HOST DEFENSE

SMALL GROUP PROBLEM SOLVING SESSION

ATOPY AND ALLERGY

Small Group Classrooms

LEARNING GOALS
Integrate the concepts of IgE Immunology with their clinical consequences.

BACKGROUND READING
Janeway: Same assigned reading for the Immunology of IgE lecture-Janeway 8th edition: 174-75; 421-23; 571-594; 600-603 AND 718-719 & 601-603. The latter cover 2 new topics are not included in the lecture material. Articles posted on HD website.

DEVELOPED BY
John A. Robinson, MD

Revised: 11/08/12
Before coming to class:

1. Read assigned chapters/pages and develop answers for **ALL** the questions in the 4 clinical vignettes

During the Small Group Session:

2. Each small group *(should be 4-5 peers- please do not sort yourselves into large groups-you will learn much less)* should discuss the four case studies and decide the best solutions to the specific integrating questions associated with each case.

3. After approximately an hour of discussion by the subgroups, the facilitator will recapitulate the answers to the integrating questions by selecting a subgroup to present a synthesis of their relevant discussions to the entire group. Facilitators will select, at their discretion, a small group for the discussion of the individual cases.

4. History has shown that students who don’t contribute to the Small Groups do not do well in the Course (remember that about 25-30% of the final comes from small groups!) and also have been assaulted by their fellow group members

5. At the end of the session, a master answer sheet will be posted on the Host Defense website.

**INTRODUCTORY CASE TO HELP YOU MAINTAIN YOUR PERSPECTIVE ABOUT IgE IMMUNOLOGY.**

*ALL OF YOU SHOULD UNDERSTAND THIS VIGNETTE FIRST.*

A 24 year old woman was admitted with intermittent hives (urticaria), edema and parasthesias. She was a Peace Corp employee who had been stationed in Africa doing pisciculture research. She had been previously healthy and there were no symptoms of sweats, fever or weight loss. The physical exam revealed hepatosplenomegaly and a swollen right leg. The only laboratory data of interest were a markedly elevated total white blood count with 32% eosinophils (normal: ~1%) and a concentrated blood smear that revealed the presence of Loa Loa microfilaria (a parasitic worm). An ELISA IgG test for the parasite was positive and a serum RAST was also positive.

**QUESTIONS:**

1. The patient had a parasitic infection transmitted by flies and endemic in central and western Africa. Predict **what cytokine(s)**, if measured in her plasma, might be elevated in this patient’s blood.
2. What available laboratory data led to the conclusion you made to the question in #1.

3. Are you surprised that the antibody detected by ELISA was an IgG? Predict what other isotypes, if any, are elevated and may have parasite specificity.

4. Does this patient have an allergy or is she responding normally to an infection?
   Faculty answer: This patient does not have an allergy in the pathologic sense of the term.

Case 1a
An older physician, surrounded by a well tended summer flower garden, is relaxing on his deck. It’s hot out and he is maintaining fluid volume with diet Pepsi (actually a Dogfish Head IPA “60”). He takes a sip and feels a foreign object in his pharynx and then a sharp pain in his neck. Five minutes later he has severe abdominal cramping, diffuse itchy hives and difficulty breathing. His wife, a critical care nurse, notes his distress and treats him immediately using drugs from their home first aid kit.

Questions:

1. What happened to the doctor?

2. Describe the immunologic steps that occurred between the precipitant and the abdominal cramping and shortness of breath.
3. Are there any lab tests that could be helpful that should be done after this episode- if he survives?

4. What options does the patient have to avert this in the future?

**Case 1b**

A two-year old boy was tormenting his six-year old brother-actually the children of the victim in Case 1a). The older boy, in a fit of pique, smashed a homemade peanut butter sandwich into the little one’s face! The two-year promptly developed a swollen eye and massive itchy swelling of the cheek on the side of the swollen eye. About 20 minutes later he began to wheeze. Unfortunately the six-year old now had a way to prevent further harassment and used it on several occasions with similar results until the two-year-old was big enough to protect himself. The younger sib, now 20, when to a Thai restaurant and had a spicy cold beef salad and chicken satay. He immediately developed severe abdominal cramping, facial flushing and vomiting.

**Questions:**

1. What was the most likely etiology of his dilemma?

2. Speculate on specific sources and contributing factors that could have precipitated his
asthma but also the skin and gastrointestinal symptoms?

3. Develop a strategy for preventing this in the future. Speculate on possible vaccine preparations that could ameliorate or prevent this reaction.

**Case 1c**

The uncle of the 2 boys in case 1b and the brother of the doctor in 1a is a beekeeper. When he started his business, he has several severe reactions to bee stings, but he persevered and now gets stung multiple times a week and never has an allergic reaction.

1. Can you postulate an immunologic mechanism(s) that could explain this?
Case 1d

A 6 y/o boy with leukemia, the brother of the 2 boys in case 1b, who was known to have a peanut allergy already, had a severe anaphylactic reaction during a platelet transfusion.

1. Can you postulate an immunologic mechanism(s) that could explain this?

Case 2

A seventy-two year old female from a dairy farm in Vermont has an aortic valve replaced. Unfortunately the new valve became infected with a bacterial organism that was resistant to almost all antibiotics but, strangely, remained very sensitive to penicillin G. An alert medical student notes that the patient reported having a “rash” a long time ago after receiving an antibiotic for “strep throat”. The only other history that was interesting was that 2 siblings had “bad asthma” when they were in high school.

Intravenous penicillin was begun. About 10” after the infusion was started, the patient began to wheeze and then went into shock. Fortunately, a rheumatologist/allergist was making rounds nearby and realized what was happening and was then able to successfully treat the patient. Two weeks after discharge, the patient, feeling extremely fortunate to be alive, bought a “lucky” bracelet at a State fair and avowed to “never take it off”. To her dismay, 3 weeks later she noted intense itching and a red, bumpy rash under the bracelet.

1. What happened in the hospital? Describe the almost fatal immunophysiology?
2. What if the patient didn’t ever have a rash of any kind and had never been treated with any kind of antibiotic before? How could she have been sensitized?

3. Describe how penicillin, a very small molecule, becomes immunogenic. (Hint: Google “hapten” or go to the background reading to figure this out.)

4. Describe the unlucky immunological response caused by the lucky bracelet.

Case 3

A twenty-four year old florist developed chronic wheezing that required daily inhaler therapy and a sporadic need for high doses of oral corticosteroids to prevent severe shortness of breath. He thought there was a link between the latter episodes of severe shortness of breath and his plant repotting work assignment in the shop. A consulting pulmonologist did not feel that he had typical asthma and the patient underwent a bronchoscopy. Secretions removed at that time contained massive numbers of eosinophils and neutrophils but did not grow an organism for the first 3 or 4 days after the procedure. A complete blood count revealed an increase in the absolute number of circulating eosinophils and a very high IgE serum level. A RAST test was done for grass, mites, and aspergillus (a fungus) and found to be strongly positive for the fungus. A gel diffusion test on the patient's serum revealed strong precipitating bands between the patient’s serum and fungal antigens. The Ouchterlony or agar gel immunoprecipitin assay technique is a time revered immunologic assay that analyzes antigen-antibody precipitation in...
gels. Wells are punched into agar gel and antigens are placed in individual wells and antibodies-known or unknown-placed in opposing wells. The proteins are allowed to diffuse through the gel. If there is a specific antibody for the antigen diffusing towards it, a visible precipitation band will develop. The # of precipitin bands that form and their fusion patterns provide information on the number of specific antigens/antibody systems present and the isotype of the antibody. This technique is rarely used anymore but in this particular patient illustrates an important principle. (To the astonishment of the faculty, a question about this technique was on a recent Board exam also!)

**Agar Gel Immunoprecipitin assay**

(SEE NOTES FOR TECHNIQUE)

![Diagram of Agar Gel Immunoprecipitin assay](image)

**Questions:**

1. Interpret the RAST findings and speculate on at least one probable etiology of his pulmonary disease. What would you predict grew in the laboratory over the next 7-10 days from the pulmonary secretions obtained by bronchoscopy?

2. The gel diffusion test appears to have detected an antibody isotype that does not correlate with his clinical presentation: What is going on here? Review the first vignette in this small group for the solution.
Agar Gel Immunoprecipitin assay

(SEE NOTES FOR TECHNIQUE)

3. Two distinct immunological reactions are occurring in the patient’s lung. Describe the immunological reactions that recruit eosinophils and neutrophils from the bone marrow and culminate in their infiltration into bronchial secretions.
4. Why do corticosteroids have an efficacious effect in this type of syndrome? Reason to points in the immune response where they (steroids) could be acting. If you could design a drug that would increase the expression of transcription factors like the FoxP3 family, would it be reasonable to try them in patients with asthma?

Case 4

Three unrelated stories…or are they??

a. Crohn disease and ulcerative colitis are debilitating, chronic inflammatory diseases of the bowel. The epidemiology of these diseases is interesting in that they occur almost exclusively in industrialized societies. This observation is reminiscent of the markedly increased incidence of allergy in the western world.

b. Many children in Africa are infected with intestinal parasites, usually worms. There is a low rate of asthma in African children from the more underdeveloped countries. There have been several UN campaigns to rid the children of gut parasites. These campaigns activated the “law of unintended consequences”

c. Totally germ free mice are very susceptible to inflammatory bowel disease and asthma. If their gut microflora is restored they were no longer susceptible to the development of either disease.

Case 4a: A patient had the misfortune of having severe asthma and Crohn’s disease. He had failed all forms of anti-inflammatory and immunosuppressive therapy. He was fed live whipworm ova harvested from pathogen free pigs every thirty days for 6 months. The patient went into a clinical remission of the inflammatory bowel disease. Amazingly, he also did not have another attack of asthma for over 2 years.

A second patient had severe ulcerative colitis and infected himself with whipworms (relatively innocuous worm). The disease went into remission.

Questions:

1. Predict what type of cell response is mediating inflammation in the gut in these 2 inflammatory bowel diseases. Also postulate what cytokines and T cell subsets might be found that usually aren’t found in the gut
2. Predict what the live worms did and explain how it happened.

3. You should be able to predict what happened to some of the African children in case 4b!

4. You might be able to predict several immune cell types and cytokines that would be present in the lungs of germ free mice with asthma and how those cell types and cytokines might change after letting them graze on the floor at O’Hare airport, Terminal 3. The answer lies in the innate immunity lecture and Dr. Knight’s lecture on microbiota and the immune response.

Ref: Olszak et al. Microbial Exposure during early life has persistent effects on NKT function. Science. 336;489. 2012
LEARNING GOALS
• You will be able to contrast the mechanisms of immune fetal protection from those employed to reject foreign organ grafts.
• You will be able to identify the maternal and fetal strategies used by humans to protect the fetus from immune attack.

OBJECTIVES
You will be able to:
  - Enumerate the effector mechanisms used to reject allografts
  - Contrast the importance of MHC disparity in fetal protection versus organ rejection.
  - List the most likely points in the rejection process that are vulnerable to immunosuppressive therapy.
  - Understand the maternal and fetal logic deployed to protect the fetus

BACKGROUND READING
Janeway 8th edition: Pages 223-226; 652-663; 675-677 & articles posted on the HD web site.

LECTURER
John A. Robinson, MD
I. The Immunology of Allotransplantation

A. Definitions:
1. **Allogeneic** - denotes transplants between members of the same species who differ genetically
2. **Allograft** - a tissue transplant between allogeneic individuals
3. **Xenograft** - transplants of organs between members of different species
4. **Recipient** - the patient receiving the organ transplant
5. **Donor** - individual providing the organ. In bone marrow transplantation and selected forms of liver, lung and kidney transplantation the donor may be a living, usually but not always related, individual.

B. Histocompatibility
1. The gene cluster within the major histocompatibility complex (MHC) (also designated the HLA complex in humans) is nature's way of providing a unique, immunological signature for each individual that provides the means by which the immune system can demarcate self from non-self, provide effective immune surveillance and identify altered or infected host cells. Presumably this complex immune response was not initially set up to frustrate transplant physicians but was devised for prevention of parasitism by other multicellular organisms. Although not devised as such, the MHC has proved to be the primary barrier to successful organ transplantation. In the randomly outbred human being, an organ transplanted from one individual to another (other than identical twins) will be immediately recognized as non-self or foreign and this event sets in motion multiple immunologic pathways of destruction that culminate in rejection of the transplanted organ.

C. Laboratory Assays Relevant to Transplantation 1. Solid Organ
1. **Transplantation** - with the advent of many new immunosuppressive agents and a better understanding of the genetics of transplantation, solid organ (heart, lung, liver, kidney, pancreas, small intestine, face, etc) transplant procedures increased until recently. However, the need for cadaver organs has far outstripped their availability and significant future increases in transplantation will not occur until either permanent artificial support, xenorgans (different species) or a national change in donor strategy (presumed consent and/or organ reimbursement) are available for replacement. While permanent electrical pump support of left ventricular function in patients with heart failure is now a reality, other artificial organs such as islet cell substitution for whole pancreas or hepatic cell infusion for liver failure are not ready yet. This reality increases the pressure to use available donor organs judiciously. In order to do this one must understand the genetics of the major histocompatibility loci - in clinical parlance, the HLA system - and how these genetic loci influence the outcome of an organ transplant.
2. Assessment of histocompatibility

a. HLA (MHC) cell typing methods. MHC or histocompatibility (HLA) typing techniques are used to detect MHC specificities on mononuclear nucleated cells. The number of matched MHC loci, especially the Class 2 ones (also known as DR), between donor and recipient can be used to predict graft acceptance. The more loci matched the better, but **MHC Class 2 (DR) matches are the most strongly predictive of graft survival**. Originally, the source of the antibodies used to detect HLA specificities were usually from women with multiple pregnancies who reacted to a paternal HLA antigens. More recently, tailored monoclonal antibodies are available against specific MHC loci.

b. Lymphocytotoxic Antibodies - The classic way to determine whether a potential organ recipient has MLC (HLA) antibodies present in their serum is to incubate their serum with a panel of lymphocytes of known HLA specificity in individual wells. If there are HLA antibodies in the serum they will bind to their MHC antigen on the lymphocyte cell surface. Complement is then added to all the wells. Lymphocytes that have bound antibody on their MHC will be lysed. Since the MHC is known for each lymphocyte well, the exact anti-MHC specificity can be determined.

c. Tissue Typing Laboratories are now using flow cytometry, ELISA and molecular methods to decrease time of analysis and increase sensitivity and decrease costs but you need to understand the fundamentals of detecting HLA antibodies.

d. The Mixed lymphocyte reactions ("In vitro") transplants:
Mixed Lymphocyte Culture (MLC)- a transplant in vitro.

The primary concept of this assay is that differences in the HLA (MHC) class I & II (particularly HLA-DR) antigens between the donors of the cells used in the assay will stimulate T lymphocytes to synthesize DNA and divide. The MLC is designed to quantitate the amount of cell division as measured by newly synthesized DNA in responder lymphocytes when exposed to irradiated stimulator lymphocytes.

The MLC requires peripheral blood lymphocytes from two individuals. Responder and irradiated stimulator lymphocytes are mixed together in the MLC. The stimulator lymphocytes are irradiated to prevent them from acting simultaneously as stimulator and responder cells (think of them as “paralyzed. They look normal but can’t proliferate if activated). A MLC consisting of a mixture of responder lymphocytes and irradiated responder lymphocytes is conducted simultaneously. The cultures are incubated for a period of 5 days at 37°C, at which time a radioactive precursor of DNA, tritiated thymidine ($^{3}$H-TdR) is added to each of the cultures. After 18 hours of additional incubation, the cellular DNA containing the incorporated $^{3}$H-TdR is assayed for radioactivity. As shown in the example in this diagram, the responder lymphocytes recognize the irradiated stimulator lymphocytes and incorporate a significant amount of $^{3}$H-TdR. In contrast, the responder lymphocytes do not respond to irradiated responder lymphocytes and do not incorporate radioactive thymidine. This typing assay thus measures the ability of responder lymphocytes to recognize non-self-HLA-D antigens on stimulator cells and is a measure of the HLA-DR antigen difference between the donors of the responder and the stimulator lymphocytes.

D. Mechanisms of Rejection

1. Alloimmune responses involve both naive and memory lymphocytes. In the graft and the surrounding tissues, dendritic cells of donor and host origin

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become activated and migrate to T-cell areas of secondary lymphoid organs. There, donor antigen-bearing dendritic cells engage alloantigen-reactive naive T cells. Naive T cells are optimally triggered by dendritic cells in secondary lymphoid organs, but antigen-experienced memory T & B cells (perhaps by past blood transfusions or pregnancy) may also be activated by other “non-professional” APC such as endothelium of the transplanted organ.

2. The mammalian immune response has multiple effective pathways to prevent engraftment of foreign organs.

a. The MHC of the transplanted organ, especially the MHC on the "passenger" leukocytes, function as alloantigens and are recognized by the immune system in two ways:

b. **Direct** - activation of the immune system by the foreign MHC marker itself **without any form of MHC processing or presentation**.

The latter is, of course, a heresy; it doesn't obey the MHC restriction concept of antigen presentation in the context of one's own MHC but the phenomenon has been repeatedly observed experimentally. Large numbers of CD4 T-cells will react with foreign MHC, possibly on the process of the molecular mimicry; however, strict evidence for the actual mechanism is not yet available.

c. **Standard recognition by the "indirect" method** - alloantigens are phagocytized, processed and represented in the context of Class II MHC antigens by antigen presenting cells to CD4 T cells.

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D. Once either indirect or direct alloantigen activation of the immune system occur, classic Th4 amplifying pathways are activated and it is likely that ALL T helper subsets will ultimately become involved.

1. CD4 Th-cells are **mandatory** participants in all forms of cellular rejection.
2. IL-12 driven T Cell mediated Macrophage immunity (delayed hypersensitivity) occurs early
3. Activation and clonal expansion of CD8-alloantigen specific T-cells follows, driven by CD4 Th1 cells and IL-21.
4. Recent evidence strongly suggests that the Th17 cell and IL-23 will rewrite the story of rejection immunology. Th17 is a very pro-inflammatory cell and if it is the predominant cell in a rejection episode, graft damage may be severe.
5. Th2 cells provide co-stimulatory signals, IL-4 & 21, for alloantigen specific B-cells which then produce alloantibody
6. NK-cells participate in varying degrees in transplant rejection, however, they are not mandatory for a successful rejection of a transplanted organ.
7. The cumulative effect is: 1) activated macrophage mediated graft destruction, 2) CD8 antigen specific graft cytolysis, 3) Th17 mediated inflammation and, 4) antibody mediated, graft destruction either by complement and/or Fc receptor activation of cell death mechanisms.
8. Induction of T regulator cells- this highly dependent upon individual “immune response” genes of the recipient and the disparity of the MHC antigens between donor and recipient

E. The intensity of rejection will depend upon the ratio of Th1, Th 2 and Th17 cell activation counter-balanced by T regulator inhibition. For example, if the dominant cell is Th17, the rejection might be more destructive (more neutrophils) than if B cells were the dominant cell.

Specific Rejection-Step 1
Specific Rejection-Step 2

Transplant Rejection

Legend:
1. NK cytotoxicity against non-self MHC
2. NK antibody mediated cytotoxicity via Fc receptors
3. Th1 activated macrophage killing
4. Th1 amplified CD8 cytotoxicity
5. Th2 driven graft antibody
6. Complement mediated cytotoxicity

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7. **Th17 activation by DC**

F. **Rejection is defined clinically by its temporal relationship to the actual transplantation.**

1. **Hyperacute rejection** is defined as **accelerated** rejection, usually within the first 48 hours after transplantation. In many instances, it occurs **immediately** after graft emplacement. This catastrophic event, although not lethal for the kidney recipient because of back-up dialysis therapy, is the ultimate disaster in heart & lung transplantation unless a mechanical device can be immediately substituted. This type of rejection is mediated by recipient alloantibody directed against donor antigens that were present **prior to transplantation**. This can also occur, but never should, if there is a major mismatch across the erythrocyte blood group ABO system of the donor and the recipient of the organ being transplanted.

2. The setting for hyperacute rejection is usually: a patient who is blood group O normally has circulating antibodies to blood group A and B antigens. These antigens are not only on erythrocytes, but are widely distributed on all tissue cells. Transplantation of an A, B, or AB organ into an "O" individual will result in an immediate reaction between the transplanted organ's A or B determinants and recipient antibodies.

3. **Antibodies with reactivity to histocompatibility (MHC) antigens** can also be present in some patients **prior to transplant**. These patients have usually been sensitized by repeated blood transfusions or multiple pregnancies.

4. The pathology of hyperacute rejection is characterized by **widespread vascular injury brought about by alloantibody mediated endothelial damage.**

   a. Rapid binding of antibodies to endothelial alloantigens causes an immediate loss of endothelial integrity and initiates platelet aggregation at the sites of the damage.

   b. When platelets aggregate, they release multiple vasoactive mediators that activate the coagulation cascade and initiate fibrin deposition. The net result is **severe graft ischemia.**

   c. Simultaneously, neutrophils are attracted to the areas of ischemia and release their multiple, highly destructive enzymes that degrade vascular barriers and cause further necrosis.

   d. The **complement system** is also activated and that escalates intravascular clotting.

   e. The clinical events occur rapidly, the graft will turn pale immediately from loss of blood supply and never function. If the allograft is not immediately lost, rapid infiltration of the organ by neutrophils, lymphocytes and monocytes usually leads to necrosis, edema and loss of the organ.

   f. In emergency situations, successful transplants have been
carried out in spite of performed alloantibodies, if they can be removed, blocked or suppressed prior to transplant.

5 Acute rejection is defined by the sudden appearance of anti-donor organ immune effector cells during the **first three weeks after grafting**. The rejection sequence, especially early after grafting, is initiated when host recipient CD4 T-cells react with alloantigens, either directly or indirectly, and mediate a DTH response. Simultaneously, alloantigen specific CD8 cells attack the graft, TH17 cells promote an inflammatory reaction and B-cell alloantibody appears after alloantigen specific B-cells take up residence within the graft.

6. The mix of cell populations, the vigor of their response, and frequency of rejection is orchestrated by a complex interplay between MHC differences, especially between HLA-D (MHC-II) loci of donor and recipient. One can find in a biopsy of the transplanted organ, a wide spectrum of cells and histologic changes that range from sparse cuffing of blood vessels by mononuclear cells, massive infiltration of the graft by cells, to actual necrosis of myocytes (hearts), tubules (kidneys), or hepatic (liver) cells to areas of resolving rejection with fibrosis. Acute rejection may become easier to diagnose with array technology and has become more manageable with the advent of new immunosuppressive agents.

7. Chronic rejection is a slow process of graft attrition caused by immunologic mechanisms that are probably distinct from those of acute rejection. The hallmark of chronic rejection is diffuse, widespread arteriolar narrowing caused by intimal thickening of the vessel. The actual immunological mechanism of intimal thickening that eventually leads to graft ischemia and malfunction is unknown. Chronic rejection is a formidable problem in solid organ transplantation since all known forms of anti-rejection therapy will not reverse it. It is a leading cause for retransplantation of hearts and gradual loss of function in transplanted lungs and kidneys.

8. Current and future strategies to prevent rejection:
   a. Optimal MHC, especially MHC-II (D) Loci, matching
   b. Blunt T-cell responses to alloantigens by immunosuppression.
   c. Provide inhibitory second signals (CTLA-4) or cytokines (IL-10 & TGF-β) to override Th-1 and CD8 responses
   d. Induce specific tolerance to the organ (also known as the HOLY GRAIL of transplant Immunology)- this is becoming a potential reality with the increasing understanding of how CD4, 25, FoxP3 regulatory cells enforce tolerance and how they can be induced in the transplant patient.
G. Bone Marrow/Stem Cell (CD34) Transplantation
Hyperacute, acute, and chronic rejection are forms of host versus graft disease. The reverse situation, graft versus host disease (GvH), is unique to bone marrow transplantation or inadvertent transfusion of immunocompetent cells into an immunodeficient host. In this setting, the host has been rendered (or is already) immunologically bankrupt in order that the infusion of new bone marrow will not be rejected. The strategy is a two edged sword, since the recipient bone marrow is now populated with effective immune effector cells that may attack a defenseless host. Graft versus host disease can be acute and result in loss of the graft, or chronic. There is a wide array of smoldering disease in the latter situation since the transplanted lymphocytes can attack skin, gut or other organs.

H. Xenotransplantation - A Special Case not ready for prime time
1. Humans, apes and old world monkeys inactivated, about 2.8 million years ago, the expression of the α-1, 3-GT gene - presumably due to evolutionary selection pressures that provided a survival advantage during the emergence of new pathogens that could bind to α-galactosyl epitopes on cell surfaces.
2. These higher primates now produce exuberant amounts (up to 1% of circulating IgG) anti-α GT antibodies after exposure to α-GT epitopes on gastrointestinal bacteria.
3. Non-primates, for e.g., pigs, have anatomically suitable organs for xenografting but unfortunately have rich endothelial displays of the α-gal epitope. Thus the attempt to transplant these organs into humans is thwarted by hyperacute rejection. Heroic attempts to reduce host anti-α GT antibodies, including the development of α-GT knock-out pigs, can blunt hyperacute rejection but these KO pigs still develop non α-gal antibodies and also undergo conventional rejection.
4. Genetic engineering that can insert a the human decay activating factor gene (See Dr. Mathews C’ lecture) in pigs has been done but only blunts hyperacute rejection also.
5. After all the above is solved, the potential transmission of pig viruses will have to be solved.

II. The Pregnancy paradox- the fetus is semi-allogeneic but survives in utero.
A. Introduction. Human reproduction is relatively inefficient. The incidence of pregnancy loss in the first trimester is at least 30%. Not all the loss is secondary to genetic abnormalities. Actually, it is amazing that the loss is not much higher because the fetus is a transplant with a partially incompatible paternal MHC. Think about it-if humans laid eggs we could skip this part of the lecture! However evolution required a placenta with all its benefits like nutrient transfer and gas exchange so the mother and the fetus collaborate to circumvent rejection by invoking ingenious adaptations of their immune systems.
B. Both the mother and the fetus prevent rejection by evoking transient but quite specific tolerance mechanisms in several very unique ways. Tolerance strategies include systemic and local ones at the feto-maternal interface:

1. Local maternal/fetal strategies.
   a. Trophoblastic (fetal origin) tissue (the maternal-fetal interface) does not display "classic" HLA-A, B, or C fetal molecules because the MHC at the interface is down regulated.

   b. Trophoblastic (fetal origin) tissue (the maternal-fetal interface) displays only a "non-classical" HLA system, HLA-G, that has an inhibitory motif for maternal NK cells. Wherever trophoblastic tissues invade the uterine wall to establish and maintain pregnancy these HLA-G molecules are displayed.

   c. Maternal NK cells
      i. in the non-pregnant uterus they increase towards the end of a menstrual cycle and appear to be somewhat similar but not identical to peripheral NK cells
      ii. in the gravid uterus under the influence of progesterone and especially uterine produced TGF-β, they convert to a markedly different NK cell and ultimately will comprise about 70% of all lymphocytes in the endometrium during pregnancy.
      iii. They do NOT express CD16, the Fc receptor necessary for antibody-directed cytotoxicity (ADCC) and their cytotoxic receptors are inhibited by HLA-G. Their cytokine profile becomes inhibitory also. They are found in increased numbers at all trophocytoblastic interfaces and are believed to have regulatory and tolerogenic functions that prevent immune cytotoxic attack on fetal structures. Pregnancy NK cells also begin to produce angiogenic factors that support successful pregnancy.

   d. There are increased numbers of maternal γδ T cells, macrophages and paternal antigen specific CD4, 25 regulatory cells in the uterine wall during pregnancy. They secrete large amounts of immuno-suppressive cytokines, especially IL-10 and TGF-β, and thereby actively inhibit maternal αβ CD4 and CD8 T cell attack on the fetus. Fetal trophoblastic tissue also produces IL-10 and TGF-β. These cytokines strongly promote the continued presence of Treg cells.

   e. It has been recently shown that some maternal lymphocytes do gain access to the fetus and home to fetal lymph nodes. The fetus, has a predominant T regulatory environment that converts the infiltrating maternal cells to T regs.
f. The fetal trophoblast upregulates a gene complex that prevents alternate and classic complement activation by maternal anti-fetal antibodies.

2. Systemic (maternal)

a. Pregnancy induced immunoregulatory hormones, especially **progesterone**, strongly suppress local and systemic Th-1 type responses. Placenta-derived factors also decrease Th-1 responses both in the uterus and systemically. IL-10, a suppressive cytokine, is also increased in the peripheral blood uterine during pregnancy. The net result to the mother is that she has suppressed Th1 responses but relatively normal Th2 responses.

b. Increased numbers of paternal MHC antigen specific maternal T regulatory cells can be found circulating in maternal blood and concentrated in the uterus during pregnancy. The paternal specific T regulators rapidly decline postpartum but persist as memory T cells that can rapidly expand in a subsequent pregnancy with the same male.

c. Progesterone also stimulates the surface cells of the uterine endometrium to display decay accelerating factor (DAF or CD55-see complement lecture) which in turn inhibits complement mediated cell death

III. Clinical Implications for the mother. Th1/Th2 imbalance is a critical concept that has serious implications for the mother during the pregnancy (think:
H1N1 influenza virus maternal mortality). Systemically, the maternal immune state during pregnancy also can be characterized as Th2 dominant with strong humoral responses but **suppressed TMMI, Th17 and cytotoxic responses**. Alterations of this state can strongly influence pregnancy outcomes.

1. **Conversion of a maternal-fetal Th2 bias** to a Th1 bias with dominance of INF-γ at the fetal-maternal interface (or systemically) is associated with **inability for successful implantation and/or fetal resorption**.

2. The TH-2 bias dominant during pregnancy can lead to **exacerbation** of maternal diseases dependent on Th-1 for suppression - tuberculosis, leprosy, toxoplasmosis and influenza are examples.

3. Inability to generate T regulatory cells (in animal pregnancy models) is correlated with fetal loss. Reconstitution of Tregs in a murine abortion model leads to successful pregnancy.

4. Expansion of paternal-specific Tregs during subsequent pregnancies may determine a successful outcome and decreased pregnancy complications - if the father is same or similar to the initial father. Conversely, subsequent different fathers may not induce rapid expansion of maternal paternal specific Tregs and this may be associated with adverse outcomes.

5. The immune system has evolved multiple toleragenic mechanisms to permit the survival of the fetus. Tumors have exploited many of them to permit survival of malignant cells. Understanding the maternal mechanisms evoked by the fetus is bound to shed light on new ways to treat malignancy.

**IV. Clinical implications for the fetus**

1. Infection of the fetus may lead to **tolerance** to the infecting pathogen and that could subsequently prevent the infant or later, as a child then adult, from responding appropriately to infection with that pathogen.

**STUDY QUESTIONS**

1. Diagram both methods of antigen presentation to the recipient immune system after allografting.

2. List the multiple pathways of graft rejection.

3. Predict the cellular patterns of acute rejection that would be found on a biopsy 21 days after transplantation. Do the same for a biopsy done one year after transplantation.

4. Devise ways to protect a pregnancy in a patient with strong Th1 responses.
Types of Transplants

Only other definition to know for now

• DONOR-
  – living-related,
  – living nonrelated,
  – cadaver

Barrier to Rejection

• IN A WORD……..MHC
• THE SYSTEM THAT ALLOWS US TO DISCRIMINATE SELF FROM NON-SELF
• PRESUMABLY AS DEFENSE AGAINST MULTICELLULAR ORGANISMS AND
• POSSIBLY SEXUAL SELECTION
Mixed Lymphocyte Culture

The classic way to measure transplant compatibility

Assessment of histocompatibility
MHC typing

Assessment of histocompatibility
Lymphocytotoxic antibodies
New approaches to histocompatibility

- Flow cytometry
- ELISA
- Molecular
- All the above decrease analysis time and costs, increase sensitivity
- But be sure you understand the fundamentals!

Mechanisms of Rejection

- Both donor and host DC get involved
- Almost all, if not all, immune effector cells will get involved
- The intensity of the rejection will depend on multiple factors, including MHC disparity, host immune response genes, physician interventions

IMMUNOLOGIC HERESY!
DIRECT VS INDIRECT ANTIGEN PRESENTATION
MECHANISMS OF REJECTION
DIRECT

Specific Rejection-Step 1

Specific Rejection-Step 2

DC are “passenger leukocytes”
Transplant Rejection

Clinical Definitions of Rejection - Hyperacute

CLINICAL DEFINITIONS OF REJECTION - HYPERACUTE

Healthy tissue grafted into patient with defective adaptive and pre-existing antibody against donor tissue

Rejection against donor blood

Graft rejection and acute rejection of graft, leading to infarction and increase in number of infections

Donor organ

INF-γ

MAC

Th17
CLINICAL DEFINITIONS OF REJECTION

• HYPERACUTE
• ACUTE: Defined by sudden (10–90 days) appearance of effector cells in the graft. Vigor depends upon Dr mismatch, gender, intensity of immunosuppression
How arrays are changing the concept of rejection

- The journal reading for today clearly shows that different patients reject organs in different ways:
  - Gene expression patterns from biopsies of organs after transplant can distinguish between acute and chronic rejection and drug toxicity
  - Also identify a previously unrecognized fact that in some biopsies, B cells predict severe rejection!
  - FoxP3 (and CD4,25 Tregs) is involved in the control of rejection and can be used to detect it
  - Next step—controlling Tregs and controlling rejection!

TRANSPLANT REJECTION
CLINICAL DEFINITIONS OF REJECTION

• CHRONIC...insidious and caused by mechanisms probably different than acute rejection, usually caused by intimal thickening that leads to graft ischemia. The major problem in solid organ transplantation today
ULTIMATE GOAL: TOLERANCE

Strategies to prevent rejection

• Optimal MHC matching, especially DR
• Block T-cell responses to alloantigens
• Provide inhibitory second signals (CTLA-4), T regulatory cells (CD4, 25) or cytokines (IL-21, IL-23, IL-10 & TGF-β) to override Th1, Th17 and CD8 responses
• Induce tolerance by manipulating Tregs
BONE MARROW/SCT TRANSPLANTS

Please remember that this situation can occur when an immunoincompetent host receives a transfusion (if it is not depleted of lymphocytes first).

GRAFT VERSUS HOST

XENOTRANSPLANTATION

- Higher primates deleted an α-1,3 GT gene to gain a survival advantage
- These primates now produce anti-α-1,3GT in response to gut bacteria
- Xenotransplants undergo hyperacute rejection
- If they survive hyperacute rejection, they then are subject to standard acute and chronic rejection
- Xenovirus transmission also a potential problem
ARE GENETICALLY ENGINEERED PIGS THE ANSWER?

- Done so far:
  - Human complement regulating proteins inserted on pig cell surfaces;
    - inhibits hyperacute rejection at least in part
    - Very recent, removal of the α-gal determinant from pig cell surfaces
- Problem remaining:
  - Anti-pig, non-gal antibodies and conventional acute and chronic rejection

Nature’s way

pregnancy

HUMAN REPRODUCTION

- Is inefficient
- Amazing that it happens at all because an allograft is in utero
- Not all loss is genetic
- The mother and fetus have devised ingenious ways to get around graft rejection
Local (in utero) strategies to protect the fetus

- Trophoblastic (fetal) tissue at the maternal-fetal interface does not display MHC class I & II.
- Only displays a “public” MHC complex-HLA-G that has an inhibitory motif for maternal NK cells
- Pregnant uterine NK cytotoxicity is inhibited by HLA-G, they do NOT express the FcR and promote regulatory effects by producing TGF-β & IL-10 (Fetal trophoblastic tissue does the same!) and produce angiogenic factors
- HLA-G & TGF-β in turn promote differentiation of T regulatory cells that suppress regional immune responses

Baby or Cancer?

THE PREGNANCY PARADOX

UTERINE WALL

FIG. BY J. ROBINSON
More Local (in utero) strategies to protect the fetus

- Maternal γδ T cells also home to the uterus and are presumed to be promoting tolerance to the fetus
- Epigenetic silencing of utero-placental chemokines that could attract effector lymphocytes (this is brand new data—not in your notes)
- The fetal trophoblast upregulates a gene complex that prevent complement activation by anti-fetal antibodies
- Lastly, when maternal T lymphocytes (ly) come in contact with fetal ly (maternal & paternal MHC) at the placental interface maternal ly are converted by fetal TGF-β to paternal specific T-regs.

FETO-MATERNAL IMMUNOLOGY

Systemic (maternal) strategies

- Pregnancy induced hormones, especially progesterone, strongly suppress uterine and systemic Th1 reactions
- Placenta produces IL-10 which acts globally
- Progesterone also promotes increased display of DAF on uterine cells which further inhibits complement reactions
- T regs generated locally in the uterus circulate systemically
**PREGNANCY-CLINICAL IMPLICATIONS**

- Maintenance of a Th2 bias during gestation is associated with success but......
- Increases the risk of disseminated intracellular infections that require TMMI and CD4 help (think H1N1maternal mortality) and Th17 responses to fungi and other pathogens.
- Maintenance of a local Th1 bias during the implantation phase decreases the possibility of successful pregnancy.
- Promotion of maternal Th2 or T regs could enhance the chance of a successful pregnancy.
- Conversely, failure of maternal ly conversion to paternal specific T regs at the placental interface might lead to spontaneous abortion (NEJM 367;1162, 2012).

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**Clinical Implications for the fetus**

- Infection of the fetus could lead to tolerance to the pathogen and could lead to subsequent inability to respond appropriately to a future infection!
Regional/System Specific Immune Systems:
Bone, Adipose Tissue and Central Nervous System

Date: Wednesday, May 8th, 2013
8:30 AM
LH190

LEARNING GOALS
The student will be able to understand that not only is the immune system important for resistance to infection but regional immune systems have developed additional functions like maintenance of bone, control of energy homeostasis and protecting the brain from “collateral” damage.

OBJECTIVES
You will be able to:
- Understand how three cytokines regulate a balance between bone breakdown and formation.
- Realize that dysequilibrium between the three cytokines is associated with clinically important bone and musculoskeletal diseases.
- Appreciate the importance of adipose tissue cytokine systems and their control of energy metabolism.
- Understand the logic supporting attempts to manipulate adipocytokines to control obesity.
- Understand the clinical implications of activating the innate immune functions of microglia in the brain.

BACKGROUND READING
One page overview in Science that is posted on the HD web site.

LECTURER
John A Robinson, MD

Revised 10/24/12
I. Introduction to Osteo-immunology (interactions between the immune and the skeletal systems)

A. Bone Homeostasis

1. Formation and remodeling is a dynamic, constantly changing but tightly controlled, process orchestrated by osteoclasts (OC), osteoblasts (OB) and immune cells. OB, OC and immune cells talk to each other mainly by signaling through the RANK (receptor activator NF-κB) – RANKL (receptor activator NF-κB ligand) system.

2. RANK - the target for RANKL is expressed on pre-OC and OC cell surfaces

3. RANKL is the key cytokine that directs terminal differentiation of pre-osteoclasts to osteoclasts, thereby driving bone resorption.

4. OPG (osteoprotegerin) is secreted by mainly by OB, but also stromal and immune cells, and acts as a decoy receptor for RANKL, thereby blocking RANKL effect on cells expressing RANK.

5. The ratio between these 2 critical proteins, RANKL and OPG determine bone loss or bone increase. Normal bone homeostasis, including remodeling, requires a normal balance between RANKL-RANK-OPG.

6. Any condition that alters the ratio between RANKL-RANK-OPG will be associated with bone pathology - the most common one being osteoporosis (see below)

7. OB also secrete macrophage colony stimulating factor (M-CSF), a cytokine that has a receptor on pre-OC that when activated promotes differentiation, proliferation and survival of OC.
Normal (balanced) Osteoclastogenesis

B. Regulation of RANKL-RANK-OPG equilibrium is dependent upon multiple factors, including cytokines, receptors and interactions of cells common to both skeletal and immune systems.

1. Osteoclasts have many properties similar to macrophages and dendritic cells and can function as APCs for T cells.

2. RANKL is expressed by monocytes, neutrophils, DC, and T & B cells. All these cells can therefore drive OC differentiation by activation of RANK on pre-OC.

3. Pro-inflammatory macrophage derived cytokines like IL-6, TNF-α and IL-17 induce RANKL and promote OC differentiation and proliferation.

4. Anti-inflammatory T cell cytokines like IL-4, 10 (among others) inhibit OC differentiation and proliferation.
How T cells can dictate bone remodeling-I

C. A general picture of how T cells could dictate bone remodeling.

1. A dominant Th1 response in and around bone is associated with IFN-\(\gamma\) and its activation of macrophages would have a pro-clastogenic effect and would promote bone resorption.

2. Th17 T cells are major producers of IL-17 and this cytokine is a potent promoter of OC activity.

3. In health, a balance between Th1 and Th2 responses strongly suppress Th17 dominance because either IL-4 or IFN-\(\gamma\) suppress IL-17 production and block its effects. A dramatic example of how important lack of Th17 suppression can be is found in rheumatoid arthritis, a devastating disease that destroys joints by eroding bone. These patients have significant infiltration of their joint spaces with Th17 cells driven by inappropriate DC release of IL-23 and uncontrolled OC activity.

4. T regulator cells also modulate bone remodeling by expression of CTLA-4 that inhibits B7 (CD80, 86) on pre-osteoclasts (remember that OC have strong similarities to and can function as DC)
How T cells can dictate bone remodeling-II

D. Estrogen and T cells and Bone

1. Estrogen is osteoprotective by promoting osteoblastic activity and inhibiting OC bone resorption. It can directly target OB & OC and also modulate the display of RANKL on T cells. The postmenopausal decline in estrogen leads to decrease in bone mass and osteoporosis.

II. Introduction to Adipo-immunology (interactions between the immune system and the adipose tissue system). This subfield of immunology is becoming highly relevant to understanding many metabolic abnormalities, especially insulin resistance and obesity.

A. White adipose tissue (WAT) is composed of pre-adipocytes, adipocytes, stromal/vascular cells and macrophages. WAT is a super-neuroendocrine organ and a critical player in general homeostasis. It is a dynamic, complex, multifaceted tissue that produces a wide array of hormones, chemokines and cytokines that regulate energy storage, expenditure of energy, body mass, cardiovascular responses, reproduction and immune responses by sophisticated cross talk mechanisms. Since adipocytes are stationed not only in adipose tissue and share a common cell precursor with osteoblasts and glial cells, there is potential for cross talk between multiple organs and fat.
B. Communication molecules of WAT

1. Adipocytes produce 5 major adipocytokines that have a broad spectrum of inter-actions with the hypothalamus, skeletal system, immune cells, liver, pancreas, gut and multiple hormones that control energy metabolism.

   a. **Leptin.** Decreases appetite and is a pro-inflammatory molecule that can increase the production of IL-1, 6 and TNF-γ by a wide variety of cells but especially macrophages.

   b. **Adiponectin.** Has a major anti-inflammatory effect, predominantly by suppression of TNF-γ, and is the major antagonist to leptin.

   c. **Macrophage inhibitory cytokine (MAC-1)** is a member of the TNF superfamily, is anorexigenic and serum levels have a negative correlation with body mass. It is a major stimulator of adiponectin production.

   d. **Visfatin-** is strongly expressed in WAT and is pro-inflammatory and pro-angiogenic.

   e. **SFRP.** Antagonizes WNT signaling and is anti-inflammatory.

2. Macrophage derived cytokines in WAT

   a. **Osteopontin.** Produced by activated macrophages and is pro-inflammatory and is strongly chemotactic for monocytes and macrophages. The importance of this activity in obesity will become evident shortly.
c. **Resistin.** Direct antagonist of adiponectin and promotes TNF-α and Il-6 production.

b. The usual players, **IL-1, 6, 8 and TNF-γ**

C. In the WAT of the normal, non-obese individual:

1. There is a yin/yang relationship between the pro-inflammatory adipokines (leptin and visfatin) and the anti-inflammatory adipokines (adiponectin, SFRP and macrophage inhibitory cytokine).

2. The macrophage population is M2 phenotype- These cells upregulate production of IL-4 & 10, attenuate NFκB, and promote repair of tissue.

3. The net effect is a slightly anti-inflammatory milieu.

D. In the obese individual (and it is a problem-almost 40% of USA adults are overweight/obese) the agonistic/antagonistic balance is lost.

1. Obesity and its attendant metabolic pathologies result from an imbalance between energy intake (food) and energy expenditure that causes an increase in WAT. Expansion of WAT leads to dys-regulation of adipocyte function characterized by chronic activation of pro-inflammatory pathways and then insulin resistance.

2. Food excess, weight gain and expansion of adipose tissue triggers:
   
a. “stuffing” of individual adipocytes with fat activates a complex signaling program that eventuates in a pro-inflammatory milieu and insulin resistance. To date, no one knows why this happens.

   b. “stuffed” adipocytes release chemotactic and pro-inflammatory cytokines that recruit large numbers of monocytes from bone marrow and macrophages from tissue to WAT. The most potent monocyte attractant is CCL2 (aka monocyte chemoattractant protein-1) that is secreted by adipocytes, the monocyte receptor is CCR2. The aggregate pro-inflammatory stimulus is potent and up to 40% of all cells in WAT in obese people can be macrophages.

   c. The marrow derived macrophages are the M1 inflammatory phenotype that initiate a pernicious paracrine loop of inflammation characterized by the classic pro-inflammatory cytokines, the production of visfatin, leptin and resistin, decreased production of adiponectin, additional recruitment of more macrophages from blood by osteopontin, and ultimately local

Revised 10/24/12
insulin resistance and excess free fatty acids. (weird fact: 20-30 million macrophages accumulate with each kg of excess fat! Who counted them?)

d. The portal vein drains WAT. The free fatty acids and pro-inflammatory and adipocyte cytokine soup flows to the liver where it triggers hepatic and systemic metabolic effects that you will (or already have) learned elsewhere.

The normal state of adipose tissue

![Diagram of the normal state of adipose tissue](image)
Monocytes, macrophages and fat in obesity

E. The implications are obvious.

1. The strong link between obesity and inflammation will lead to therapeutic attempts to suppress the inflammatory component and normalize weight.

2. “fatty” arteries can be viewed as local obesity and there appears to be compelling evidence that cholesterol crystals in an artery attract macrophages that reproduce the self perpetuating accumulation of fat and inflammation in very dangerous places like a coronary artery. In coronary plaques, macrophages use the chemokine CCL2 to attract circulating monocytes to the plaque. Once there, they are prohibited from leaving by another chemokine system. Mice that are deficient in either CCL2 or CCL2R do not get atheromatous plaques! Much more on the pathology of this problem next year.

III. Primer on Central Nervous System (CNS) Immunology.

A. Initially thought that the CNS was “immune privileged” because it tolerated foreign grafts for long periods of time.

1. The “privilege” was based on no lymphatic drainage and the blood brain barrier.
2. Now shown that the quiescent immune status of the brain is maintained by active suppression of immune responses by cells and cytokines.

B. Microglia- the phagocytic cells of the CNS (they presumably also have other supportive functions in the healthy brain state).

1. Two subsets in the brain.
   a. One migrates early in development from bone marrow and is a self renewing population with macrophage & dendritic cell characteristics. They are highly branched and display very low concentrations of MHC and receptors.

   b. The other population is comprised of perivascular, bone marrow derived cells typical of circulating blood monocytes.

C. Innate immune response in the CNS

1. Both microglial subsets are maintained in a down regulated state similar to M2 macrophages. They produce TGF-β, IL-10 and IL-1Ra (IL 1 receptor antagonist).

2. Activation of the glial system by injured neurons (DAMPS), infection (PAMPS) or directly by peripheral cytokines converts them to M1 status and they secrete pro-inflammatory cytokines. They also change their morphology as they rapidly up-regulate their surface receptors.
3. This has serious implications for the host because IL-6 especially has potent effects on many neural functions. A classic example is acute delirium during serious systemic infection.
Regional & System Specific Immunology
John A Robinson, MD

Bone Immunology
Osteo-immunology

Bone Homeostasis
• Formation and remodeling is a dynamic, ever changing process orchestrated by osteoblasts (OB), osteoclasts (OC), hormones and immune cells
Osteoblast Differentiation

Modified from Glass et al., Endocrinology, 2007

Adipocyte
Mesenchymal Stem Cell
Osteochondral Progenitor
Chondrocyte

Osteoclast Lineage

RANK-  RANK+  RANK+
Monocyte precursor  Fusing monocyte precursor  mature osteoclast
Multiple signals  RANKL  RANKL

Bone Homeostasis

• OB, OC and immune cells communicate with each other through the RANK-RANKL system.
• RANK expressed on preOC and OC
• RANKL is the cytokine
• OPG (osteoprotegerin) mainly from OB is a decoy receptor
• The ratio between OPG and RANKL determines bone formation
• OB releases M-CSF that promotes OC survival and proliferation
Regulation of RANKL-RANK-OPG

- Dependent on multiple factors
- RANKL can be expressed by many innate and adaptive immune cells, including neutrophils, monocytes, DC, T & B cells
- Proinflammatory cytokines, 1,6,8, TNF and 17 induce RANKL and thereby promote OC
- Anti-inflammatory cytokines like 4 & 10 inhibit OC

Abnormal (unbalanced) Osteoclastogenesis
T cells can dictate bone remodeling

- A dominant Th1 response in and around bone is associated with INF-γ and activated macrophages. Result: pro-clastogenic and bone resorption
- TH17 cells, if dominant, are potent OC activators
- A normal Th1 or TH2 response or balanced TH1/Th2 responses would strongly inhibit IL 17. Result: a good thing
- Tregs can modulate remodeling by expressing CTLA4 that will block pre-OC development

Pannus of RA

Many, many Th17 cells

How T cells can dictate bone remodeling-I

Bone

M-CSF

Pre-osteoclast

RANKL

RANK

OPG

OC

RANKL

T & T17

CTL

Pre-OC
Abnormal (unbalanced) Osteoclastogenesis

How estrogens can dictate bone remodeling

**Abnormal (unbalanced) Osteoclastogenesis**

- Pre-osteoclast
- M-CSF
- Estrogen
- RANK
- RANKL
- Pre-osteoclast
- osteoclast
- B & T LY
- osteoblast
- osteoclast

**How estrogens can dictate bone remodeling**

- Estrogen
- RANK
- RANKL
- Pre-osteoclast
- osteoclast
- B & T LY
- osteoblast
- osteoclast

- Mac
- Pro-inflammatory cytokines
- IL-17
- T-reg
- CTLA/B7
**Why is this important?**

- Obesity is firmly linked to major adverse health outcomes, especially DM-2 and insulin resistance.
- Adipose tissue is the largest endocrine organ that produces a wide array of hormones that act like cytokines and vice-versa.
- Any stimulus that increases inflammation in adipose tissue is a bad thing.

(adapted from Oh & Olefsky. Science, 329, p 307, 2010)

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**White adipose tissue (WAT)**

- Super endocrine organ and critical for homeostasis.
- Produces hormones, chemokines and cytokines.
- Regulates energy storage and expenditure, body mass and immune responses.
- Composed of pre-adipocytes, adipocytes, stromal/vascular cells and macrophages.

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**WAT communicators**

- There are 2 major types of macrophages:
  - M1(proinflammatory)
  - M2(anti-inflammatory)
- Macrophage cytokines in WAT
  - Osteopontin- pro-inflammatory and potent chemotactic signal for monocytes and macrophages
  - The usual suspects: IL-1,6,8 & TNF-α
WAT communicators

- Adipocytes produce at least 6 adipocytokines (APK) that act like hormones and have broad spectrum of effects
  - Leptin is pro-inflammatory
  - Adiponectin is anti-inflammatory (Mistake in notes! suppresses TNF-α, NOT TNF-γ)
  - Macrophage inhibitory cytokine is TNF like but promotes adiponectin release
  - Visfatin is pro-inflammatory and pro angiogenic

Normal (non-obese) WAT

- Yin/yang relationship between inflammatory and anti-inflammatory adipocytokines with a net effect of slightly anti-inflammatory milieu

Obese WAT

- Yin/yang relationship between inflammatory and anti-inflammatory adipocytokines is not present
- Expanding fat in adipocytes triggers a very dangerous signaling program that makes WAT very pro-inflammatory by recruiting large # of monocytes and macrophages into it
- The portal vein carries the problem into the liver and (probably) also into atheromatous plaques.
There is fat in surprising places

Adipose tissue in the non-obese state

Monocytes, macrophages and fat in the non-obese state
Monocytes, macrophages and obesity

Implications of inflammatory macrophages in WAT
- In theory, suppression of inflammation should lead to weight loss
- A fatty artery is “local obesity”.
  - Cholesterol in the plaque attract M1 macrophages
  - Macrophages secrete CCL2 to attract monocytes and then prohibit them from exiting.
  - Big plaque to ruptured plaque is clinical problem of huge dimensions

Unique features: Central Nervous System
- Innate immune responses in the brain are held in active suppression by microglia and Il-10 and TGF-β
- 2 subsets of microglia in CNS:
  - migrate early in development and have DC and macrophage characteristics and act like M2 Macrophages
  - They are highly branched and display little MHC
  - Other subset is perivascular and derived from circulating monocytes
Unique features: Central Nervous System

- Activation of the glial system by DAMPS/PAMPS converts the M2 to M1 and then they secrete pro-inflammatory cytokines
- They upregulate MHC and change morphology

Clinical Implications

- Pro-inflammatory cytokines, especially IL-6, have potent effects on neural function and survival.
- May be important cause of delirium, long term traumatic brain damage
- Will be a lot of research on this in the coming years
Manipulation of Immune Responses

Date: Wednesday, May 8th, 2013
Time: 9:30 AM
LH 190

Learning Goals:
You will understand that the complex pathways that a normal immune response selects to react to specific pathogens provide the physician/scientist with multiple points at which the response can be altered or co-opted for the benefit of the patient.

You will realize that:
- The ability to manipulate the system mandates that a normal immune response must be understood first.
- The critical influence of genetic polymorphism must be taken into consideration.
- The pathogenesis of the disease has to be known before a logical intervention can take place.
- Biotechnology can then exploit this new knowledge and provide opportunities to diagnose and treat disease.

Background Reading.

Janeway `8th edition: Pages 222-223; 682-691 and posted articles on HD website (same reading that will be needed for the small group on lymphocytotoxicity.

Lecturer: John A Robinson, MD
Preamble: Once you understand normal immune responses, you can try to manipulate them for the benefit of patients and public health. A major caveat for anyone trying to manipulate such a complex system is that interference with one component almost always is associated with unanticipated effects downstream of the intervention. This is especially true when host immune response genes are polymorphic. Polymorphism is generated by evolutionary pressures and is a good thing for most, it can be a bad thing however for a few.

The following is a prime example of how powerful gene polymorphisms/mutations can be: A significant number of people, especially in Europe, have either homozygous or heterozygous alleles for a defective co-receptor that is required for HIV infection. If one is homozygous for the allele, it is basically impossible to get HIV. That would seem to be a good thing but, if that same individual becomes infected with the West Nile virus, there is a high possibility that either fatal or severe central nervous disease will ensue because the same receptor prevents West Nile virus entry into the brain.

I. Gene polymorphism dictates immune responses.

A. MHC-antigen presentation dictates how the host responds to pathogens.

1. Mammals have devised an ingenious system by which they can distinguish self from non-self. MHC Class I and II gene determinants are closely linked on the genome and have been throughout evolution-clear evidence of their critical importance to our survival. The MHC complex, located on chromosome 6 in man, is a cluster of genes that by virtue of their extreme polymorphism provide a unique immunologic signature on an individual’s cell surface.

2. Initiation of an immune response is dependent upon presentation of antigen either in the context of MHC Class I or II products to antigen-specific cells.

   a. If MHC does not present antigen, NO immune response will occur

   b. If antigen is presented, the extremely polymorphic MHC generates unique peptide binding repertoires in its antigen presenting grooves that provide almost infinite flexibility on what type of and how a peptide is displayed to reactive T-cells. Recently it has been shown that 5-6 amino acid long genetically determined peptides in the binding groove of APC provided some fortunate HIV infected individuals the ability to control their HIV infection without drugs.

B. TLR engagement and DC response to TLR engagement have critical bearing on the expression of disease
1. Both the duration and intensity of an immune response are strongly dependent on not only which TLR is engaged but “how much” it is engaged and whether the DC responds appropriately to TLR engagement.

2. The affinity and number of TLR displayed on a DC are determined genetically. The wide disparity of symptoms experienced by a large population of humans after being infected with a virus reflects TLR polymorphisms:
   a. No, too few or mutated TLR- no response to pathogen and death
   b. The “wrong” TLR- ineffective or pathologic response
   c. “too many” TLR- hyper-response and collateral tissue damage

3. Dendritic cells control the type of immune response to pathogens by determining the pattern of cytokine secretion during antigen presentation to T cells. That cytokine pattern can then dictate vigorous responses or induce tolerance to the antigen

C. Cytokine and TLR gene polymorphisms strongly influence the type and intensity of immune responses.

1. In a spectacular example of how the fusion of basic and clinical research culminates in clinical advances, it has recently been shown that patients who have IL-10 gene promoters that produce large amounts of IL-10 have a low incidence of significant graft versus host disease after bone marrow transplants (See posted article). This observation, based on strong experimental evidence that IL-10 suppresses Th1 responses, will lead to IL-10 gene typing in patients prior to transplant and provide a basis for more precise tailoring of their therapy.

2. A second example is the recent finding that mutations of the IL-10 receptor are associated with inflammatory bowel disease

3. A third example is that patients with a specific TLR polymorphism increased the risk of an invasive fungal infection in patients after stem cell transplantation (see posted article for details).
The cascade effect of genetic polymorphism

II. Microbiota and the Immune System - the “biggest” new idea in a long time.

A. Bacteria were here 2 billion years before an amoeba even thought of existing.

B. It turns out that bacteria probably drove the evolution of multi-cellular organisms and simultaneously realized that these multi-cellular organisms were a great environment. Most bacterial species have formed symbiotic relationships with us in specialized niches like the gut, respiratory tract, vagina and skin.

C. There is increasing evidence that the bacterial symbionts (ones that live happily with us and not against us) drove the evolution of the adaptive immune system to their advantage and taught it how to recognize what might be harmful to both the host and the bacteria that needed the host to thrive.
   1. Best evidence for this is that germ free animals have very defective immune system development and functions that are corrected by populating the gut with bacteria.

D. Why is this in the “Manipulation” lecture?
   1. Bacteria that normally live in the human gut have trained (manipulated) TLRs to force DC to be tolerant of them
   2. Normal populations of bacteria in the gut in concert with the individual’s genome dictate the ratio of T regulator cells to Th17 cells
3. Changes in the gut bacterial population will change the ratio and are associated with autoimmune diseases and inflammatory bowel disease.

E. The full development of these concepts can be found in a recent review in Science (336:1268-1273, 2012) that is posted on the HD website. **OPTIONAL reading.**

### III. Manipulation of the immune response in the real world- Infectious Disease Vaccines

A. To date, the most successful- in terms of people benefited- medical exploitation of immunology is vaccination. ( the scariest bit of recent survey evidence ever: a poll of residents (medical, surgical, etc. revealed that 15-20% of them were “skeptical” about vaccines! Couple that with the recent outbreaks of measles and whooping cough and it is clear we have a problem.)

1. The basic strategy of a vaccine is to provide the host with long-lasting, specific resistance to an infectious disease.
   a. But first things first. In order justify a vaccine, the infectious threat to the population has be defined in terms of morbidity and mortality. This is done by **epidemiologic** studies. Epidemiology is the scientific study of epidemics and epidemic diseases, especially the factors that influence the incidence, distribution, and control of infectious diseases; the study of disease occurrence in human populations
   
   b. Epidemiology provides the way to calculate mortality and morbidity, identify those at risk, and will provide clues to cause and dissemination.
   
   c. One gold standard involves "Mr. fix-it" and a pump handle but a good recent example is the H1N1 pandemic and identification of groups at highest risk.

2. While the threat is being defined, how the pathogen causes the disease (next year!) and how the host confronts the pathogen has to be known
   a. Identification, microbiologic analysis and propagation
   b. Determine the portal of entry of the infection
   c. Determine what structural component mediates entry/pathology
   d. Look "forward" in animal studies. While these are helpful, they almost never are sufficient. Mice are not humans.
   e. Look "backward and forward" in human post-infection
   f. Determine the effective immune mechanism invoked by the host to survive.
3. So what does the first year Host Defense student need to know? Only the CONCEPTS on how to “trick” the immune system into mounting a “normal” response to a pseudo-infection.

   a. An example of a genius way ahead of his time involves Sarah Nelmes and James Phipps and a country MD named Jenner.

   b. He observed that milkmaids had mild or no smallpox during epidemics.

   c. He inoculated a boy with fluid from a lesion on the skin of a cowpox-infected milkmaid. The boy developed mild cowpox.

   d. Then 6 weeks later he inoculated the boy with infectious smallpox fluid. The boy did not get sick, never developed clinical smallpox during subsequent epidemics.

   e. Jenner also gave us the word “vaccine” (from vaccinia=cowpox) and virus. In the spirit of “no good deed goes unpunished”, he was met with great skepticism but persisted and smallpox fatalities plummeted after the use of the vaccine.

4. And then **be sure** to understand the principles of Vaccinogenetics:

**The cardinal rule of infection:**

*Widespread infection of an outbred population results a genetically driven wide spectrum of antigen presentation, TLR and Cytokine activation, all of which lead to a highly diverse set of clinical symptoms and disease outcomes—from no symptoms to death.*

**The cardinal rule of vaccinogenetics mimics that of infection:**

*Widespread vaccination of an outbred population results a genetically driven wide spectrum of antigen presentation, TLR and Cytokine activation, all of which lead to a highly diverse set of immunological responses and immunity outcomes—from complete immunity to limited immunity to no immunity.*
G. How to go about making a vaccine. (you will do specific examples in a small group) **The mechanism(s) that causes the disease or syndrome must be known.**

1. If the disease is one that stimulates a TMMI and/or CD8 cytotoxicity response in natural conditions, then the vaccinologist has to devise a vaccine that activates TMMI without causing real infection.

2. If the disease is mediated by toxins or organisms that cannot hide inside cells, then we need a vaccine preparation that stimulates a B cell response.

3. If the disease is mediated by organisms that require an IL-17 response, many fungi for example, we need a vaccine that promotes Th17 cell dominance. (a reverse principle here might apply in autoimmune diseases-you will see the logic in the next lecture)

4. If the disease is a viral disease, the vaccinologist will need to devise a strategy that not only prevents dissemination of the virus but also facilitates T-cell responses that limit cellular infection.

5. What about a drug addiction? Since it is known that cocaine causes its effect by binding to dopamine transporters and blocking their ability to clear dopamine, a vaccine has been created that induces antibodies that bind to cocaine and make it a bigger complex that cannot fit through the junctions of the blood brain barrier.

5. In ALL cases, memory will have to be generated.
6. In contrast to Jenner’s time where cowpox lesions were ground up and given to susceptible humans, there are a very wide range of options for the production of safe vaccines.

a. What type of preparation and route of vaccine delivery will be most efficacious? Live attenuated, killed, recombinant? And do you need to give the antigen a “boost”? These questions can be resolved by structural analysis of the pathogen, knowing the portal of entry and the type of response elicited by the pathogen (Th1, Th2 etc.)

b. Although in theory the natural route (respiratory, GI, vaginal) should be best, this is not always true. The newest vaccine, HPV, is a good example.

c. Accept the fact that a vaccine will not be effective 100% of the time and will have side effects. Two predictions, obvious by the principles of vaccinogenetics, are that a few vaccinees are going to have a significant reaction to the vaccine and a few will not respond to it

III. Tumor Immunology and Tumor vaccines

A. We have limited opportunity to study immunity to tumors in this course so I am sneaking in the concepts here.

B. Not all tumors arise because of defects in immunity that have developed with age. In fact, there are multiple causes that include DNA methylation or mutation, environmental factors, viruses, etc. It is highly likely that most cancer is multifactorial in origin.

C. The basic tenets of tumor immunology are:
1. some, but not all malignant cells look "different" to immune cells.
2. The ones that look like “self” don’t invoke a rejection response. The ones that look different can, in theory, be attacked by immune effector cells.

3. Three ways a tumor cell can look “different” to the immune system:

![Diagram of tumor cell interaction](image)

4. Evolution has developed multiple immunologic ways to suppress or eradicate a mutant cell if it can be recognized as such.
   a. Example: some mutant cells acquire excess chromosomes (hyperploidy). The latter stimulate mutant cell display of an “eat me” signal on the cell surface which then provokes phagocytosis by macrophages. They, in turn, degrade the mutant cell and present its cancer antigens to T cells.

5. The survival of a "recognizable" tumor cell implies that either it is not recognizable to the immune system or it has developed a mechanism(s) to thwart activation of cytotoxic responses to it.

6. The tumor immunology paradox: Why aren’t “recognizable" tumor cells eliminated by the immune system?
• TSAg = Tumor specific antigen

Figs. by John A Robinson, MD

D. Tumors have ingenious ways of manipulating the immune system: All involve manipulation of their environment by exploiting regulatory cytokines and cells in some ways that resemble those by which the mother protects her developing fetus.
1. Immune effector cells are coaxed into the tumor by tumor secreted chemokines and converted to tumor protector cells.

2. The tumor “reorganizes” the surrounding stromal tissue, especially the fibroblasts, into a structure that promotes immune tolerance to the tumor by supporting the growth of Tregs.

3. Some tumors display a ligand that activates an inhibitory receptor (PD-1) on cytotoxic T cells.
One way that tumors manipulate their environment

There is only one defense mechanism left when Tregs are turncoats

• Fig. by John A Robinson, MD

E. How to make a tumor vaccine- in theory -will also be done in the Lymphocytotoxicity Small group

IV. Targeted Strategies based on understanding the pathogenesis of a disease. Some examples follow…
A. Monoclonal antibodies- remember these from an early small group?
   1. Anti-TNF specificity for diseases with pathologic inflammation. Rheumatoid arthritis is the prototype.
      a. the side effects may be predictable. Increased risk of a Th1 disease

2. Asthma. Monoclonal with the ability to block IgE binding to a mast cell

3. Transplantation. Monoclonals that effect T cell rejection:
   a. block cytokine production
   b. down-regulate cytokine receptors
   c. inhibit co-stimulatory signals

4. B cell antibody mediated diseases
   a. Monoclonals that deplete B cells by binding to B cell CDs

5. Anti-PD-1 or anti PD-1 receptor, anti CTLA4 for anti-tumor therapy

6. And the best manipulation for last. There are strokes of genius and then there are real strokes of genius. The ultimate way to manipulate the immune system is change it out for a new one. Stem cell transplants offer that opportunity. Recently a patient with HIV developed acute leukemia and needed a SCT to survive. He had no compatible related donors and so an unrelated donor was needed. A compatible donor was sought but not just one with compatible stem cells but one who also had an inherited deficiency in the CCR5 receptor. The latter receptor is required for HIV infection. The transplant was done and the recipient not only survived his leukemia but also cleared the HIV virus completely and was able to stop all his anti-HIV medications. (for full details see the New England J Medicine article posted on the HD website).

This is just the beginning: bright minds make the future of medicine bright and in the near future stem cell transplants will be tailored to provide survival advantages for the recipients by providing TLR that resist fungi, etc.!
Manipulation of Immune Responses
Also known as: why I need to understand Host Defense

Facts for the day
• Females with the highest IQs measured between 8-12 reach menopause later or earlier?
  • Later
• Males with 4th fingers that are longer than their 2nd fingers are happier or more depressed?
  • Depressed
  • Why?
  • Too much fetal testosterone

Let’s talk more about fingers
• Females with shorter ring fingers relative to their 2nd fingers are more or less fertile?
  • More fertile
• Now back to men with longer 4th fingers—more or less fertile?
  • More fertile
Pair-bonding

- Evolved from the same brain systems that are implicated in drug addiction
- In Voles-ONE genetic polymorphism switched the rodent from a promiscuous life style to monogamy
  - vasopressin1A increased expression was associated with monogamy

Reproduction Jeopardy-guess the hormone

- Attachment
  - Vasopressin/oxytocin
- Romantic Love
  - Dopamine/norepinephrine
- Lust
  - testosterone

Preamble

- Polymorphisms dictate host responses. Some promote effective response, others lead to ineffective responses
- Once you understand immune responses, you can try and manipulate them for the benefit of patients and the public health
- When you try and manipulate a complex system, there can always be unanticipated downstream effects.
- This is especially true when the genes running the system are polymorphic
An example of polymorphism/mutation power

- A significant # of Europeans have either homozygous or heterozygous alleles for a defective co-receptor for HIV
- It is basically impossible for a homozygous individual to get infected with HIV
- But... if that same person gets infected with West Nile, high likelihood of severe infection or death

Polymorphism

- MHC- antigen presentation matters!
  - Extremely polymorphic
  - Type of response based on Class 1 or Class 2 presentation
- TLR-the switch matters
  - Activation determines type and intensity of response
- Cytokine- the signals matter
  - Polymorphisms are linked to quantitative differences in production

Gene Polymorphism

- MHC-antigen presentation dictates how the immune system responds
  - If doesn't display Ag, NO response
  - If the antigen IS presented, the polymorphic MHC generates multiple unique antigen binding grooves that guarantee almost infinite variety on how the peptide is displayed by the DC
How MHC determines susceptibility versus protection

TLR Polymorphism

- The duration and intensity of the response is dependant on “how much” a TLR is triggered.
- The affinity and number of TLR are determined genetically. We know this by the wide spectrum of clinical responses to a virus.
  - From death to asymptomatic infection
  - DC are rich in TLRs and they will do what the TLR tells them to do - that means the “right” cytokine and in the “right” amount

There are lots of TLR on lots of different cells
Cytokine Polymorphism

Three examples

1. Patients who produce lots of IL-10 to a given stimulus do better after a bone marrow transplant because they suppress graft versus host reactions better than those who produce low amounts of IL-10
2. Mutations of the IL-10 receptor are associated with inflammatory bowel disease
3. Patients transplanted with a specific TLR polymorphism had increased risk of a specific type of fungal infection

The cascade effect of genetic polymorphism

Microbiota and the Immune System

Biggest new idea in a long time

- Bacteria were around 2 billion years before amoebae
- Bacteria drove the evolution of multicellular organisms
- Many bacterial species have symbiotic relationships with us
- "Friendly" bacteria drove the evolution of our immune system
Why are we talking about this in a “manipulation” lecture

- Bacteria manipulate our DC to be tolerant of them
- Bacterial populations in the gut dictate how we respond to various antigens by manipulating our T regs
- If we alter gut bacteria with medical treatments, we risk the development of autoimmune diseases!
- More on this in a Dr Knight lecture

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**IMMUNOGENETICS**

- NO ILLNESS OR VERY MILD
- LIMITED ILLNESS
- SEVERE ILLNESS OR DEATH
- CARRIER

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Real World Manipulation of the Immune Response- Vaccines

- Epidemiology
  - The factors that influence the incidence, distribution and control of infectious diseases
- History
  - Pumps, Sarah and James
  - Understand the pathogen
    - You have to understand what causes the pathology and that will happen next year
- Immuno-Epidemiology
  - host responses in the field
- Vaccinogenetics
  - If you don’t get this, you will be disappointed with your results
- Construct the vaccine
  - This was done in small groups
A contemporary example of how epidemiology can explain things

A classic example of epidemiology

- John Snow, MD (1813-1858)
- Studied water supply and urban distribution of patients in London during cholera epidemic
- Then drew a map
A classic example of epidemiology

- From the map he was then able to postulate what the source was and curtailed the epidemic by?
- Removing the pump handle!

Another classic example that integrates immunology with epidemiology

Jenner was country MD
- Observed how people that worked with cattle had mild or no smallpox during epidemics
- Inoculated a boy with fluid from a cowpox infected milkmaid
- The boy developed mild cowpox
- Then 6 weeks later inoculated the boy with smallpox fluid
- The boy did not get sick, never developed clinical smallpox in subsequent epidemics
- Jenner also gave us the word “vaccine” (from vaccinia=cowpox) and virus
- He was met with great skepticism but persisted and smallpox fatalities plummeted after the use of the vaccine

This wouldn’t happen today!

Dr. Jenner infected a boy with cowpox to protect him from smallpox.
Normal T& B cell response to vaccine antigens and induction of active immunity

Reaction to vaccine based on hyper B/T cell response secondary to manner of MHC/TLR presentation and cytokine polymorphisms

No immune response to vaccine antigens because of MHC/TLR lack of effective presentation/activation and/or cytokine suppression

Cardinal Rule of vaccinogenetics

- Widespread infection of a population results in a wide spectrum of antigen presentation to polymorphic TLR and Cytokine genes that then dictate the clinical manifestations and course of the disease

Ground rules of vaccinogenetics

- Devise proper type of response strategy—one must know the mechanisms causing the disease/syndrome
  - TMMI for intracellular infections
  - IL-17 for fungal infections (what could you do if Th17 cells were causing a disease?)
  - B cell response for toxins and organisms that resist phagocytosis
  - T & B responses for viruses
  - Vaccine for a drug addiction? What is that mechanism?
- Generate memory cells in ALL cases
- You have done this in small groups
Cocaine

How to make a Vaccine

• Understand the immunology
• Fool the host into thinking an infection has occurred
• Understand that not all people are going to respond

Real World Manipulation of the Immune Response-Tumors

• The basic tenets of tumor immunology are:
• Some but not all malignant cells look "different" to immune cells. The ones that look different can be attacked by immune effector cells.
Real World Manipulation of the Immune Response-Tumors

- Evolution has developed multiple immunologic ways to suppress or eradicate a mutant cell if it can be recognized as such.
- The survival of a "recognizable" tumor cell implies that either it is not recognizable to the immune system or it has developed a mechanism(s) to thwart activation of cytotoxic responses to it.
The devious tumor cell
(think about Scylla and Charybdis)

One way that tumors manipulate their environment

This leaves only....
Targeted manipulation strategies based on pathogenesis

- Anti-TNF
- Anti-IgE
- Anti-Rejection
- Anti-B cell
- Anti-CTLA4

You will learn specific disease examples in MHD next year but you are already ahead of the game because now you can predict what to develop based on what immune response should work!