Things are changing with the single gene idea of disease. miRNA. Transcription, translation, protein! DNA is transcribed into a mRNA molecule which then is translated into a peptide chain of amino acids at the ribosome. Studies in eukaryotes showed that the mRNA can be edited after it is transcribed (post-transcriptional editing. introns vs exons) and the peptide chains made at the ribosome can also under go modification, with the addition of sugars or other groups. (post-translational modification).

Today it appears that small microRNAs participate in the regulation of gene expression, before the transcript mRNA ever reaches the ribosome. Post transcriptional silencing of genes! What is the DNA called that makes miRNA? Its not a gene...

Side story: An example of Post-translational changes in a peptide/proteins is erythropoeitin (Epo) Epo is a natural human hormone that signals for increased red blood cell synthesis. It is sometimes abused by athletes looking to temporarilyincrease the oxygen carrying capacity of their blood for increased endurance during sports activities. The Epo gene has been cloned and a recombinant form of Epo exists, but it cannot be prduced in genetically modified bacteria, as other recombinant proteins are. The Epo peptide undergoes post-translational modification, most noteable the addition of sialic acid sugar groups. Epo is therefore produced in genetically modified chinese hamster ovary cells (a mammalian cell line that can be grown easily in tissue culture) and the CHO cells modifiy the Epo peptide with sialic acid residues, producing functional Epo. The arrangement of sialic acid residues in recominant Epo, raised in CHO cells is slightly different than that in humans, and it is these slight differences that can be detected in anti-doping analysis via protein electrophoresis.

Reversible

irreversible-cell death Glycolysis acidifies the cytoplasm, Calcium leaks from mitochondria and the smooth ER. Some proteins denature and clump together, lysosome and endosome proteins/enzymes can now work in the cytoplasm. Loss of Ca++ control, activates or inactivates other enzyme processes. Free fatty acids combine with Ca++ to form insoluble salts. OR apoptosis occurs.

adaptation Hypertrophy, hyperplasia, metaplasia, cellular inclusion

death? mitochondrial dysfunction, plasma membrane leaks

Inflammation? vascular tissue response 1) wall response, 2) effector proteins

No vessels? No inflammation! Cartilage has no blood vessels, think sharks. Articular (hyaline) cartilage is amazing stuff, but infection is bad news for the joint. The eye ball is another compartment which is "priveledged" in its lack of immune cells.

Side story: This actually allows some modern antibody treatments to work well, where as system

monoclonal antibodies has had poor success. Treatments for macular degeneration (anti-VEGF) prevent new blood vessel growth. Also allows fr some biochemical measurements of the post-mortem interval (potassium concentration in the vitreous humor)

acute inflammation: 1)vessel caliber change, 2) vessel permeability 3. Leukocyte migration.

Flow change allows WBC to roll along vessel wall and emigrate through it. ICAM differences explain influenza pathology. Common cold relies on VCAMS? URI.

regulation of the acute immune response: Dysregulation leads to organ pathology Key actors Histamine, serotonin released from Mast cells, Tissue factor released from ECs, Platelets

The liquid part:

plasma proteins: 1) compliment 2) kinin 3) clotting cascade

Who? Arachadonic acid, leuktrienes TNF-alpha, interleukin 1, 2

Compliment C3a C5a opsins, membrane attack complex

Kinins bradykinin similar vascular effects as histamine, dry cough from ACE inhibitors?

Clotting cascade: Virchows Triad-endothelial dysfunction, stasis and hypercoagulability: endothelial dysfunction,(exposure of vessel collagen) (atheroma), stasis (AFIB, DVT) cause hypercoagulopathy, thrombus, emboli. In addition to the state of pregnancy, Birth control (both estrogen related), smoking

Aside : Trauma TCCC hypothermia, hypotension and coagulopathy (dilution coagulpathy too?)

The cellular part: Innate vs adaptive immunity. A very brief background introduction

Innate -a rapid (minutes-hours) but not especially specific response.

APCs , I think they are all macrophages,(granulocytes)myeloperoxidase present antigens on MHC 2 (HLA). Have Toll Like Receptors (Das is Toll) TLR that recognize PAMPS. Amazing evidence for evolution. Best known PAMP is lipopolysaccharide (think E.Coli 0147:5). Discovered when sterile IV solutions produced high fever (innate immune response). Many medical supplies labelled "Pyrogen free". Newer PAMPS are purines! ATP, Urate, Adenine Adenosine???

ATP, Purines, Gout. Colchicine blocks cytoskeletal movement. Allopurinol shunts urate elsewhere.

LPS can cause systemic inflammation and shock, in a completely sterile environment!!! Rodents are 1 million times less sensitive to LPS!!! Can you vaccinate yourself against LPS, gout? Maybe, but the

APCs will still activate and will still create the shock.

Ebola and Dengue are unique in their affinity for APCs---Fever!

Adaptive (the stuff of vaccines)

Lymphocytes form against any and all antigens, anything recognizing self is eliminated in the thymus (over heart, involutes with age) or deactivated by anergy in the periphery. Small molecules (not protein) (think aspirin size) are poor antigens but researchers can get around this by attaching a small drug molecule to a bigger molecule. Problem is the non-specific antibodies to the big molecule. This may occur in rheumatic fever and heparin HIT? (fractioned vs non sizes)

A B-cell, when activated, turns into a plasma cell. Producing huge amounts of the antibody it is made for.

Helper T-cells enable ibteraction between APCs and B cells

NK cells, CTL

cytokines activate ECs and cause vasodiltiation. Congestion. Net result is fluid loss out of vessel compartment. Low Albumin also seen in liver disease, burns, nephrotic syndrome. Increased blood flow but slower movement and leaky vessels allows leukocytes to attach to, roll along, and exit capillary.

Endothelial cell activation and dysfunction is a significant source of pathology. Ebola, Sepsis, atherosclerosis, DIC, Vasculitis,(lupus). Hemorrhagic E.Coli. Shock (profound hypoperfusion) due to trauma, transfusion reactions. Leads to hypercoagulability and inappropriate activation of clotting cascade. This leads to thrombus (a pathological clot) and embolism (the fate of the clot). In DIC, a consumptive coagulopathy, microthrombi cause ischemia in small vessels and all clotting proteins are consumed.

E.Coli 0145:7 causes endothelial dysfunction and kidney failure.

Connection: hepatitis. Chronic liver disease (and repair) leads to a lack of clotting factors and serum albumin. Scarred liver has poor flow and result in passive congestion. (Stasis) No albumin leads to water leaving vessels, plus passive congestion, leads to pitting edema (unlike inflammatory edema)

Endothelial cells contain von willdebrand factor,nitric oxide synthase. Nitric oxide, a neurotransmitter

discovered in 1990s but whose effects were well known prior is a major actor in vessel diameter. Nitrates given for angina (heart pain) relax smooth muscle, increasing flow in cornary arteries, but lowering blood pressure. Esophagial pain will be relaxed too!!! Erectile function in men is dependent on nitric oxide and Viagra acts upon the second messenger pathway. Nitrates cause erectile dysfunction, as well as other vessel disease, including diabetes.

Here, problems of infection+ sepsis, trauma, large burns, transfusions overlap in the response they elicit and the pathology that develops---Shock.

Chronic (longterm) inflammation.

hypersensitivity 1234

1) Ige mediated allergy/anaphylaxis. Preformed antibodies activate mast cells, release histamine. Endothelial activation leads to leaky vessels, edema. Histamine release and kinins cause flushing of skin vessels, but contraction of lung smooth muscle, angioedema. itching.

Airway compromise and hypovolemic shock from systemic vasodilation and fluid loss across capillaries.

2) Antibody, Opsinization, Fc fragments

TTP,

hemolytic anemia,

pemphigus,

goodpastures,

rheumatic fever,

myasthenia gravis,

Graves disease,

pernicous anemia?

3) Immune complex. (antibodies and their target antigen protein)

serum sickness, vasculitis, lupus, anthus

In the old days, diphtheria anti-toxin, tetanus antitoxin was made by inoculating large animals like horses. Adaptive immune response would produce antibodies. Collect blood, remove solid matter and cells, inject the plasma with the antibodies (and every other horse protein). Circulating immune complexes invite inflammation, cannot be filtered through kidney and also inspire glomerulonephritis. Leading to nephrotic syndrome, leaky vessels, coagulopathy etc

vasculitis: polyarteritis nodosa, Lyme disease? Lupus

Lupus forms immune complexes with the patient's own proteins

Transfusion reactions are an unfortunate situation the immune system was not equipped for. Rh factor incompatibility between mommy and baby too! Who gets blood inside? Nobody, unless you get a transfusion.

4) cell mediated GVHD, TB Needs CD4 cells and CTL TNF alpha (cachectin)

Your immune cells may reject the donor tissue, but don't forget that the donor tissue still has immune cells from the donor! Those cells will reject the recipient.

Methotrexate is folate antagonist,=NO new DNA!!!! Methotrexate can be used to treat autoimmune disease, such as rheumatoid arthritis, Lupus, pemphigus etc. due to preventing the proliferation of lymphocytes. This a form of immunosuppression. Larger doses of methotrexate are used to treat cancer, again, by limiting the proliferation of cells, by making folate and hence, new DNA scarce. Profound immunosuppresion occurs as an unfortunate side effect, unless you have an autoimmune disease in addition to cancer, in which case, your autoimmune symptoms improve.

Sulfonamide, the first antibiotic, is a bacterial Methotrexate! Antibiotics such Bactrim (trimethoprim+sulfamethoxazole), Silver sulfadiazine (Silvadene cream), Sulfamethoxazole (in Pediazole) prevent bacterial growth and proliferation through inhibiting folate metabolism. The bacterial version of tetrahydrofolate is a bit different than the human version and result from distinctly different, enzymes.

Vaccines are hard to make! Only about two dozen vaccines are in common use. ie these vaccines produce lasting immunity without a multitude of booster shots. Check out the CDC pink book. http://www.cdc.gov/vaccines/pubs/pinkbook/index.html

Inactive germs or fragments of germs often function poorly as antigens, necessitating multiple booster inoculations or adjuvants to be combined with the vaccine. The germs which are exceptions to this were the first few (easy) vaccines. Small-pox, Measles, Mumps, Rubella, tetanus etc. Notice, almost all of those are bacteria (big germs). Biologically active germs cause infection, and will eventually produce enough antigen to elicit immune response Its own booster dose. The live polio vaccine is a good example of this, though the killed virus (Mr. Salk) worked well enough as a vaccine. Freunds+ Alum containing adjuvants

Got sick? APC activation! Polio vaccine (RNA virus) is a live vaccine. Small pox, chickenpox are DNA viruses. Hint hint. The multiplying microbe with serve as its own booster shot, however indifferent you immune system may seem.

TLR4 receptor may explain why adjuvants work. Toll-like-receptors are the molecules which connect the innate (APCs) and adaptive (lymphocytes) immune systems.

Infections that cannot be cleared: HIV, Lyme disease, TB, Herpes, polio (cd4+, fibroblasts, ghon complex, herpes and nerve roots, gut.

Cancer and paraneoplastic syndrome. Parathyroid hormone mimic, and the calcium perturbation

Carcinoid tumors and serotonin syndrome

The transfusion reactions

Lactimal and Stevens Johnson syndrome

Anaphylactoid drugs like opiates.

Post-operative fever? Its not uncommon for patients to develop a fever in the first few days after a surgery, generally abdominal or thoracic, where breathing can be somewhat painful during recovery. It has been said that this fever develops from underinflated regions of lung (atelectesis) but there isn't a lot of science to back this up. It IS a convenient reminder of the role in fever, that antigen presenting cells , such as the resident macrophages of the lung have in acute inflammation. Pleural effusion and pulmonary emboli can also be sources of fever, further adding to the myth?

When cells die:

Started with resources: Pubmed, Aclands, Robbins, Alibris, Audible

Patterns: Granulomas Quiet cells with granular nucleus (euchromatin) Cells with large nucleus to cytoplasm ratio (lymphocytes, clue to chronic inflammation)

The cartoon cell came from histology of 1800's and EM of the 1970's. Two colors Hematoxylin and Eosin. Hematoxylin stains DNA, Eosin stains protein (Red is dead). DNA and RNA are acids and attract basophil dye. Proteins often negatively charged save for lysine, proline(+)

DNA is associated with histones, which has been demonstrated to be crucial in gene expression (epigenetic)

Beautiful metachromatic dyes such as Toluidine blue, and the Romanovsky Geimsa, Papanicolou stains show whole spectrum.

What's a cell to do?

Adaption

Reversible injury

Death-irreversible change. The outside becomes the same as the inside.

Adaptation: hyperplasia, hypertrophy, metaplasia, cellular inclusions ,molecular change such as inducing smooth ER production in the liver cell.

Hyperplasia--Lymphoid tissue such as the spleen, lymph nodes, proliferate during infections, becoming larger and more cellular. Leukemia, anemia. Liver after most damage (only remaining regenerative organ in humans)

Psoriasis and thickening of skin, HPV warts too. Gingival hyperplasia from phenytoin use.

Hypertrophy-heart in response to hypertension. Though the root cause of hypertention is unclear, the heart as a post-mitotic organ can only get bigger. There is some contraversy over c-kit+ cells, likely fraud. The kidney will hypertrophy post removal for transplantation,

metaplasia--border between squamous and columnar epithelia in the esophagus/stomach junction, upper airway and bronchial respiratory epithelia and the vagina/cervix junction

Barrott's esophagus from GERD, alcoholism. Do mice get Barrett's??? What is the pH in a mouse stomach?

Smoking and the upper airway (Carla Kim Brochoalveolar stem cells?)

HPV and the cervix (columnar) and vagina (squamous) junction

inclusions-- amyloid protein, viral proteins, hemosiderin pigment from blood often found in spleen, where red blood cells go to die and be recycled or turned into bile pigments. "Heart failure cells"-hemosiderin laden macrophages in the lungs result from passive congestion of blood. Leads to

RBC debris. Lipofushin? A wear and tear pigment? Iron. The human body doesn't have any terrific means of regulating iron absorbtion+excretion. Repeated blood transfusions (RBC overload) or genetic disorders such as hemachromotosis result in much hemosiderin pigment found at autopsy.

Lipid (fatty change) Neiman Pick disease. Choline deficient diets.

Reversible: Hydropic change, loss of basophilia. Life is about keeping the outside different from the inside. If the two equilibrate, you are dead. A number of ion pumps keep sodium, potassium, chloride and calcium in check. Examples include the Na+/K+ ATPase & ATP synthase in the mitochondria. This regulates the osmosis of water in the cell, with a small additional contribution made by cytoplasmic proteins. Calcium is sequestered in the mitochondria and the endoplasmic reticulum, and is often a control for many enzymes as well as a signal molecule between neuron synapses. Endosomes are a compartment that maintains an acid pH for digestive enzymes kept away from cytosol.

As cell runs out of ATP, ions pumps start to fail, cell is stuck with glycolysis/anaerobic respiration. Ion concentrations change, water starts leaking into various compartments, and cell accumulates acid products of glycolysis (lactic acid) which normally would go through the citric acid cycle and the electron transport chain in the mitochondria. The "switch" to anaerobic respiration does not solve any problems. It is not a survival strategy. The only exception is skeletal muscle, but this is also fatigable muscle (it doesn't work when hypoxic but recovers with rest ie. when oxygen demand=oxygen delivery)

Acid pH causes denaturation of proteins, ribosomes detach from the ER, causing a loss of any cytoplasmic basophilia, which indicates a problem with new protein synthesis. Whatever faint blue cytoplasm you could see from the H&E stain is gone, since those ribosomes are no longer "concentrated" on the rER. You see this most distinctly in liver and neurons of the brain. This basophilic rough ER is Nissl substance! With no rough ER, the cell is stuck with diminished capacity for protein synthesis. This is not good. Protein synthesis is one of the key processes that distinguish the inside from the outside of the cell.

Acid pH allows enzymes from the endosome be catalytically active in the cytosol, allowing degradation of cytoplasmic proteins into free amino acids. Normally the endosome compartment is the only area of such acid pH (due to an ATP fueled H+ pump). The cells appear acidophilic, also called eosinophilic, as the acid cytoplasm stains more heavily with the orange/red eosin dye. Muscle cells demonstrate this eosiniphilic hange very well.

What you get is a swollen cell with membrane blebs in nucleus and plasma membrane, loss of basophilia and resulting eosinophilia. Histones and DNA collapse, and condense, causing a shrinking and darkening of the nucleus (pyknosis)

Worst of all, calcium seeps from hiding and starts activating or inhibiting proteins, causing metabolic chaos. Worsening protein function and enzyme dysregulation. Free fatty acids (released as the cell membrane is digested) and calcium can combine to form insoluble salts (soap scum) also adipocere (grave wax)

IF problems can be reversed, by reestablishing blood flow, elimination of the poison etc, cell can recover or enter point of no return Mitchondrial transition response? The formation of the mitochondrial transition pore is one definitive sign of crossing the line from reversible injury to point-of-no-return. A protein pore forms allowing mitochondria contents to equilibrate with the cytoplasm. Not good. The MTP has been studied, mostly in the context of heart and brain ischemia. ie heart attack and stroke.

Cell death. Contents leak out. Karyolysis Necrosis

A number of diagnostic tests rely on identifying specific proteins released from cell death with a tissue. In liver disease/injury -the transaminases (AGT, ALT). The heart? CPK MB form. The pancreas? Amylase

Sometimes called coagulative necrosis (empty ghosts), liquifactive (involving bacteria and APCs neutrophils, macrophages) and caseaus (combination cheese like TB)

The wrinkle of cell death: Apoptosis. Deliberate cell death. In normal development (think tadpoles changing to frogs) or significant DNA damage. Radiation or alkylation from chemotherapy agents. Genome DNA is chopped up (detected with TUNEL stain), cell contents for small vesicles that do not leak, and therefore do not induce inflammation. Caspase cascade. In particular, caspase 3.

phosphatidylserine on the inner surface of the plasma membrane is flipped outwards.=”I am apoptotic, please eat me “

Escaping apoptosis is a mechanism leading to cancer. p53 is a well known "cancer gene"

Like the tadpole-to-frog analogy, removal of growth factors signal apoptisis.

Example: The endometrium, of the female uterus, undergoes cycles of hypertrophy and atrophy, leading to monthly menses. These cellular and tissue changes are signaled and maintained by the sex hormones, in particular estrogen and progesterone. Endometriosis (when endometrial tissue is found out side the uterus, anywhere in the abdomen) causes profound inflammation and pain. The ectopic endometrium will grow or atrophy in synch with the menstrual cycle. How does the endometrium get outside of the uterus? Nobody knows. Its a weird mystery.

ovarian cysts too. Inducing a sort of chemical menopause prevents these "problem" tissues from growing, but produces symptoms of menopause in women still in their child bearing years. Vaginal atrophy, and hot flashes (flushing) are common side effects of these treatments.

Cancer?:BPH benign prostatic hypertrophy. Remove or inhibit the testosterone growth factor and the prostate shrinks.

Cancer: estrogen receptors. Some cancers will have their growth regulated by hormones. The discovery of the estrogen receptor in some breast cancers lead to the development of chemotherapies that are uniquely specific in the types of cells they disrupt. Some new evidence for COX-2 in dog bladder cancers. Possibly in colon cancer.

Neurons undergo apoptosis and do not divide ever, regardless of the manipulation. Ever hear of brain cancer? Those are gliomas, not neuron tumors. Only example of a neuron cancer is retinoblastoma (young young babies) Researchers can play games like fusing cells with a b-cell lymphoma cancer cells (using PEG or electricity) to form an immortal cell line. Can't be done with neurons. They apoptose .

TNF and IL2 cause inappropriate apoptosis as well as the CTL and NK cells. (The death receptor)

The picture tour: Myocardial infarction, insulitis, infarct (new, old) atherosclerosis, true clots vs postmortem. Granuloma from foreign body (showed in beginning) tattoos, asbestos, pulmonary fibrosis/pneumoconiosis. Macrophages in lungs cause fever!

MI Acute, healed, 10 day blow out macrophages! The dead heart tissue recruits inflammatory cells, and eventually gets gobbled up by active macrophages. Clearing this dead tissue and debris can actually degrade the integrity of the ventricle wall, causing rupture. Scar tissue isn't deposited as fast as the debris is cleaned up. Most risky ~ 10 days post infarct.

infarcts kidney, liver, intussusception. In solid organs, infarcts are often wedge shaped, reflecting the branching geometry of blood vessel. Organs with a "dual" blood supply will become congested and engorged with venous blood rather than becoming pale bloodless infarcts. The intestines and the liver are the best examples of this. Most other organs, you can think of blood coming in one side and flowing out the other, but these organs "in the middle" of the body's blood circuit receive venous drainage from various distal parts of the body, resulting in more than one exit/entrance the organ. Block something, and the pipes don't run dry, stuff seeps in from the collateral plumbing.

Diabetes insulitis. lots of lymphocytes. Chronic inflammation of the pancreatic islets with eventual loss of the insulin producing beta cells. Thought the be a form of autoimmunity.

Atheroma foamy macrophages! Cholesterol clefts-needle like crystals of pure cholesterol!

Granuloma angry, constipated macrophages! When acute inflammation can't be readily resolved. Large activated " epithelioid " macrophages are desperately trying to devour the foreign body, and are encircled by lymphocytes driving the reaction. The so called foreign body reaction. –wood splinters.

sutures, pencil graphite form granulomas, The angry macrophages often form multinucleate "giant cells"

connection??: Osteoclasts, specialized cells which resorb bone and multinucleate giant cells derived from the same bone marrow lineage as macrophages. Dissolving bone is hard angry work?

Post-mortem clot "Currant jelly and chicken fat." is the classic description of post-mortem clots No lines of Zahn. Layered clots result from flowing blood.

reperfusion injury: *contraction band necrosis* in the heart and whales! Rhabdomyolysis, myoglobin from crushed or dead muscle causes nephrotoxicity, and subsequent kidney failure (red or dark urine), also free potassium and calcium causes muscle contractions and heart arrhythmias ROS from reperfusion cause more injury to the cell and organ. Remember that the sarcoplasmic reticulum is actually modified endoplasmic reticulum.(smooth ER) Ca++ sequester. The mitochondrial Transition Pore (MPT) function/dysfunction? is implicated in reperfusion injury.

Another term is "tourniquet shock", the concern being rhabdomyolysis and systemic inflammation, but aggressive hydration can protect the kidneys. Again, free ATP, purines like uric acid, (all things that belong inside cells) activate APCs and drive the innate immune system crazy---fever, endothelial cell activation and dysfunction, leading to blood vessel dialation, hypovolemia due to fluid leakage, septic like shock. Activating compliment cascade, clotting cascade...

connection: capture myopathy has *contraction band necrosis* as well as rhabdomyolysis. Captured wild animals, in particular, large muscular animals like deer, horses, etc. acquire a muscle myopathy that cannot be explained by struggle alone. Beached whales, and other beached cetaceans (dolphins, porpoises) show signs of contraction band necrosis in their heart tissue. Is this similar to Takotsubo cardiomyopathy???

Dark neurons. Described by Jan Cammermeyer 1962. Purkinje cells are odd. Look for a a paper called "Return of the dark neuron" Just touching the brain can cause this necrosis artifact, despite chemical fixation that would adequately preserve other tissues. Brain tissue must be left fixing in formalin or paraformaldlehyde, undisturbed in the skull for at least 24hours before being handled. Or else the Dark neurons form, mimicking degenerative changes/necrosis.

Notice the darkened. slightly shrunken neurons. The nucleus and is rER rich cytoplasm has condensed into a dark mess. Though very dark, you can see how reddish the dyeing cells have become. Adjacent to the injury you can see healthy neurons, with their dispersed chromatin, and distinct nucleoli. The pale basophilic (blueish) nissl substance and clear cytoplasm.

Squames from skin, mouth. Is there DNA here? No? CSI is crap. The little purple guys are bacteria.

Ending side story: The Big Bang Theory. A female physicist comments on a colleagues pupil response, when he looks at his secret love interest. "Unless you are a heroin addict...."

They got the pharmacology wrong. Heroin makes for pinpoint (miotic) pupils, and a slow moving gut. Opiates known for causing constipation, and the effective antidiarrhea medication, Imodium (loperamide) is an opiate receptor agonist in the gut but not the CNS.