors that further accelerate the production of leukocytes.
   d. also has neuroendocrine effects that act through the pituitary axis to cause the release of ACTH and adrenal corticosteroids in response to stress. (later in Course by Dr. Mathews)
   e. is critical in the early portion of immunologic reactions where it facilitates T-cell responsiveness to IL-2.
   f. acts in autocrine fashion to stimulate antigen presenting cells to be more efficient antigen presenters
   g. IL-1ra is the naturally occurring antagonist of IL-1 and does so by competing for IL-1 receptors. It clearly is synthesized to prevent the severe morbidity of an unbridled IL-1 response and this property is now exploited clinically.
2. **IL-6** demonstrates many extremely redundant, broad spectrum biologic effects characteristic of IL-1. In many circumstances, IL-6 is actually synergistic in the presence of IL-1, induces IL-2 responsiveness in T-cells and is a major requirement for certain Th subset activation, especially Th17. IL-6 has potent central nervous system, hepatic and hematological effects. IL-6 differs from IL-1 in that it has growth and differentiation effects on B-cells and bone calcium metabolism.

3. **TNF-α**, a cytokine polypeptide produced by activated M/M (and many other cells), plays a **central role** in the immune system.
   a. It has very broad and predominantly pro-inflammatory effects.
   b. Under normal, **non-inflammatory** conditions, TNF-α is critical for **maintaining immunologic homeostasis**, presumably by regulating apoptosis. Its pivotal role can be inferred by the fact that the TNF gene complex is located within the MHC complex.
   c. This cytokine is an extraordinarily potent M/M activator that confers the ability to kill tumor cells and many microbial pathogens.
   d. TNFα activates endothelium, promotes vasodilatation and increases MHC expression.
   e. During systemic infections, TNF-α, in concert with IL-6, can produce important clinical morbidity that ranges from flu-like symptoms during viral infections to septic shock, the adult respiratory distress syndrome, severe muscle wasting and overall inanition.
   f. The use of anti-TNF biological agents has proven very effective in diseases with uncontrolled inflammation, especially rheumatoid arthritis and psoriasis.

4. **IL-8** is classified as a chemokine, but functions as a cytokine.
   a. It is a major, but not sole, stimulus for proliferation, mobilization and recruitment of **neutrophils** to a site of infection. It is produced by neutrophils, macrophages, and many other cell types.

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**ACTIVATION OF MACROPHAGES**

Figure by John A. Robinson, MD
C. What does “delayed hypersensitivity” mean?

1. **Delayed hypersensitivity (DH)** – is nothing more than an archaic term for **T-cell mediated macrophage immunity (TMMI)**. Unfortunately, DH is firmly entrenched in both the experimental and clinical literature. It is commonly applied to clinical situations where macrophage activation is a central component of the disease pathology, for example, tuberculosis.

2. The simplest way to clinically visualize TMMI/DH might be the following: If an intern is exposed to a patient with tuberculosis, the intern may become infected with tubercle bacilli transferred by the coughing patient. If the intern is healthy, the tuberculous infection is usually localized to a small area of the lung because it is **self-limited by a successful defensive TMMI (DH) reaction that was initiated by**:

   a. **Infected Macrophages and DC migrating to regional lymphoid tissue (hilar nodes in this case)** and then presenting tuberculous antigens in the context of MHC Class II to CD4 T-helper cells.

   b. The CD4 T cells specific for the tuberculous antigens are activated (armed), proliferate and **migrate back to the site of infection where** they encounter TB antigen, secrete the appropriate cytokines and activate macrophages which are then able to kill the intracellular tuberculosis organisms.

3. The intern now has an expanded pool of TB reactive Th1 cells circulating in the blood and soft tissue. If **non-living** tuberculosis antigens are placed underneath the epidermis at some later date, a large, raised, red and tender lesion at the site of the antigen insertion will appear in approximately 48 hours (hence, the delayed terminology). This would be a positive TB skin test. The general immunologic principle governing this type of skin testing can be an effective in vivo way to measure T cell immunity to specific types of previous infections.

   a. A recent much needed improvement in TMMI/DH testing is the **Quantiferon Gold assay** which is both highly specific and sensitive. Mononuclear cells (mostly lymphocytes) from peripheral blood are incubated with highly specific TB antigens. If the patient has or has had TB, IFN-γ is released and can be detected.

V. **Lymphocyte Cytotoxic Immunity.** There are two major ways that lymphocytes can **directly** kill pathogens—one way is antigen specific, the following is not. Let’s digress a bit and get the non-T helper pathway done first

A. **Natural killer cells:**

1. NK cells are large granular lymphocytes that are evolutionarily “older”
than T cells and a key member of the innate lymphoid cell (ILC) system.

2. They express **no CD3 complex**, α, β, γ, δ chains and not only produce interferon-γ but are also strongly responsive to it and the “natural” interferons α and β that are produced by viral infected cells.

3. NK cells have no antigen specific receptors, are not MHC restricted and can provide immediate defense against virus or neoplasia transformed cells - thus buying time for antigen specific CTL to clonally proliferate.

4. The primary role of NK cells may be one of front line, initial defense and they probably represent a transitional cell bridging innate and adaptive immune responses. The response time differential between NKs and activated antigen specific T cells in the figure below emphasizes their ability to rapidly respond to a virus.

5. NK cell cytotoxicity is **suppressed** by killer cell inhibitory receptors (KIR) on their cell surface that recognize normal self MHC-Class 1 markers. In the absence of a self-MHC, the KIR will be turned off ONLY if the target cell has an NK activating receptor. How smart is evolution?- imagine what would happen to your erythrocytes if they had an NK activating receptor!

6. Not only can NK cells kill directly by cytotoxicity mechanisms similar to CD8+ cells but they can also mediate **antibody dependent cell mediated cytotoxicity (ADCC) via their Fc receptors**. This mechanism is similar to one used by M/M to kill antibody coated target cells.

7. Very recently, it has been found that NK cells may “remember” some pathogen encounters and, under certain conditions, may have regulatory functions.
B. CD 8 cytotoxic T-cells (CTL).

1. The CD3, 4+, Th1-cell, IL-12 activated subset is predominantly concerned with macrophage activation and optimizing CD8 cell functions. The CD3, 8+ subset has evolved a highly specific defensive capacity that enables this lineage to DIRECTLY kill foreign, infected or mutated cells by exquisitely antigen-specific cytotoxic mechanisms.

2. CD8 Cytotoxic T-cells (CTL) recognize endogenously synthesized antigenic peptides complexed with compatible MHC-1 molecules on target cells.

3. Activation of CD8 Cytotoxic T Lymphocytes (CTL).
   a. CD8 cells evolved as a defense against viral infections and probably also tumor cell emergence. The first activation step is endogenous (within the cell) production of a viral or tumor antigen, transport to the cell surface and presentation in the context of MHC Class I determinants.

   b. The antigen binding CD8 cell initially requires a co-stimulatory signal and has evolved multiple ways to receive it.
i. IFN-γ and IL-2 secreted by neighboring NK cells that have been activated during viral infection are critical activation signals.

ii. The most important influence on the development of a CTL is the parallel development of antigen specific CD4-T helper cells. Activated CD4 cells produce IFN-γ, IL-2 & IL-21 -There is no question that CD4 cells are vital to control of viral infection. HIV, a virus that eliminates CD4 cells, is associated with severe, often fatal viral infection.

iii. IL-21 acts as a potent proliferative stimulus to antigen activated CD8 cells. IL-21 also enhances CD8 killing mechanisms. Subsequent expansion of antigen specific CD8 cells by IL-21 during viral infections is massive and can approach 50% of all CD8 cells at the peak of infection.

iv. Once activated, CD8 cells do not require further co-stimulatory signals to maintain cytotoxicity. This makes sense: most viral infected cells are somatic (lung, gut, skin,etc) and will not have B7 on their surface.

v. Most APCs are not infected by viruses but they can and do recognize viruses via a TLR and phagocytize viral particles (especially antibody coated ones) and virus in the remnants of broken down viral infected cells. They display these processed antigens in MHC class II just like bacterial antigens with one important difference: the APC exports some of the antigen into the cytosol and loads them on to MHC Class I. Presentation in this way by an APC maximizes CD8 activation. The downside of cross-priming is eventual APC death.

Four mechanisms that initiate and enhance CD8 cytotoxicity
4. Specific Mechanisms of cytotoxicity

a. Recognition step: First, recognition of a cell that needs to be killed must occur either via the CTL MHC-class I peptide with its antigen specific TCR and accessory molecules or by a NK cell reacting to absence or altered MHC Class-I.

b. Rapid adhesion ensues (conjugate formation). Extensive contact develops between the target and CTL/NK.

c. The final killing steps are common to both CTL and NK cells:
   i. Pore formation in the target is induced by perforin. Perforin is a CTL intracytoplasmic protein with strong homologies to C9 - a complement component (future lecture)-that opens an actual physical pore in the target cell membrane that leads to osmotic instability.

   ii. Co-injection of CTL serine esterases (granzymes) may also be important. Both the esterases and perforin are prepackaged in the intracytoplasmic granules that are characteristic of all NK cells and become prominent in maturing CTL.

   iii. Apoptosis or programmed cell death is another important killing mechanism. This mechanism is induced by switching on death genes in the target cell via Fas/FasL signaling and activation of the CASPASE system.

d. The CTL or the NK then disengages, refreshes its killing capacity, and can bind to another viral infected cell.

e. Cytotoxicity must be controlled. What turns it off?
   i. successful elimination of antigen (usually viral) eliminates infected targets

   ii. Activated CD8 cells, in the absence of antigen expressing targets, cannot maintain expression of CD8 and become CD3+, 4- & 8-. This switches on apoptosis programs and the cells commit suicide via Fas/FasL

   iii. This eliminates about 90% of activated CD8s, the remainder switch to a memory mode (later in Course)

VI. The “classic” Th2 reaction
A. KEY CONCEPTS:

1. If the constellation of underlying factors that include type of antigen, type of epitope and its presentation and the genetic makeup of the individual designate that the initial or early cytokine profile is dominated by IL-4, The Th0 cell will differentiate as a CD4 Th2 cell. A committed Th2 cell can be identified by the transcription factor GATA-3.

2. If a Th1 (remember there are unique TLR for different classes of pathogens) Toll like receptor is not involved in the activation of the DC, IL-12 is not produced and Th1 response does not occur.

3. TLRs, appropriate for the threat, promote IL-4 production by DCs and Th2 differentiation when activated.

4. A cytokine profile dominated by the early appearance of IL-4 is the primary determinant of efficient development of high affinity specific antibodies and memory cells.

B. Activation of a Th2 reaction

1. Soluble antigens circulating in the blood, and extracellular pathogens (can live outside a host cell) promote Th-2 responses, most likely because they do not engage an appropriate Th1 TLR. This is the converse of Th1 activation when intracellular antigen or intracellular infection activates a response.

2. Pivotal cells that drive Th0 cells to the Th2 subset are:
   a. Dendritic cells under specific gene influence and unique antigens that produce IL-4
   b. committed Th2 cells that are producing IL-4
   c. B cell presentation of antigen promotes IL-4 by Th0 cells
   d. Mast cells/basophils- future lecture- that produce IL-4 & IL-13

3. Involvement of any or all of the above cells stimulates IL-4 dominance which then commits Th-O cells to the Th-2 subset. IL-4 simultaneously inhibits Th-1 TMMI and Th17 development.

4. IL-4 is the major B cell growth initiation cytokine and the principal driver of immune responses that require antibody formation.

5. The major, but not sole, source of IL-4 is the Th2-cell.

6. Activated Th2 cells also produce IL-21, IL-5, IL-6 and IL-10. These cytokines promote B cell cell growth and isotype switching.

7. IL-21, in the absence of IFN-γ and in the presence of IL-4, has a central role in the proliferation, differentiation and survival of B cells

11/29/12
8. IL-4 and IL-21 have potent effects on the differentiation characteristics of B lymphocytes and is an isotype switch factor that induces these IgM-producing B-cells to synthesize IgG and IgA.

9. IL-4, in conjunction with allergy promoting genes and IL-13, is an absolute requirement for an IgE response to parasites and other antigen specific IgE (allergic) responses- future lecture

10. Interleukin 4 and interleukin 13 have a very similar ancestry and structural homologies that are reflected in their many redundant and overlapping biologic activities

11. IL-13, like IL-4, is an antagonist cytokine produced by activated T-cells that down regulates cytotoxic and inflammatory functions of M/M and DC.

12. IL-10 produced by Th2 cells, DC & probably some T regulatory cells (future lecture) is a strong promoter of B cell differentiation, isotype switching and proliferation. **IL-10 is also a very potent suppressor of Th1 responses** and has a poorly defined but very important role in controlling inflammation in general.

13. Not **all** Th2 cells produce **all** Th2 cytokines in a given situation

**TH2-B CELL RESPONSE**
C. Functions of the Th2 Response

1. The role of the Th2 response is to enhance B-Cell function and ultimately antibody production.

2. Antibodies are used by the immune system to:
   a. make pathogens, including viruses, more attractive to NK cells, macrophages and neutrophils.
   b. recognize and mount effective immune responses to antigens that are not efficiently taken up by phagocytic cells.
   c. bind toxins for efficient elimination.
   d. target mutant and infected cells for killing.

THE LOGIC OF THE RESPONSE

General concepts of T-cell reactions should now be very evident:

- In general, the dominant T helper response is dictated by the type of infection, the type of TLR activated and the dominant cytokine(s) present.

- The biologic effects of IL-12 activated Th1, IL-23 activated Th17 (see below) and IL-4 activated Th2 subsets are unique to each subset and interference in the balance between the generation of these subsets has important implications on the manifestations of infectious, autoimmune and malignant diseases.

VII. Th17 helper cells.
A. Maintain your perspective and remember that the type of infection dictates the type of immune response!

B. Some bacteria that can live outside of cells (extracellular) and many forms of fungi trigger TLRs that in turn instruct DCs and macrophages to produce not IL-12 or 18 but a trio of initiating cytokines-TGF-β, IL-6 and the signature cytokine of the Th17 pathway, IL-23.

C. When the latter trio is the dominant cytokine milieu, a Th0 cell, in the presence of its antigen, will differentiate to a Th17 cell. The unique transcription “master regulator” factor for the Th17 cell is ROR-γt (just remember the ROR).

D. The differentiated Th17 cell produces IL-17. This interleukin is a potent recruiter of neutrophils, especially to skin and mucosal surfaces.

E. If either IL-4 or IFN-γ becomes the dominant cytokine in the immune reaction, Th17 activation and differentiation is strongly inhibited.
VIII. CD4, Th1 Regulator subsets are characterized by their ability to modulate/suppress immune responses. In the main, T regulators are CD4, 25+ and have a unique transcription factor “master regulator”- FoxP3.

A. T regs are generated in at least 2 different sites.
   1. Thymus where the AIRE gene complex influences their autoantigen specificity - Dr Le’s lecture last week

   2. Post-thymus - presumably in the peripheral lymphoid tissue where uncommitted Th cells, under the influence of regulatory cytokines like TGF-β and IL-10, and in the absence of significant concentrations of IL-6, develop the T reg phenotype CD4,25, FoxP3.

3. Dependant upon Il-2 for survival and proliferation

4. The T reg is the focus of intense research because it is possible that controlling their function may be beneficial in treatment of tumors and autoimmune disease.

5. More on T regs later in Course

IX. The newest T helper cell - the T follicular helper cell (Dr Clancy will discuss later)
   1. Restricted to B cell follicles in lymphoid tissue

   2. Can be identified by CD278 display and upregulation of IL-6 and
Il-21 receptors

3. In contrast to Th2, regulates antigen specific B cell responses

STUDY QUESTIONS:
1. List the important functions mediated by an IL-12 activated Th-1 cell.
2. List the important functions of a Th-2 cell.
3. Define the cytokine profile characteristic of the four Th1 subsets.
4. Describe the process by which an activated, antigen specific Th-1 cell can mediate TMMI
5. List the factors that dictate the cytokine profile of a T helper cell response.
6. Name and characterize critical interleukins produced by macrophages during a Th-1 response.
7. Define the pivotal role of INF-γ in cell mediated T-cell responses.
8. Contrast the antigen processing and presentation mechanisms of MHC class I and II cell.
9. Diagram two mechanisms of T-cell cytotoxicity.
10. Understand how IL-17 responses are controlled by other T cells

EXAMPLE OF TEST QUESTION
A specific TCR reaction with a peptide-MHC class I complex:
A. Generates only a TMMI response.
B. Will cause predominant Th-2 cell proliferation.
C. Begins the activation sequence of a CD8 cell.
D. Inhibits cytotoxic responses.
E. Promotes IgE responses in non-allergic individual.

Correct answer to above question: C
Where are we?

• The hard part—B cells and their genetics, MHC—are behind you
• The interesting part—understanding the complexity of the normal system—is in front of you.
• Down the line is extrapolating from the normal situation to clinical situation(s)

Cardinal Rules of T Cell Immunity

1. T cells are the orchestrators of immune responses
2. T Cells are necessary for optimal functioning of both cell mediated immunity and B cell immunity
3. T cell responses are regulated by specific cytokines and T regulator cells (Tr)
4. Antibodies recognize 3D conformations of antigens, T cells recognize peptides in the context of MHC.
5. Antigens presented in MHC-I are recognized by CD8 T cells
6. If an antigen is processed & presented by MHC-II by an APC to a naive CD4 T cell, one of 4 responses can occur: Th1, Th2, Th17 or T reg.
7. Commitment to a subset depends upon host genetics, type of infection and which type of TLR and cytokine profile dominates the early phase of T cell activation
8. Each subset has specific functions and is associated with specific cytokine profiles
Cardinal Rules of T Cell Immunity

8a. The Th1 subset enhances and amplifies cellular mediated immunity by activating macrophages and/or promoting cytotoxic responses by CD8 cells
8b. The Th2 subset promotes optimal antibody production
8c. Th17 subset promote chronic inflammation
8d. T reg subset modulates or suppresses immune responses

The universal rule of the immune response

• The type of threat and the genes that drive the TLR and cytokine expression dictate the type of immune response. This concept is critical for understanding how a wide spectrum of infectious and autoimmune diseases occur in humans

KEY CONCEPTS

• One of four things can occur when antigen is presented by Class II MHC to a CD4 Th0 cell
• Either a Th1(helper),Th2, Th17 or T regulatory response will ensue.
• Commitment to a subset will depend upon which TLR system is activated and which cytokine profile becomes dominant at the time of presentation
• The following initiation and activation steps occur in secondary lymphoid tissue
The CD4 family

T Cell mediated macrophage immunity (TMMI)-the “classic” T cell reaction

- Infections by organisms that require phagocytosis (uptake of a complex antigen) and intracellular killing provoke ‘classic’ Th1 responses.
- The trigger for TMMI always involves a TLR on a DC.
- Complex antigen phagocytosed by an DC will be presented in a modified form by MHC-II.
- The genetic background of the host will dictate the type and intensity of TLR activation.
- The recognition and uptake of a living pathogen or complex antigen by a DC triggers conversion of an ‘immature’ DC to a mature DC which then:
  - Processes the antigen to peptides
  - Upregulates its MHC-II
  - Upregulates co-stimulatory molecules
  - Migrates to lymphoid tissue
  - Upregulates production of cytokines IL-12 and IL-18

T Cell mediated macrophage immunity (TMMI)

- IL-12 (obligatory Th1 helper initiator) & IL-18 initiate the commitment of a Th0 cell to the Th1 subset and also have potent effects on NK cells.
- The committed Th1 cell can be identified by induction of the transcription factor T-bet.
- Antigen-activated Th1 cells in the presence of IL-12 & IL-18 upregulate CD28 and CD154 (40L).
  Note: delete subset. These co-stimulatory signals are required during T cell mediated reactions.
T Cell mediated macrophage immunity (TMMI)

- The Th1 cell then provides the cytokines to propagate the TMMI response (IL-2 and INF-\(\gamma\))

Th1 Initiation steps

Interferon-\(\gamma\)

- Produced by activated Th1(CD4), NK and activated CD8 cells
- Powerful activator of macrophages
- Signature cytokine of the Th1 helper reaction
- Powerful upregulator of MHC II and endothelial receptors
- Potent suppressor of the Th2 and Th17 response
- IL-12 activated Th1 cells produce IL-21- a potent promoter of CD8 killing activity.
- IL-21-in the absence of interferon-\(\gamma\), is a potent promoter of B cell growth and development
IL-21

- IL-12 activated Th1 cells produce IL-21 - a potent promoter of CD8 killing activity. (coming up)
- IL-21-in the absence of interferon-γ, is a potent promoter of B cell growth and development (coming up)

IL-2

- Critical growth cytokine produced by activated Th1 and CD8. T-regs are strongly dependant on it
- Acts in autocrine and paracrine modes
- IL-2R expressed as a functional unit after antigen activation
- The high affinity IL-2 receptor is expressed as a functional unit after specific TCR-Ag reactions
- Genetic defects in its assembly result in severe immune deficiency diseases.
Why do we have Th1 reactions?

- Th1 helper activation was developed to provide an antigen specific, efficient way to recruit highly activated macrophages to a site of infection.
- A small number of Th1 cells can recruit vast numbers of macrophages.
- The Th1 cell exploits the ability of INF-γ to activate macrophages while simultaneously suppressing a Th2 response which would not be helpful in the type of infection that provokes a Th1 response.

The End Result of TMMI-the activated macrophage

- The classic tetrad of macrophage produced pro-inflammatory cytokines are IL-1, IL-6, IL-8 and TNF-α.
- These cytokines have a wide range of autocrine, paracrine and systemic effects that promote inflammation.

IL-1, A pro-inflammatory cytokine

- Prototype primordial cytokine that facilitates host responses to stress.
- Produced by a wide range of cell types.
- Promotes neutrophil growth and emigration from the marrow.
- Acts with IL-6 on CNS to cause fever, depression.
- Neuroendocrine effects on adrenal gland.
- Stimulates APCs to increase Ag presentation.
- Antagonist is IL-1Ra.
IL-6, A pro-inflammatory cytokine

- Many effects redundant with IL-1
- Primary cause of fever and other constitutional signs of infection
- A T-Cell "vitamin"-it promotes responsiveness to IL-2, accelerates antigen activation
- Distinguishing characteristic is its strong growth and differentiation effects on B cells in the presence of other B cell cytokines and effects on bone mineral metabolism where it activates osteoclasts
- It is also be required for optimal Th17 development

TNF-α, A pro-inflammatory cytokine

- Plays a central role in the immune system
- Potent Macrophage activator
- Potent activator of endothelial homing and adhesion molecules
- Potent upregulator of MHC and other cytokines
- Potent inducer of apoptosis and angiogenesis
- Has systemic effects that range from flu-like symptoms to death
- The availability of anti-TNF biologicals provides a way to manipulate its effects clinically.

IL-8

- Is the most potent stimulus for mobilizing and recruiting neutrophils to the site of infection
- Produced mainly by macs, neutrophils and during intense inflammation by endothelial cells
What does “delayed hypersensitivity” (DH) mean?
- Archaic term for TMMI
- Entrenched in the literature
- Commonly applied to clinical situations where macrophage activation is a central component of the disease pathology
- DH or TMMI can be exploited to detect past infections

TB skin testing and why TMMI is also called DH
- Patient with TB coughs on tired first year resident
- First year resident inhales organism and it is subsequently phagocytized by a lung macrophage/dc which migrates to the hilum
- TB antigens presented to T cells
- TB specific CD4 Th1 cells migrate to site, activate macs that can now kill or suppress TB
- Many years later......physician skin tested with dead TB antigens which are phagocytosed by mac/DC at site and presented to passing TB specific CD4
- CD4 recruit more macs which then form a papule over next 24-48
TMMI is a very efficient T cell response

![Diagram of TMMI response]

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T cells continued

But Wait! There is even more about sweat!...

- Androstadienone, a male chemical signal, is found in sweat.
- 48 female undergraduates at UC Berkeley took 20 sniffs from a jar of andros.
- Serial blood samples were then taken
- What happened?
  - The women reported improved mood, higher sexual arousal and had increased BP, PR and RR
  - What did the blood show?
    - Serum cortisol went way up and stayed up for 60''
  - What happened when the same group sniffed yeast 20 times?
  - Nothing!
  - Food, at least bread, is not the way to a woman’s heart!
Lymphocyte Cytotoxic Immunity

- One way is antigen specific, the other is not.
- NATURAL KILLER CELLS
  - Large, granular, "older" than T cells
  - NO CD3 or αβ or γδ chains-no Ag receptors
  - Are not MHC restricted
  - Produce INF-γ
  - Responsive to natural interferons
  - Killing mechanisms suppressed by normal MHC-I and activated by altered MHC-I in combination with activating ligands on the target cell
  - Food for thought: why don’t they attack red cells?
  - Buy time for the CD8 cell to develop
  - Recent evidence that NK can "remember" some pathogens and also have strong regulatory activity
NATURAL KILLER CELLS

• Also kill when pathogen complexed with antibody binds to their Fc receptors (ADCC)

Antigen Specific Cytotoxicity

• The CD8 cell evolved to specifically recognize and kill foreign, mutated and viral infected cells
• The recognition is via display of endogenously produced antigen in a MHC-I determinant
• Optimal activation of CD8 cells requires parallel activation NK cells, antigen specific CD4 helper cells and/or the presence of memory cells (later). Preferably all three……
• Critical initial cytokine signals are provided by activated NK cells that produce IL-21, IL-2 and IFN-γ
• Antigen activated CD4 cells continue to produce IL-21, IL-2 and IFN-γ and promote a continuing CD8 response

Two ways cytotoxicity can be initiated
Four mechanisms that initiate and enhance CD8 cytotoxicity

- INF-γ, IL-2, IL-21
- CD28/B7
- MHC-I & II
- TLR

Cytotoxicity

- INF-γ, IL-21
- CD8
- CD4
- NK

T Cell cytotoxicity

- CTL recognize and bind virus-infected cell
- CTL programs target for death, inducing DNA fragmentation
- CTL migrates to new target
- Target cell dies by apoptosis

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What turns off cytotoxicity?

- Activated CD8 cells do not require co-stimulatory signals and can kill repeatedly. Why is this?
- But are dependant on viral display on targets
- If CD8 cells are successful, targets at some point disappear
- Activated CD8, in the absence of specific targets, activate their own death genes by Fas/FasL.
  - This deletes about 90% of the expanded CD8 population
  - The remaining ~10% become memory cells

The CD4 family

Cardinal Concepts of a Th2 Reaction

- Any infection or antigenic stimulus that causes IL-4 to be the dominant cytokine at the onset will lead to a TH2 reaction
- This will occur when:
  - a Th1 TLR is not engaged and IL-12 not produced
  - There are TLRs that induce DCs to produce IL-4 instead of IL-12
  - when B cells present antigen
- B Cells recognize and bind extracellular or soluble antigens
- A cytokine profile dominated by IL-4 is the primary determinant of the production of high affinity specific antibodies and memory Th2 cells
Activation of a Th2 Response

- Soluble antigens and extracellular pathogens promote Th2
- Pivotal cells that drive Th0 to Th2 are:
  - Committed Th2 cells
  - B cell presentation of antigen
  - Mast cells (future lecture)
  - DC and TLR under specific gene influences
- IL-4 initiates and is an absolute requirement for a Th2 reaction
- IL-4 is the growth hormone of a Th2 reaction—its major source is the Th2 cell
- The activated Th2 cell also produces IL-21—a potent B cell stimulator in the absence of IFN-γ
- IL-5, IL-6, and 10 are the major drivers of B cell differentiation and isotype switching—major source is the Th2 cell
- IL-4, IL-10 & IL-13 suppress the development of a Th1 reaction
- IL-13 has a similar ancestry as IL-4 and is a critical player in IgE responses (later lecture)

Functions of the Th2 response

- Enhance B cell function and ultimately antibody production:
  - Make pathogens more attractive to Macs and Polys
  - Bind toxins
  - Target mutant/viral infected cells for killing

TH2-B CELL RESPONSE
The Th1-Th2 concept (usually called a paradigm-a word I hate almost as much as "robust")

- IL-12 and IL-4 activated subsets are polarized is the concept and it no longer holds sway in the world of immunology.
- Instead, the dominant T helper response is dictated by the type of infection, the type of TLR activated and the dominant cytokine(s) present.
- The biologic effects of IL-12 activated Th1, IL-23 activated Th17 (coming up) and IL-4 activated Th2 are unique to each subset and...
- Interference in the balance between the generation of these subsets has implications on the manifestations of infectious, autoimmune and malignant diseases.

The CD4 family

The biologic effects of Th1, Th2 and Th17 include interactions with other immune cells and cytokines.
Th17 helper cells

- Remember that the type of infection dictates the type of immune response
- Bacteria and fungi that live outside of host cells trigger TLRs that instruct DC to produce NOT IL12 but a trio of cytokines, TGF-β, IL6 and the signature cytokine of the Th17 pathway, IL-23
- The trio prompts the Th0 cells to differentiate to Th17 cell with the unique transcription factor ROR
- The Th17 cell produces multiple types of IL-17 which is a potent recruiter of neutrophils
- If either IL-4 or IFN-γ is a dominant cytokine, Th17 reactions are strongly inhibited

CD4-Th17 subset

- Cardinal Characteristics (more on them later)
  - Produce IL-17- an inflammatory cytokine
  - Induced by DC production of IL-23
  - Unique nuclear receptor is ROR
  - Suppressed by either IL-4 or IFN-γ
  - May have innate defense role
  - Central role in autoimmune diseases

The EXPANDING CD4 family
CD4 Th1 regulator subset

- CD3,4,25+ and FoxP3 +
- Arise in at least 2 sites
  - Thymus
  - Peripheral lymphoid tissue
- Strongly influenced by TGF-β
- Also discussed in several future lectures
  - Thymus
  - Peripheral lymphoid tissue

The Ever-EXPANDING CD4 family

T Follicular helper cells

- Recently confirmed to be a distinct lineage
- Restricted to B cell follicles in lymphoid tissue
- Probably acts as a regulator of antigen specific B cell responses
- Can be identified by CD278 & upregulation of IL-6 and IL-21 receptors (Dr Clancy will address these in future lecture)
Innate Immunity-too important to neglect.

A. A historical vignette that supports the notion that when all is said and done, the medical profession is still the most innovative one…..and here’s why.

1. Let’s go back to about 1891. There was a bone surgeon (as orthopedic surgeons were called in those days) in New York named William B Coley. He had a 17 y/o patient, Bessie Dashiell, who had a rapidly growing tumor on her right wrist. He amputated her forearm but the sarcoma relentlessly metastasized and killed her about 10 weeks later in early 1891. Coley was profoundly affected by her death and resolved to not let this happen again. He scoured patient hospital charts to see what happened to other patients with sarcomas. To his amazement he discovered a patient who had had a similar sarcoma resected from his face but whose post-op recovery was complicated by a streptococcal infection at the site of the removal. Amazingly, the non- resectable portion of the tumor melted away in the course of the bacterial infection. Coley searched Manhattan for the patient, eventually found him, and confirmed his tumor free survival.

Coley became convinced that infection in some way stimulated a host response to a tumor that could destroy it. Soon, he was referred another patient with a bone tumor. He injected the tumor directly with live streptococci and noted rapid shrinkage of the tumor. Unfortunately, his use of live bacteria caused severe side effects and even death in some of his subsequent patients and he soon converted to using killed organisms but he was
still able to show that a significant number of his treated patients had anti-tumor responses. This led to his life long quest to treat cancer with bacterial “toxins”.

2. So what was really happening to Coley’s patients? Be ready to discuss, in contemporary host defense terms, the mechanisms that most likely led to tumor shrinkage in his patients over a hundred years ago.

3. There are other lessons to be learned from this that will make you better clinicians:
   Coley was an avid reader of the literature and had noted even before Bessie was referred to him that many surgeons thought some patients did “better” if they had an infection after tumor surgery. This is why we have journal clubs that will give you the ‘prepared mind’ ready to rationally act on a new idea. And now medical informatics makes past observations and clinical studies easy to find and read.
   Coley was courageous and willing to try something new, yet based on scientific observation, that might help his patient
   Coley had both excellent deductive and intuitive reasoning skills. The best clinicians you will encounter in training will have those skills-develop them to the utmost
   One last thing: a physician never knows all the possible ramifications that can ensue from taking care of a patient. It turns out that one of Bessie Dashiell’s closest friends happened to be none other that a young John D Rockefeller. He was so devastated by her death that he developed a life long interest in cancer. This interest led directly to the creation of the Rockefeller Institute and the Memorial Sloan-Kettering Cancer Center.

B. Bedside to Bench back to Bedside and back to Bench.
How clinicians anticipated the way to treat a weird syndrome, tried a logical treatment but only partially succeeded. Translational clinicians returned to the lab and were able to discover why the treatment was only partially successful.

1. In 1957, a single patient with very high fevers with no demonstrable cause that occurred sporadically and resolved spontaneously was reported by a general practitioner.

2. In 1982, a large Irish family with Scottish ancestors had multiple members that had periodic bouts of very high fevers, severe muscle aches (myalgias) and erythematous (red) rashes that occurred without warning but resolved without treatment in about 2 weeks. Frequent and extensive laboratory and microbiologic lab testing, even exploratory surgery, shed no light on the cause. An enlightened internist (probably a rheumatologist)
figures out that these patients might have severe inflammation without infection and called it the “X” (you will figure out what the “x” is soon) syndrome.

Question: what immunologic pathways, when activated, could lead to this syndrome? What type of host defenses could be involved and postulate a few pathways that could be involved.

The astute clinician meanwhile directly attacked the inflammation with a drug known to be broadly anti-inflammatory. ……and it worked but had lots of potential side effects.

3. Now we are in the mid nineties and the large Irish family previously reported was even larger. This family was restudied-pedigree below:
Question: What do we learn from this, if anything? Speculate on another genetic analysis the investigators did with these families (Hint: was discussed in Dr. Mathew’s lecture) Was the latter rational and was it helpful?

4. Now we are in the late nineties. A specific mutation was identified.

To maintain your perspective- “most of the time things are not what they seem to be” (unknown author but probably Twain or Berra)
Questions:

a. With your new HD knowledge, predict which cytokine(s) could be involved in the “X” syndrome that is characterized by fever, severe muscle pain and rash.

b. Now predict the most obvious type of genetic mutation that should be present.

c. Using the logic applied in b, several biotech companies produced monoclonal antibodies that could either bind to and block the culprit cytokine “X” before it bound to its cell receptor or could act as a decoy receptor and prevent the cytokine from activating its cell receptor.
d. Did they work? Oddly enough, to the surprise of many rheumatologists -not well in many patients with the syndrome- and this led back to the bench.
Normal Cytokine X signaling

5. The above is normal “X” signaling. The following is signaling is the “X” syndrome:
Please do not memorize any term in any of the figures. You will not be tested on specific terms, only on concepts.

5 (continued).
Study the X syndrome figure closely. The extracellular portion of the mutated receptor appears non-functional. How can that be? If true, there should be less cytokine “X” activation of the cell. The answer is in the lower right panel.
e. The last piece of the puzzle is the fact that when mice have a homozygous mutation of “X” they don’t exhibit the syndrome. When they have a heterozygous mutation of “X”, they do have the syndrome! We will discuss the reasons for this and how they meet the criteria for “things ain’t what they seem to be”

C. Cigarette Smoking and Innate Immunity

Read the posted article from *Inflammatory Research* 57 (2008), 497-503. Don’t get overly compulsive about the minutiae of signaling etc, but dwell on the innate immunity concepts.

Your facilitator will select lucky individuals from the group to discuss each of the following questions- How does chronic exposure to cigarette smoke alter…?

1. a. effective **clearance (think mechanical problems)** of bacteria from the pulmonary tree.
1. b. performance of the key innate effector cells like macrophages and neutrophils

2. the profile of inflammatory cytokines in the lung

3. not only innate immune response, but ultimately adaptive immune responses to infection in the lung

4. It is clear that cigarette smoking is not a good idea. Recently, you have learned that an effective response to a viral infection requires a highly coordinated and regulated response to the infecting virus. Be ready to explain to the group why influenza A, a virus that directly infects alveolar macrophages, is associated with serious morbidity and mortality in smokers.