LEARNING GOALS

- Understand how gene profiling by array technology is fueling fundamental changes in the way scientists and physicians think about genes, diseases, and therapeutics

- Understand how a synergistic fusion of biologic concepts, physico-chemical engineering and therapeutic advances is going to produce a quantum leap forward in patient care

BACKGROUND READING: Many of the immunologic concepts have been covered in previous lectures. The critical material is posted on the Host Defense web site.

DEVELOPED BY
John A. Robinson, M.D.

I. Introduction

A. I know that you have already had lectures and small groups elsewhere on array techniques but it very important that you understand how critical this technology and its offspring are to the practice of medicine in the 21st Century. Another way of gaining
perspective on their importance is to realize that the use of molecular immunology and pathology technology (DNA and tissue arrays are just the beginning) are going to displace almost all traditional biochemical and histological study of disease. It is not fanciful to suggest that medical school curricula within a decade will have almost no resemblance to current ones. The new curricula will be rich in, among other things, bioinformatics and artificial intelligence applications, customized gene profiling of patients and their responses to drugs, gene profiling that redefines many diseases entirely, and also predicts their prognosis and response to therapy. All the aforegoing will be taught in the context of how a physician can assemble and interpret vast amounts of data and correlate it with the individual patient. But I digress…this doesn’t mean you shouldn’t study "classical basic science" for Boards next year!

B. A good example of how advanced technology can be a blessing and a new burden to bear is mass cytometry (MC). MC takes flow cytometry (you remember flow cytometry I hope because you will be tested on it, you won’t be tested on MC)) to a new level. Flow cytometry can analyze up to 10-15 different fluorescent color tags on a cell and is a workhorse research and clinical tool for understanding all kinds of biological processes.

MC can simultaneously detect over a 100 different markers on a single cell. This will lead to completely new ways to understand cell biology. The latter is the blessing, the need for the current generation of physicians to learn how to apply the new understandings will be the burden.

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C. Close behind the application of array technology to biologic processes and clinical medicine is the rapidly developing field of nanomedicine. More on it later in this small group.

II. MICROARRAYS—the future is now

The new science of genomics provides a powerful way to measure the expression of thousands of genes simultaneously in very small amount of tissue, even single cells. This represents a powerful new technology created by a synergistic intersection of biology, computers and automation. Already artificial intelligence techniques are already being used to “mine” the vast amounts of data generated by arrays. Not only gene expression per se can be measured but also, more importantly, associations between gene groups can be detected. Although microarrays are not really immunological techniques per se, you need to understand not only how they will rapidly expand the understanding of basic immunologic responses but also revolutionize the diagnosis, prognosis and treatment of many inflammatory and neoplastic diseases. The applications and implications of the technology are vast and compelling.

A. Fundamentals of a DNA microarray: (you should know this already)
1. Large numbers of target DNAs are attached to a solid support. In some instances the genetic function of the DNA is known. More often however the DNA is simply a gene fragment or expressed sequence tag developed from cells or tissues.
2. mRNA is extracted from the sample to be studied—for example, a tumor.
3. The mRNA is reverse transcripted.
4. The resultant cDNA is labeled with a fluorescent dye
5. The spotted DNA is probed/hybridized with the labeled DNA
6. All the “spots” on the plate are then scanned to measure the presence or absence of fluorescence—hence the presence or absence of gene expression.

There are many variations of the fundamental technique. For example, cDNA can be spotted and mRNA from a tumor can be labeled and studied.

I. FIRST STEP: Study the 2 diagrams below that demonstrate the stepwise array process used to shed new light on disease causation, best ways to construct vaccines and improve diagnostics. Two journal articles posted on the HD web site for this small group will provide more information if you need it.
Students: To prepare for the remainder of this small group, you must go to the Host Defense website and read the posted articles. This is necessary because the arrays will not reproduce well by standard copying techniques.

**IMPORTANT**—When you read the articles, do **NOT** be concerned with the specific methodology and most of the reagents, physical/chemical details and gene terminology—read them for the concepts only

**Article # 1** provides a beautiful example of how gene profiles of dendritic cells confirm that these critical APC detect specific pathogen molecular patterns and then tailor the appropriate innate immune response to a specific pathogen—remember the innate response being driven by a spectrum of TLRs? Cells that first encounter potential pathogens could discriminate between three major pathogen groups (bacteria, fungi and viruses) and then initiate appropriate innate immune reactions to counter the threat. Not only can gene expression be detected but also the sequence of expression can be studied. The combination of detection, sequence of expression and associations of multiple genes provide powerful insights into how the immune system works. The gene profiles also emphasize the extreme redundancy built into the immune system as a safeguard.

The varying gene expression during innate responses could be detected on a gene chip that contained 6800 immune cell genes. This type of fundamental research was impossible before the availability of array technology and guarantees that exponential leaps in understanding immune responses are at hand.

Questions related to Article #1:

1. Do the results in the article confirm that the innate immune response to a virus like influenza is different than to a bacterium like *E. Coli*? Does the difference seem significant and support the notion that the immune system had to invent new ways of dealing with viruses?
2. How does the data in this article support the clinical observations made for over a hundred years by physicians that individual patients vary widely in "how sick" they get when infected with a given organism. For example, if the entire class of 2015 was infected with virus "X", many would have no symptoms, some a mild illness, others would stay home for a few days and maybe one would end up in ICU! Later in the course, you will discover what is behind the heterogeneity of clinical disease expression and how it will show up in an array.

**Article #2 & 3** These studies demonstrate how array technology will significantly change the way medicine is practiced **by the time all of you are residents**!

Questions related to Article 2 & 3 and related editorials

1. The lymphoma study posted on the HD site emphasizes that studying cells and subcellular phenomena in isolation can be very misleading. Tumor cells are so interesting that we forget to look at where they live (and don't live). Intuitively one might think that the genetic characteristics of the tumor cells would be the most valuable information to guide care of the patient. How does this study convince you this is not the case?

2. Discuss the clinical implications of how a "single" type of tumor defined by classic histologic methods, when analyzed by DNA microarray, is revealed to be not so "single". (Hint: read the breast cancer article posted on the HD site)

3. If microarray chips can predict the response to chemotherapy in women with breast cancer and molecular probing of biopsies can predict which ones will become malignant, be ready to discuss the implications for oncologists, pathologists, patients, pharma companies and economists.

The following section is for the sake of knowledge only and will give you the basis for exciting dinner conversations with family, loved ones and non-medical people. **YOU WILL NOT BE TESTED ON THIS SECTION.**

**III. NANOMEDICINE** - the future is almost now!

A. What is it? Need to understand nanotechnology first. Nanomaterials are defined as the "intentional design, characterization, production and application of materials, structures, devices and systems by controlling their size and shape in the nanoscale range- 1-100 nm. This is a similar range within which many molecular and pathologic processes operate.

B. Nanomaterials are being designed for transport, insertion and accumulation of diagnostic and therapeutic agents into tissue and individual cell sites that have been very difficult to access by traditional vehicles. Tumors are a prime example- delivering
effective doses of chemotherapy to a tumor currently forces an oncologist to use toxic
dosages that limit use in humans.

C. A common feature of nanomaterials is a marked increase of surface area relative to
their volume and those expanded surfaces can be coated with a high density of molecules
of interest. Nanomaterials can be composed of metal ions with magnetic, electronic and
optical properties that can be “turned on” once they have gained access to a site of
interest to produce local heat that can kill a tumor cell. They can be constructed in a way
that allows loading the surface or inner core with potent cytokines or anti-tumor drugs
that become active after delivery

D. Each student group, after reading the following, should try and conjure up some exotic
way to use nanomedicine in a patient.

E. nanomaterials may also enhance diagnostic imaging, especially MRI, where their
magnetic properties can be exploited.

1. An example of diagnostic and/or therapeutic nanotechnology:
These nanobeads can be infused into patient and target a tumor with tumor specific antibody localization, then release chemotherapeutic drugs into the tumor. They can also carry an MRI imaging agent so tumor can be found.

F. In vitro Nanodiagnosis

A. Two areas of high interest are using individual cells as diagnostic probes and gold nanoparticles that can replace PCR technology for the highly specific detection of infectious agents.

B. Some examples follow:

1. B lymphocytes have been engineered as pathogen sensors by inserting genes for:
   a. bioluminescent jellyfish proteins that emit light when their surface antibody is cross-linked.
   b. and using monoclonal antibody technology to produce antibody to the specific pathogen of interest.

2. The sensitivity and speed of the assay is spectacular. Anthrax specific B cells, when exposed to as few as 50 units of the organism from a nasal swab, emit light that can be easily detected with available sensors within 5"!
C. Nanotechnology uses minute iron and gold particles that increase the sensitivity a million fold over current assays.

1. The technique exploits magnetic properties of iron particles that are encased in plastic coat that has been coated with a monoclonal antibody against a protein of interest.

2. The coated iron particles are reacted with the protein and also with gold nanoparticles coated with a polyclonal antibody to protein (the sandwich technique similar to ELISA assays) and short strands of DNA that act like a "bar code". The iron particles that have migrated to the magnetic field are isolated, the DNA snipped off and the "bar code" read by ultrasensitive DNA techniques.
Nanomaterials Used as Labels to Amplify Detection Signals in Diagnostic Devices.

T-CELL IMMUNITY 1 and 2

Date:  Friday, March 30 & Wednesday, April 4, 2012  
Time:  10:00 AM Friday  
       8:30 AM Wednesday  
       LTH 190

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 on 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. 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 which is 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.

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

11/26/11
a. IFN-\(\gamma\) is produced **predominately by CD4 subset (Th1) lymphocytes and natural killer cells** but also by activated CD8 cells and \(\gamma\delta\) T cells

b. IFN-\(\gamma\) receptors can be found or induced on almost all cell types

7. The introduction of INF-\(\gamma\) into the sequence is the pivotal step for amplifying T cell mediated macrophage activation.

a. IFN-\(\gamma\) is a powerful activator of macrophages and the signature cytokine of an ongoing Th1 reaction.

b. IFN-\(\gamma\) 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-\(\gamma\) is its ability to control the display of MHC-I and II determinants on APC such as DC, M/M and endothelium. Thus, IFN-\(\gamma\) 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-\(\gamma\) 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**

![Th1 Propagation and Maintenance Diagram](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 hepatic and hematologic 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
   e. TNF-α activates endothelium, promotes vasodilatation and increases MHC expression.
   f. 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.
   g. 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.

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 immune 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.

![Reprinted with permission](image)

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.
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 virus) eliminates 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 design 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.
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 prone 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.

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**TH2-B CELL RESPONSE**

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**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.
The EXPANDING CD4 family

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-γτ** (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 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 (aka ICOS) 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 Tr reg.
7. Commitment of a Th0 cell to a Th subset is determined by the type of TLR, DC and its dominant cytokine present at the time of presentation
8. The dominant cytokine profile is determined by the type of infection and host genetics
9. Each subset has specific functions and cytokine associations
10. Th1 helpers amplify macrophage immunity and antigen specific cytotoxicity, Th2 promote antibody formation, Th17 promote inflammation and Tr reg. regulate!
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

- Th0
  - IL-23
  - IL-4

- Th1
  - T-Bet
  - IL-12

- Th2
  - GATA-3
  - IL-4

- Treg
  - FoxP3

- Th17
  - ROR
  - IL-23
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:
  - No longer can phagocytose
  - 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 &18 upregulate CD28 and CD154 (40L) 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-γ).
Interferon-γ

- 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-γ, 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, CD8, and Tregs
- 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

IFN-γ: when dominant, down regulates Th2 and Th17
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
- It may 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

ACTIVATION OF MACROPHAGES
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 macrophages and presented to passing TB specific CD4
- CD4 recruit more macs which then form a papule over next 24-48 hours
- A new & better way to measure TMMI will be discussed in a small group

TMMI is a very efficient T cell response

The end result will be less than 5% of the cells present will be TB specific T cells, the rest will be activated macrophages
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
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

CYTOTOXICITY

T Cell cytotoxicity
What turns off cytotoxicity?

- Activated CD8 cells can kill repeatedly
- 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

- Th0
- Th1
- Th2
- Th17

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, 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

[Diagram showing interactions between Th2 cells, B cells, and other immune cells, including cytokines like IL-4, IL-10, and IL-21.]
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.
- The TLR engaged will determine the cytokine profile
- TMMI forces Macs to kill intracellular organisms & down-regulates B cell antibody production because it will be ineffective against a threat inside a cell
- B cell-antibody axis does the opposite: provides mechanism to facilitate elimination of extracellular threats & and inhibits TMMI
- Interference in the balance has implications for health & disease
- The cytokine change can be a natural sequence of events or externally produced- the latter is important for the clinician
**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** IL-12 but a trio of cytokines, TGF-β, IL-6 and the signature cytokine of the Th17 pathway, IL-23
- The trio prompts the ThO 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 (much 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 role
  - Central role in autoimmune diseases

**The EXPANDING CD4 family**

![Diagram of CD4 cell subsets and cytokines](image-url)
CD4 Th1 regulator subset

- CD3,4,25+ and FoxP3 +
- Arise in at least 2 sites
  - Thymus
  - Peripheral lymphoid tissue
- Strongly influenced by TGF-β
- Dependant on IL-2 for survival and function
- Also discussed in a future lecture
  - Thymus
  - Peripheral lymphoid tissue

The Ever-EXPANDING CD4 family

Th Follicular helper cells

- Recently confirmed to be a distinct lineage
- Restricted to B cell follicles in lymphoid tissue
- Probably act as a regulator of antigen specific B cell responses
- Can be identified by ICOS, & upregulation of IL-6 and IL-21 receptors