Lecture 3: Immunity - Defenses

Evolution of Human Disease
Pascal Gagneux

Winter 2021

Today's class will be about defenses:
the many ways that we attempt to prevent and fight disease, mostly by microbial (viral. bacteria, fungal, protozoan and helminth) infection.

Defenses?

Barriers (skin, mucus, decoys, tight junctions, blood brain barrier….)

Toxins (anti-microbial peptides, defensins, cathelicidins)

Sentinels/Surveillance (white blood cells; neutrophils dendritic cells, macrophages)

Traps (Complement, innate immune receptors, neutrophil nets)

Self-surveillance: (MHC/HLA, including backup against manipulation)

Rapid reaction (innate immunity killer cells, T-cells)

Delayed and specific reaction (adaptive immunity/antibodies)

Memory (B-cells)

Off-button and ways of preventing friendly fire. (brakes)

Repair/compartmentalize (clotting)

Last resort: suicide programs for disobedient cells

Practice question:
List four different types of host defenses.
1. Mucus barrier
2. Skin
3 .Antimicrobial toxins
4. Immune cells

Too warlike?

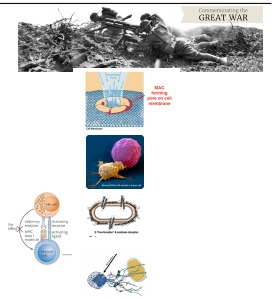
Membrane Attack Complex

Natural Killer Cells

Bactericidal compounds

Killing Receptors

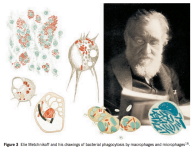
Suicide Bombers



Immune responses actually represent formidably aggressive processes, so aggressive that they also form a danger to ourselves: "horror autotoxicus"
What is *horror autotoxicus*?
The horror of having one's own powerful immune system unleashed against oneself.

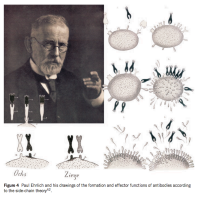
Discovery of immune responses

Elie Metchnikoff 1864-1916
Zoologist to pathologist



cells that eat……
from feeding to defense

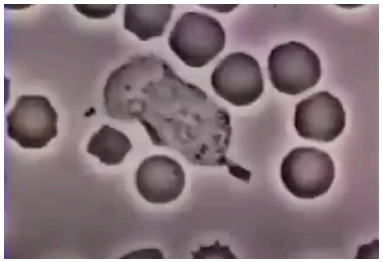
Paul Ehrlich 1877-1915
physician with the eyes of a chemist



blood/serum based immunity
antibodies and colonial selection for
best fitting probes

Cells fighting intruders by eating them (phagocytosis)
or by secreting “molecular probes” or beacons of destructions called antibodies.

Macrophage in action



https://www.youtube.com/watch?v=BDr44vLNnPY

Macrophage (rough size 20 micrometers) running after a bacterium (size 0.5 to 5 micrometers) and eating it.

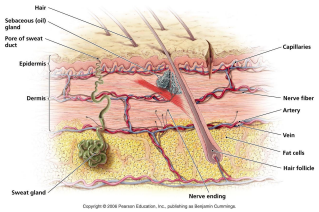
Practice question: How much larger is a human macrophage than a bacterium?

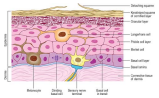
Answer: 4 to 40 tomes larger.

Practice question: How does the macrophage recognize the bacteria?

Answer: By using innate immune receptors that recognize pathogen associated molecular patterns (PAMPs).

Barriers: Skin



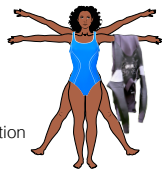


Dry skin with its layer of shedding dead cells is a very strong barrier.
Skin secretes chemical toxins (e.g. defensins) that kill bacteria.

Barriers: Skin

One of the defining features of our Human Lineage

Barrier
Protection
Defense
Thermoregulation
Energy



Vitamins
Sensory
Ecosystem
Signal
Mind

Practice question: How thick is human skin?
Answer around 2 mm.

Barriers: Skin

Calculating the actual surface of the human skin:

If the skin surface looked like this

≠

But human skin has appendage openings and looks like this

Then the standard surface area estimation of **2 m²** is correct

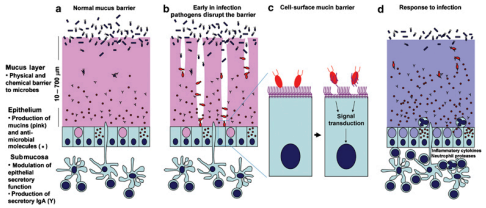
Therefore the surface area is closer to **25 m²**

Gallo, R, Human Skin Is the Largest Epithelial Surface for Interaction with Microbes . J Invest Dermatol. 2017 June ; 137(6): 1213–1214.

The surface area of skin has been miscalculated
Human skin is not a flat surface. The presence of approximately 5 million appendages such as hair follicles and sweat ducts greatly increases the epithelial surface area that is uniquely accessible to the microbiome.

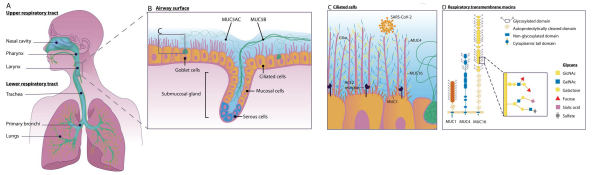
Practice question: What is the total surface of the human skin?
2 square meters without the folds, 25 square meter with.

Barriers: Mucus



Pathogens adapt to eat their way through the host mucin barrier.
Hosts can counter by secreting more and different mucus.
Practice question: What is mucus made of?
Answer: Mucus is a hydrated biogel consisting mostly of highly glycosylated mucin glycoproteins but also salts and anti-microbial proteins.

Barriers: Mucus

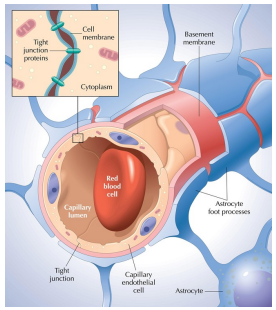


Maitrayee Chatterjee et al. ***mBio*** 2020; doi:10.1128/mBio.02374-20

Animal airways are covered with mucus. In humans, the composition of this mucus is an important information for trying to understand how viruses such as Sars-CoV2 manage to reach the host cells and infect them.
Mucosal defense in the respiratory tract during SARS-CoV-2 infection. (A) Human upper and lower respiratory tracts. (B) Respiratory epithelium with ciliated cells, goblet cells, and a submucosal gland. The soluble mucin MUC5AC is secreted by goblet cells, and the soluble mucin MUC5B is secreted by mucosal cells in the submucosal gland. (C) Ciliated epithelial cells express transmembrane mucins MUC1 (red), MUC4 (blue), and MUC16 (yellow) and the SARS-CoV-2 entry receptor ACE2. (D) Domain structure of transmembrane mucins MUC1, MUC4, and MUC16. Mucin O-glycan structures and, specifically, terminal sialic acids play an important role in virus-mucin interactions.

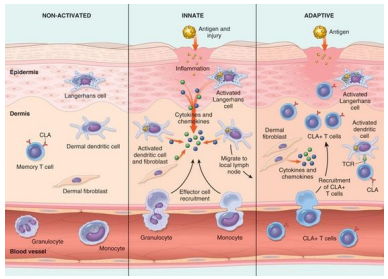
Bottle brushes are good analogies for mucus, the twisted wire would be the protein backbone and each bristle would represent a sugar chain.
The handle could represent the transmembrane domain. Many muffins are secreted and membrane anchored ones can be secondarily cleaved by enzymes (proteases) causing them to shed, with whatever intruder is attached to them…

Barriers: Blood-brain barrier



Special connection between cells (tight junctions) lining the blood vessels normal shield special compartments of the body such as the brain and the testes and the follicle is the ovary.

Breaching the Barrier



Damage to the skin barrier triggers many types of immune responses and healing processes.

Parts of the immune system

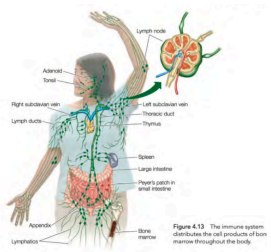
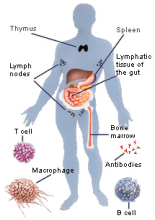
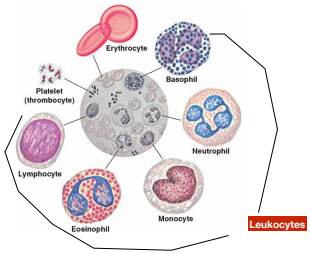


Figure 4.13 The immune system distributes the cell products of bone marrow throughout the body.



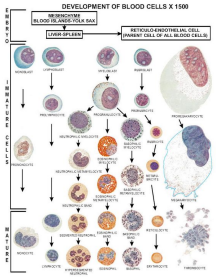
Then immune system has about as many cells as your brain:
It is a second information processing system
Unlike the brain, it is very distributed and does not have one central organ.
What kind of information does the immune system process?
Molecular information about self and non-self, consisting of composition and shape of molecules and the patterns these form.

Types of blood cells from bone marrow



All blood cells constantly form from stem cells in the bone marrow: red and white blood cells and platelets. Lymphocytes are B cells and T cells, the other white cells are often called “granulocytes” because their cell nuclei look “grainy”.

Development of blood cells



constantly regenerated from stem cells in the bone marrow:

in your lifetime:
10 times more of these cells than all the cells of your body!

All from the same stem cells (hematopoietic stem cells in the bone marrow)
red blood cells and platelets lose their DNA in the process of maturing.

Practice question:

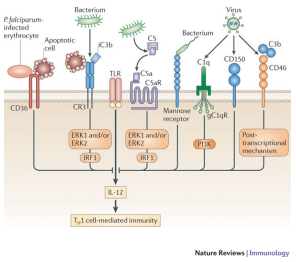
List two cell types in the body that lose their genomes as they mature:

RBC

platelets

lens cells of the eye

Innate Receptors of Non-self



Germ-line encoded receptors for non-self molecules.
These are called innate because they do not need to “learn” about novel molecular patterns but rather have evolved over generations to target tell-tale foreign molecular patterns.

Practice question:

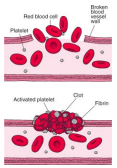
What are innate immune receptors?

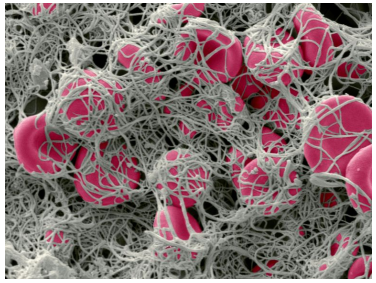
Answer: Proteins made by a host organism (even in absence of immunization) that recognize molecular patterns on potential pathogens and parasites.

What is the impact of rich diversity on alleles at many of the genes encoding innate immune receptors?

Answer: Individual humans can react very differently to the same pathogens.

Clotting: liquid blood turns to gooey clot in an instant





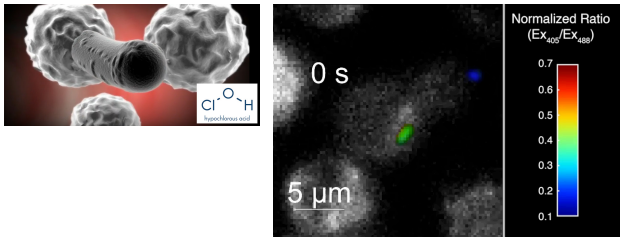
Blood is a super-charged fluid, ready to clot and form gooey plugs, whenever necessary (this too is a huge liability…as it is easy to die from spontaneous blood clots (thrombosis).

Practice question:

How can liquid blood rapidly form a clot?

Blood is super charged with proteins that can react to contact with oxygen and form mesh works of fibers that can crosslink and entrap platelets, thus forming a clot.

Making bleach where needed!

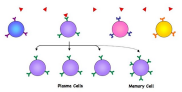


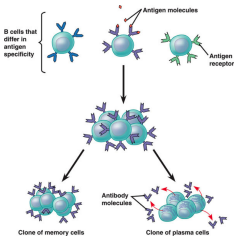
Degrossoli *et al.* ***eLife*** 2018

Immune cells can manufacture bleach with a special enzyme (myeloperoxydase) that can use hydrogen peroxide to make hypochlorous acid!

Clonal selection

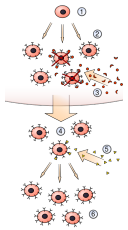
white blood cells
allowed to behave like microbes….





Only B-cells and T cells with fitting receptors are allowed to behave like a cancer” = clonal expansion
B-cells make antibodies (immunoglobulins)
T-cells make T-cell receptors (very similar to immunoglobulins, but attached to T-cells)

Selection against self-reacting cells for non-self reacting cells

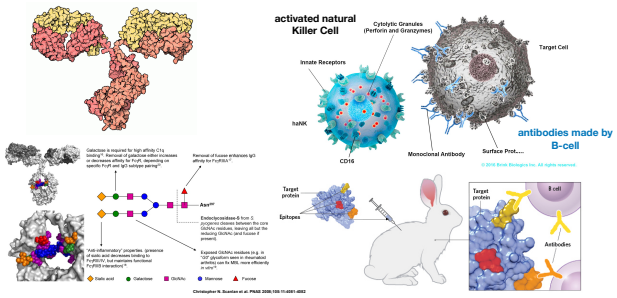


Abstract diagram of the clonal selection of B and T lymphocytes.
Legend: 1. Hematopoietic stem cell
2. Immature lymphocytes with various receptors
3. "Self"-antigens from the body's tissues
4. Mature, inactive lymphocytes
5. Foreign antigen
6. Cloned activated lymphocytes

In the thymus, the training organ of the adaptive immune system, B-cells and T-cells “learn” how to appropriately recognize self and non-self. Only those cells with appropriate recognition are allowed to live a replicate, eventually moving to the periphery of the body (lymph nodes).
How does the adaptive immune system prevent cells from reacting against self?
developing immune cells that recognize self to strongly are forced to undergo apoptosis. (controlled cell death).

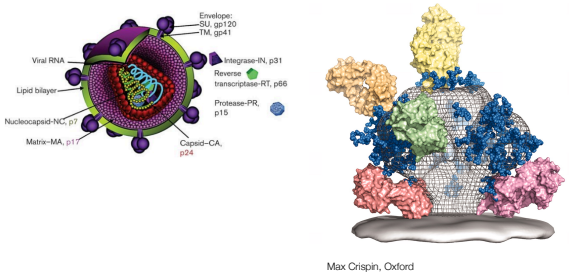
Practice question: Which mechanism is more important in the somatic evolution of B- and T-cells, negative selection our positive selection?
Answer: Both.

Antibodies



Immunoglobulin antibodies are super precise molecular probes.
They can be generated in animals by inoculating these with a particular antigen (a foreign molecule usually presented together with a danger signal, adjuvant) for later use in clinics or labs.

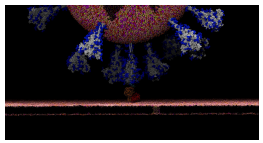
Antibodies targeting HIV glycoprotein spike

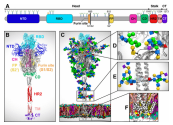


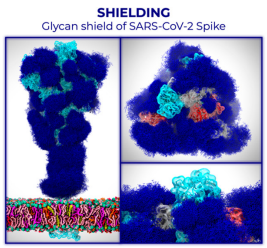
Max Crispin, Oxford

Broadly Neutralizing Antibodies to HIV target the glycoprotein (pprotein shown in grey, glycans/ sugars in blue) on the HIV viral envelope. These antibodies often target glycans or a combination of glycans and underlying peptide.

What about Sars-Cov 2 virus spike glycoproteins?



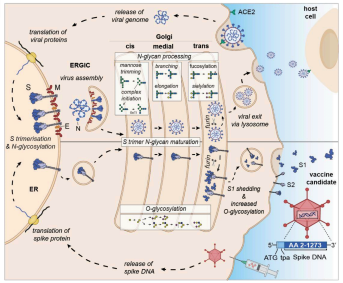




Romy Amaro , UCSD

The Sars-Cov 2 virus is covered by spike glycoproteins.
Each spike is a trimer (there individual proteins combined into one functional unit) and each spike proteins carries 22 different N-glycan chains. The dark blue “clouds” on the right represent the sugar chains (glycans) covering most of the surface of the viral spike glycoprotein.

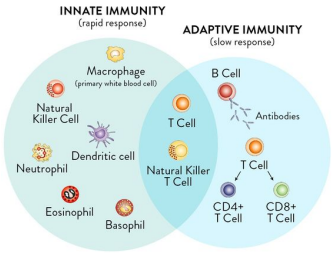
Sars-Cov 2 virus spike glycoproteins and vaccines



https://www.biorxiv.org/content/10.1101/2020.11.16.384594v1.full.pdf

Differential expression and glycan processing of virions and vaccine derived spike glycoproteins. SARS-CoV-2 binds to its receptor ACE-2 and infects cells, leading to the release of the viral genome and translation of viral proteins. Spike protein is co-translationally N-glycosylated and forms trimers in the ER that traffic to the ERGIC where they are incorporated into budding virions. Individual virions continue through the secretory pathway to the trans-Golgi prior to following a lysosomal egress route. For the vaccine candidate, spike DNA is administered via an adenovirus vector system, and spike protein is synthesized in the ER, where it is N-glycosylated and trimerises as before, but as it is not incorporated into a budding virion in the ERGIC, it continues through the secretory pathway and, via lysosomes, to the plasma membrane. In both cases the spike glycoproteins have access to both the N- and O- linked host glycosylation machinery. Upon furin cleavage in the trans-Golgi, S1 and S2 of the virus stay non-covalently associated, whereas furin cleavage of the vaccine antigen results in shedding of monomeric S1vaccine antigen. Glycomic signature analysis of these two proteins show that the N-linked glycosylation occupancy levels, which are determined in the ER, are comparable for S1virus and S1vaccine antigen whereas the attached glycoforms vary reflecting their different accessibility to glycan processing enzymes. S1vaccine antigen carries not only higher levels of complex N-glycans but is also extensively O-glycosylated after furin cleavage in the trans-Golgi, when most S1vaccine antigen is shed and secreted in a soluble monomeric form. Some S1 and S2 vaccine antigen is displayed on the cell surface, presumably as trimers.

Innate and adaptive immunity cooperate



Successful immune responses utilize both, innate (rapid) and adaptive (slower but more precise) recognition

Question: give three cell type involved in innate immunity and three in innate immunity.

Answer: Innate: Neutrophil, Macrophage, Basophil; adaptive: B-cell, T-cell, T helper cell.

Mammals have to learn about self before discriminating against non-self.

Figure 2. Leslie Brent, Rupert Billingham and technician Trevor Courtenay at University College London in 1955/1956, working out the intravenous route for the inoculation of cells into newborn mice. (Image provided by Leslie Brent).

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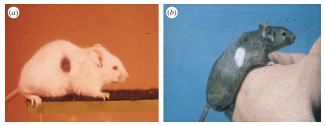
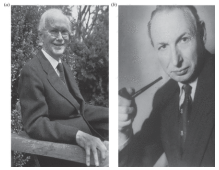
rstb.royalsocietypublishing.org PhilTrans R. Soc. B 370: 20140302

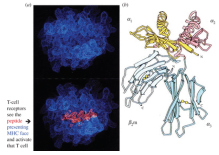
In experiments with newborn mice Medawar and colleagues showed that up until birth, young mammals cannot recognize foreign molecules yet.

Mouse to mouse transplantations



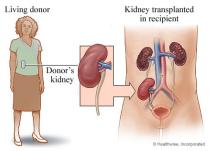
George Snell and Peter Gorer





Using mice strains of different color, Snell and Gorer discovered the recognition system that allowed or precluded xenografts: The Major Histocompatibility Complex (MHC)
“Histo”=tissue”, “compatibility”= can be compatible for transplantation

Parallels between transplantation and pregnancy?



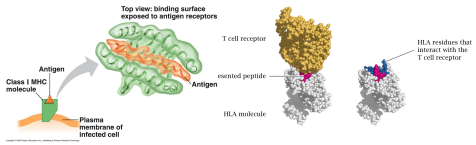
circulating antibodies
targeting donor molecules

circulating antibodies
targeting paternal molecules

Mothers were found have circulating antibodies against molecules on the surface of white blood cells of their children’s father(s): These proteins that seemed to “insult the immune system of the mother were named: “HLA - human leukocyte antigens”.
Organ transplant recipient have antibodies to the transplant (MHC).
MHC turned out to be the human HLA! All mammals have their own MHC system.

Declare Yourself! HLA/MHC

Human Leukocyte Antigen/Major Histocompatibility Complex

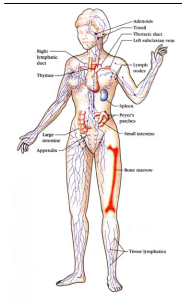


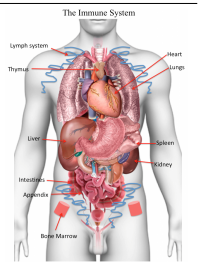
Most cells, constantly sample their insides and present what they sample to the outside

The sampling "probes" are called MHC or HLA
The probe and its cargo form an important signal to immune cells

The HLA (human) /MHC (other mammals) system is a self declaration and non-self detection system in one!
How can the immune system detect the presence of virus that has already infected a cell?
The HLA molecules of the cell will present cellular content on the outside of the cell allowing the immune system to detect viral debris, intra-cellular parasites or cancer.
Practice question: what is the difference between MHC and HLA?
Answer: HLA is the human MHC.

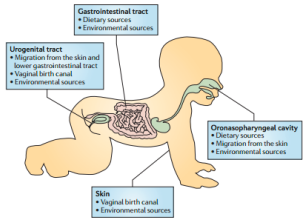
Food is non-self! eating as defense





Animals have to eat, and food is non-self, how is that possible given the animals' powerful immune systems?
The gastrointestinal tract is very well controlled by immune cells. Macrophages and neutrophils can "eat" foreign particles and microbes that may escape into the self.
Digestion by acidity, enzymes (proteases, lipase, glycosidases etc) and microbiome bacteria (and their enzymes) reduces most foreign molecules to small units: monosaccharides, fatty acids and amino acids.

Microbiome colonization of the newborn:

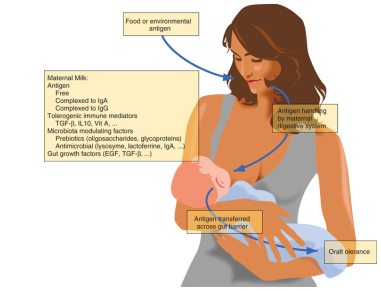


The first year of life is when the microbiome of each bay is established

Reid et al. 2011 NATURE Reviews I Microbiology

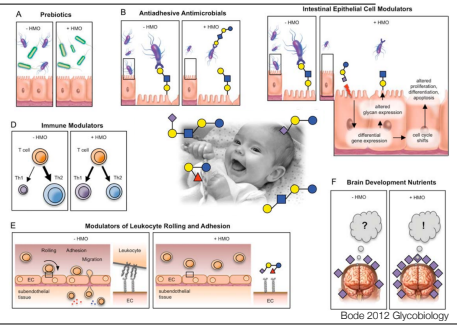
We are born almost 100% human
within seconds they are colonized by microbiota, naturally this would be our mothers vaginal microbiome and her gut microbiome. Increasingly, and in association with C-section babies can first be colonized by microbes on the hands of hospital care staff… (and *Staph*…..)..

Breastmilk and immunity



Before reaching the milk, ingested airborne and dietary antigens are handled by the maternal digestive system, which could contribute to the generation of tolerogenic peptides. Depending on the maternal antigen exposure and mammary gland permeability, various amounts of antigen will be found in breast milk. Maternal sensitization to the ingested allergens will dictate whether the transferred antigens will be found in the milk free or complexed to antigen-specific IgA and IgG. The presence of IgA will trap antigens and prevent their transfer to the child, whereas antigen bound to IgG will be very efficiently transferred across the gut barrier using the FcRn. Prebiotics, such as oligosaccharides, that are present in breast milk will lead to the development of a microbiota promoting immune tolerance induction.

Human Milk Oligosaccharides -Protective Effects



Human milk unlike cow's milk is extremely rich in milk oligosaccharides. These have various protective functions, from favoring the growth of "good" microbes, to acting as anti-adhesives for "bad" microbes to the modulation of the gut epithelial cells and the associated immune cells. Milk can also directly act as brain food.

Practice question: How does human breast milk improve infant health?

Answer: It contains prebiotics that help the infant gut be colonized by the right bacteria, it contains maternal antibodies that attenuate infections in the infant, and it modulates infant immune development.

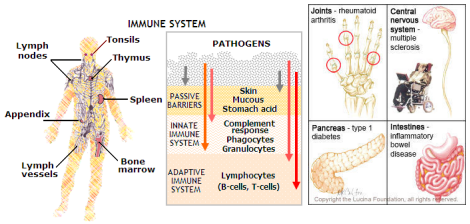
Know Thyself!

A Tsuku-bai (蹲踞) water fountain in Ryoanji Temple, Kyoto, Japan

Tsuku-bai (蹲踞)

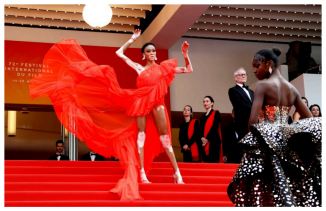
Defense is Costly and Dangerous!

autoimmune disorders



The cost of immune defenses includes autoimmunity.
Some individuals or some of their tissues come under attack by their own immune system.
Can you name two autoimmune diseases:
rheumathoid arthritis: self attack on joints
multiple sclerosis: self attack on central nervous system
type 1 diabetes: self attack on pancreatic islet cells that secrete insulin inflammatory bowel disease: self attack on gut tissue and or associated microbes

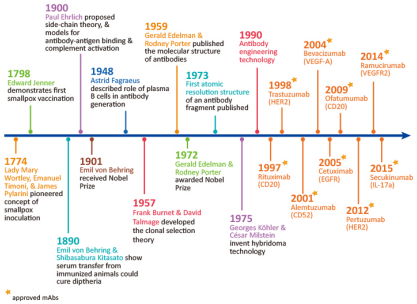
Autoimmune reaction targeting melanocytes



Winnie Harlow in Cannes, 2019

Vitilago, a highly visible autoimmune disease resulting from autoimmune attack of melanocytes, the cells that produce melanin, the pigment of hair, skin and eyes. Winnie Harlow, Canadian super model.

Key insights into Self-Nonself recognition



Timeline of discoveries in immune therapy.
Monoclonal antibodies specific for certain molecules are now routinely cloned and mass produced in mammalian cells.
Question: what is a monoclonal antibody?
Answer: A specific antibody made by one clone of B-cells
(these can be isolated and the mass-produced by introducing the DNA sequence encoding this specific antibody into a cell line).

Purity Worship:

Pushing back against infection with religious beliefs?

Hinduism

Buddhism

Judaism

Christianism

Islam

Shintoism

Taoism

Could the recurring theme of purity, cleanliness and hygiene in many religions have originated from the observation that these attributes limit infectious disease? All these belief systems originated in regions with large cities, complex societies, trade, empire and other factors that facilitate disease transmission in densely populated areas….

Grooming

Behavioral defenses such as grooming are very important for social animals. These behaviors can then also come to take on social importance.

Tibi grooming me

Summary

Barriers: skin, mucus, tight junctions

Defenses: cellular and humoral (liquids and the molecules these contain)

Surveillance: sentinel cells (white blood cells, dendritic cells)

Immune organs: thymus, spleen, lymphatic system, gut associated lymphatic tissue (GALT)

Detection of non-self molecules: innate immune receptors, antibodies

Immune cells are allowed to multiply and evolve like microbes

Stem cells continuously produce new blood cells.

The immune system its immensely powerful and dangerous!

Summary continued

Immunity requires knowledge of Self and non-self

Immune education happens after birth
and is helped by breast milk.

Defenses are expensive and dangerous.

Immune cells: domesticated cancer?

Cells and soluble molecules (Abs) are important for recognition of non-self.

Parasites have been with us since a long time.

Immunity cannot get rid of parasites (worms) well.

Worms (helminths) might represent “Old Friends”.