

## ***Immunology for the Rheumatologist***

Rheumatologists frequently deal with the immune system gone awry, rarely studying normal immunology. This program is an overview and discussion of the function of the normal immune system. Why should we bother to understand immunology? The diseases we treat are largely immune based and while our former therapies were developed almost blindly, we are now seeing targeted therapies. The TNF (tumor necrosis factor) inhibitors were developed to specifically target TNF. There are T cell and B cell targeted therapies. In order to better understand our treatments, it is important to understand immunology. We will then be better prepared to understand our diseases and current treatments as well as those treatments that will be emerging over the next several years. We realize that rheumatic diseases are characterized by inflammatory damage to tissue. The clinical features of specific diseases reflect the following three mechanisms: first, stimuli that initiate and propagate the inflammatory response; second, particular tissues that are targeted; and third, the predominant inflammatory tissues involved. Understanding these mechanisms is key to rational therapy. The immune system has multiple roles. It can provide surveillance against tumors and is important in transplant rejection; however, the primary role of the immune system is defense against infection. How do perturbations in the normal immune system result in disease? To answer this question we need to understand a bit more about the immune system.

The immune system consists of two components - the innate immune system and the adaptive immune system. The physical barriers, such as the skin and epithelial membranes, are considered part of innate immunity. Innate immunity is a non-specific response involving both immune and non-immune cells. It is an immediate response, and is highlighted by inflammation. Specific cytokines and chemokines are involved in this inflammatory response. Adaptive immunity involves only immune cells (T cells and B cells) and is a delayed response that requires clonal expansion and effector cytokine secretion. Very importantly adaptive immunity involves memory, whereas innate immunity does not. When we look at various clinical aspects of disease, there are a number of clues in the presentation that provide information as to the nature of the immune response. For example, gout is an acute inflammatory disease and is moderated by innate immunity, whereas rheumatoid arthritis is chronic and moderated by adaptive immunity. The nature of the disease stimulus might be a clue as well. For example, uric acid triggers the innate immune system whereas microbes trigger the adaptive immune system. The cellular nature of the response is important to understand as well. For example, neutrophils are characteristic of innate immunity whereas lymphocytes are characteristic of adaptive immunity.

The cells of the innate immune system include neutrophils, eosinophils, basophils, NK (natural killer) cells, and the antigen-presenting cells. With regard to adaptive immunity there are CD4 positive T-helper cells, CD8 positive cytotoxic T cells, B cells and dendritic cells. When a microbe tries to gain entry to the body its first barrier to entry is the epithelium, so that some of the mechanisms of innate immunity actually prevent infection. If the microbe is able to gain entry through the epithelial barrier, the next lines of defense are the phagocytes and NK cells. The complement system is also part of innate immunity. If the innate immune system proves to be inadequate then the adaptive immune system comes into play - B cells producing antibodies and T cells generating effector cells.

The innate immune system is the initial response to microbes. It recognizes structures that are shared by classes of microbes using germ-line encoded receptors, so there is limited diversity. Innate immunity consists of the epithelial barriers, phagocytes and NK cells, the complement system and a whole array of cytokines (TNA alpha, IL-6, IL-10, etc.). These are all defenses without memory. Physical damage and chemical insults are immune system concerns; however, the biggest danger to the immune system is infection. The human body must be able to recognize and eradicate infection. The body senses danger using pathogen-associated molecular patterns. These are patterns that are unique to bacteria. For example, bacterial cell wall components such as polysaccharides and double-stranded RNA are structures that are foreign to the healthy human body. The body recognizes these structures with what are referred to as pattern recognition molecules. The toll-like receptors are key pattern recognition molecules of the innate immune system. These receptors mediate the innate immune response. They are found on macrophages, neutrophils and dendritic cells. They recognize distinct pathogen-associated molecular patterns.

Macrophages have a wide array of receptors on their surface. When a macrophage encounters a bacterium it binds to it using these receptors. The macrophage then engulfs the bacterium and also releases a number of pro-inflammatory cytokines.

The complement system is one place where both innate and adaptive immunity come together. Microbes are able to directly activate the complement system. During an interaction between C3 and the microbe, there is complement activation producing C3A, which is pro-inflammatory, as well as C3B which leads to opsonization and phagocytosis. Subsequently, C5 is cleaved to C5A and C5B. Ultimately there is generation of the membrane attack complex which leads to lysis of the microbe. Since there is no antibody involvement, this would be part of the innate immune system; however, the classical complement pathway involves antibodies and is therefore part of adaptive immunity.

Gout is an illustration of innate immunity. There are a number of features of a gout attack that support the notion that it is a manifestation of innate immunity. It is an acute response and is self-limited. Urate is the stimulus and the attack is resolved when urate is removed. The predominate response is neutrophilic. There is no lymphocytic reaction, nor is there any antibody formation. Urate, by virtue in part of its negative charge, can activate complement. Urate can also interact with synovial cells, particularly synovial macrophages, leading to the production of Interleukin 1 beta. Urate binds to the macrophage via toll-like receptors 2 and 4, leading to activation of the inflammasome. An inflammasome is a complex of intracellular proteins that are present in neutrophils and macrophages involved in activation of the innate immune system. Urate crystals activate the inflammasome, which leads to the production of Interleukins, particularly Interleukin 1 beta, which is excreted from the cell. Uric acid crystals can also be taken up by neutrophils. There are therefore a number of ways in which the human body uses the innate immunity to deal with urate crystals.

Adaptive immunity, a delayed response to a specific antigen, demonstrates the features of specificity and memory. It consists of lymphocytes and their products and uses specific receptors which are generated by somatic mutation. While the receptors in innate immunity are germ line receptors, those of adaptive immunity are generated by somatic mutation and must be regenerated every generation. The body has three strategies to combat microbes: first, secreted antibodies bind to extracellular microbes, block their ability to infect host cells and promote their ingestion and subsequent destruction by phagocytes; second phagocytes are able to ingest and kill microbes while helper T cells enhance the killing by phagocytes; third, cytotoxic T cells directly destroy infected cells that are inaccessible to antibodies.

T lymphocytes mature in the thymus and express specific receptors that bind antigen. There are two main types of T cells - CD8 positive cytotoxic T cells and CD4 positive helper T cells. A number of subsets of CD4 positive helper T cells have been identified. The naive CD4 positive helper T cell, if exposed to IL-12 and IL-18, can differentiate into the Th1 cell. If it is exposed to IL-4, it can differentiate into the Th2 cell. If it is exposed to TGF Beta, it differentiates into what is referred to as the Treg cell, and if it is exposed to TGF Beta and IL-6, it differentiates into the Th17 cell. The Treg cell is an important control mechanism ensuring that the immune system does not become too robust, whereas the Th17 cell leads to the production of inflammation. With regard to effector function, the CD8 positive cytotoxic T cell is responsible for killing viral infected cells. The CD4 positive Th1 cell activates infected macrophages and it also provides help to B cells for antibody production. The CD4 positive Th2 cell provides help for B cell antibody production, particularly in switching to IgE production. The CD4 positive Th17

cell is pro-inflammatory and enhances the neutrophil response. The CD4 positive regulatory T cell suppresses T cell responses.

Humoral immunity is a major limb of the adaptive immune response. Immunoglobulin is structurally homologous to the T cell receptor and is also produced via somatic recombination. It provides surveillance against blood borne pathogens. B cells develop in the bone marrow and from there they migrate out to peripheral lymphoid tissue. B cells produce antibodies. Some of these might be autoantibodies that could drive an immune response such as in rheumatoid arthritis. Interestingly, the B cells are also able to produce proinflammatory cytokines. Additionally, B cells have the ability to present antigen to T cells and provide the second signal thereby resulting in T cell activation. An antibody can basically do three things. First, it is able to neutralize the microbe. The antibody coats the microbe and directly neutralizes it. Secondly, the antibody can opsonize the microbe. In other words, the antibody coats the bacteria and promotes ingestion and destruction by the phagocyte. Thirdly, ingestion and destruction by the phagocyte can be promoted by antibody-mediated complement activation.

T cell function begins with the antigen-presenting cell. An extracellular antigen is taken up by an antigen-presenting cell. The antigen is processed and presented back on the cell surface in the context of major histocompatibility complex (MHC). Antigen complexed with MHC class 2 on the cell surface is presented to CD4 positive T lymphocytes, whereas antigen complexed to MHC class 1 is presented to CD8 positive T lymphocytes. In order for antigen to be recognized by the T cell, the proper antigen specificity and the proper MHC molecule is required. In the case of the CD4 positive T cell, it is a MHC class 2 molecule and in the case of CD8 positive T cell, it is a MHC class 1 molecule.

What is needed to activate the T cell? First, the proper antigen for that T cell receptor must be complexed to the proper MHC molecule. There is a co-receptor (i.e. CD4 or CD8 needed to activate the T cell. Also needed is a second signal referred to as the co-stimulatory signal. Failure to co-stimulate results in ignorance, anergy or apoptosis. In other words, the hypothesis is that three signals are needed to activate the T cell: first, antigen binding to the T cell in the proper MHC context; second, T cell co-stimulation; and third, a cytokine signal to drive differentiation. Figure 1 demonstrates signal one. Here we have the antigen- presenting cell presenting the proper antigen in the setting of the proper MHC molecule to the T cell receptor. In this case, CD4 is acting as a co-receptor. Figure 2 illustrates signal two, the binding of CD28 on the T cell to B7 on the antigen-presenting cell. Another name for B7 is CD80/86. There are a large number of co-stimulatory signals. Some of which, such as CD28 in this case, activate the T cell. Others, such as CTLA4, inhibit T cell activation. There are therefore up-regulatory as

well as down-regulatory co-stimulatory signals. Figure 3 illustrates the third signal: the signal that drives T cell differentiation. These are cytokines that are produced by the antigen-presenting cell. If the antigen-presenting cell produces IL-12, it leads to TH1 differentiation. If it produces IL-4, it leads to TH2 differentiation. If the antigen-presenting cell produces transforming growth factor beta (TGF- $\beta$ ) and Interleukin 6, the pro-inflammatory subset, TH17 cells, occur. If TGF- $\beta$  alone is produced, the regulatory subset, the Treg cell, occurs. The drug abatacept takes advantage of CTLA4. CTLA4 is a naturally occurring part of co-stimulation that is down-regulatory. When it binds to an antigen-presenting cell it actually leads to down-regulation of the T cell. In the construction of abatacept, the external domain of CTLA4 is bound to the heavy chain constant region of an immunoglobulin molecule. When this molecule binds to CD80/86 on the antigen presenting cell it blocks T cell activation.

The adaptive immune system has the properties of specificity and diversity, which enable the immune system to respond to a large variety of foreign antigens. Memory allows enhanced responses upon repeated exposure to the same antigen. Clonal expansion increases the number of antigen-specific lymphocytes to keep pace with the infecting microbes, and contraction homeostasis lead to down-regulation after the affecting agent has been eradicated. Figure 4 illustrates naive B cells. These could just as easily be T cells. There are both anti-X B cells, and anti-Y B cells. The body is confronted by antigen X. What is seen first is a primary anti-X response. Once that response has evolved, there is down-regulation, leaving memory B cells against antigen X as well as naive B cells against antigen Y. There is then an exposure to antigens X and Y. This leads to a secondary anti-X response referred to as an anamnestic anti-X response. It is more rapid and more robust because the organism has memory cells. The response to antigen Y is a primary anti-Y response looking very similar to the primary anti-X response on the left side of the figure. In Figure 5 there is an antigen presenting cell presenting antigen to naive T cells. There are also naive B cells being exposed to the antigen. These exposures result in a clonal expansion of B and T cells. This leads to the generation of an antibody specific for that infection as well as effector T cells specific for that infection. Once the antigen has been eliminated, there is a down-regulation of the system leaving surviving memory cells. At birth, perhaps 5% of our lymphocytes are memory cells, whereas by adulthood about 50% of our lymphocytes are memory cells. Remember that B cells mature in the bone marrow whereas T cells mature in the thymus. From these sites they migrate to the peripheral lymphoid organs. There is tremendous specificity in the adaptive immune system. If the immune system is confronted by antigen X, there will be a clonal expansion of the T and B cells that are specific for antigen X. Figure 6 provides an example of the adaptive immune system in action. The person has stepped on a tack, so there is a break in the skin and bacteria are able to enter. The first thing that happens is that antigen-

presenting cells within the skin take up the bacteria that are able to gain entrance. The antigen-presenting cells then transport the ingested bacteria to a lymph node. Within the lymph node the antigen-presenting cells, which have now processed the antigen, display it on the surface in the context of the appropriate MHC molecule. On the left there are T helper cells and on the right there are cytotoxic T cells. There is then clonal expansion of the appropriate T cells. These T cells enter the circulation and migrate out to the site of infection where they are able to help eradicate that infection. Macrophages are activated leading to the killing of microbes and cytotoxic T cells kill the target cells.

To conclude, consider adaptive immunity in action, using rheumatoid arthritis as an illustration. There is strong evidence of genetic risk factors as well as environmental factors triggering autoimmunity in rheumatoid arthritis. The theory is that an unknown trigger sets up an initial focus of inflammation in the synovial membrane which attracts leukocytes into the tissue. Autoreactive CD4 positive T cells activate macrophages resulting in the production of proinflammatory cytokines and sustained inflammation. Some of these cytokines induce the production of MMP (Matrix metalloproteinase) and RANK (Receptor Activator of NF-KappaB) ligand by fibroblasts. Finally, MMP attacks tissues and the activation of bone destroying osteoclasts by RANK ligand result in joint destruction.

We believe that rheumatoid synovitis is an antigen-driven adaptive immune process. There is persistence and affinity maturation of autoantibodies, indicating somatic mutation of immunoglobulin genes followed by selective survival of B cells producing the highest affinity antibodies. In addition, there is an association with MHC class 2 molecules and up-regulation of TH17 cells. Finally, there is a lymphoid architecture of the rheumatoid synovial membrane and a limited T cell repertoire early in disease and then epitope spreading. These are all features that support the belief that rheumatoid arthritis is an adaptive immune process gone awry.

Figure 1

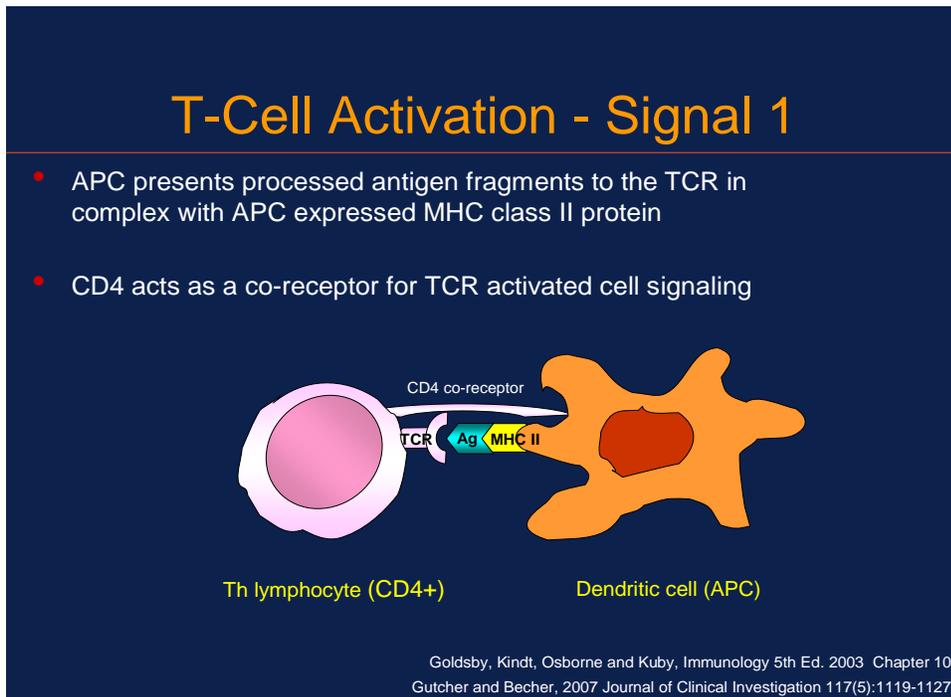
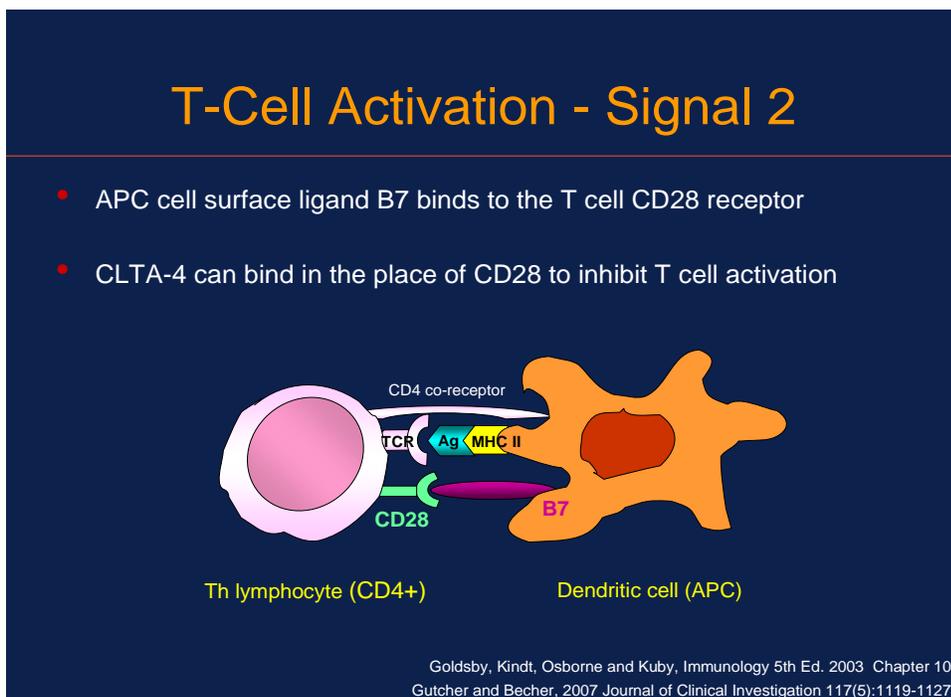
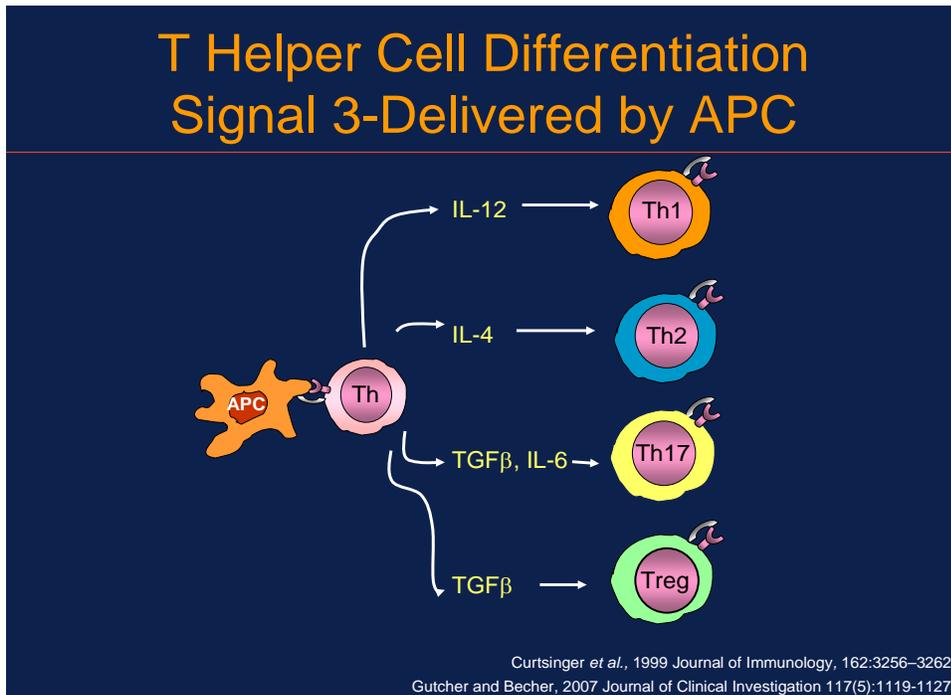


Figure 2



**Figure 3**



**Figure 4**

# Specificity of Immune Response

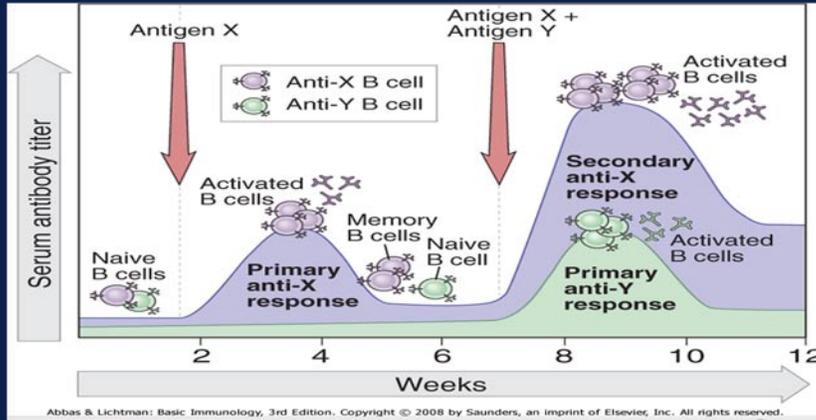


Figure 5

# Phases of Immune Response

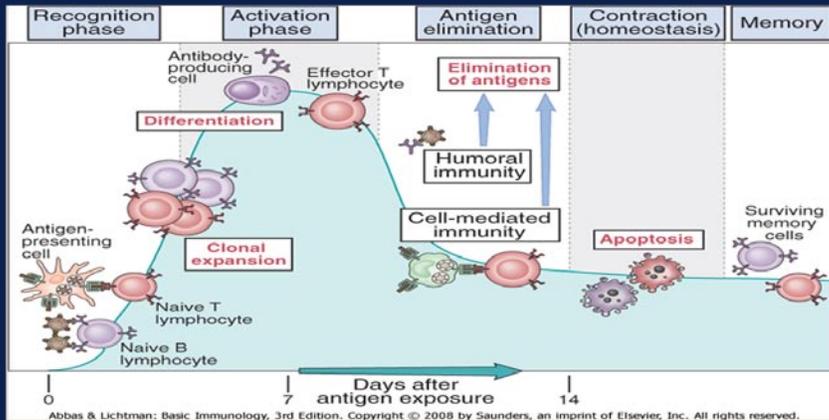
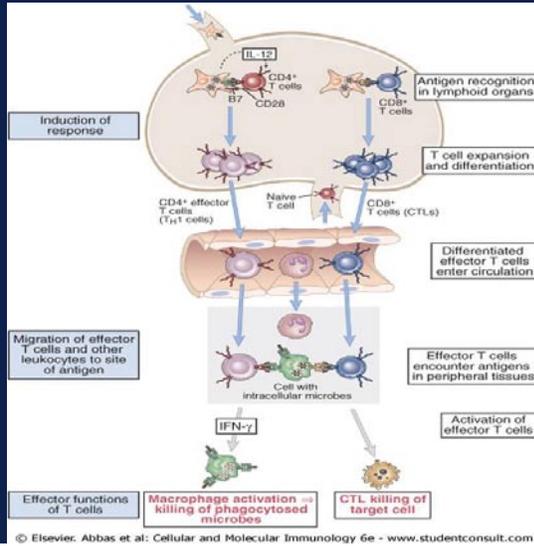


Figure 6

## Effector Cell elimination of antigen



## References

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