Cells of the Innate Immune system

**Neutrophils (PMNs):**
- Produced in bone marrow, increases during infection
- Most prevalent WBC
- Attracted to sites of immune complex deposition via complement activation; cause of tissue destruction

**Eosinophils:** increased in blood and tissue in Type I hypersensitivity diseases; eosinophil chemotactic factor (ECF) found in primary granules of mast cells and basophils

**Basophils (top) and mast cells (bottom):** Both express Fc receptors for IgE; cross-linking of bound IgE by antigen (aka allergen) triggers release of primary granules, containing histamine, eosinophil chemotactic factor (ECF), and other compounds in Type I hypersensitivity

**Monocytes:** antigen-presenting cell; found in peripheral blood; can differentiate into macrophages in tissue.

**Natural killer (NK) cells:** resemble lymphocytes morphologically and somewhat resemble cytotoxic T-cells biochemically (use perforin and granzyme B to kill targets); but there is no TCR
- Have Fc receptor; participates in antibody-dependent cellular cytotoxicity (ADCC)
- Defense against intracellular microbes, i.e., kill cells with intracellular microbes

**Macrophage (top) and dendritic cell (bottom):**
- Phagocytic and antigen-presenting.
- Possess Fc and C3b receptors which facilitates phagocytosis of opsonized (Ig and/or complement-coated) targets
- Dendritic cells transport antigen from epithelial sites (such as gut, skin, etc) to lymph nodes
- Generation of reactive oxygen species (respiratory burst) and nitric oxide occur in phagolysosome
- Specific names in tissue, e.g., Kupffer cells in liver, mesangial cells in kidney, microglial cells in brain
Acute Phase Proteins

• CRP binds PAMPs (pathogen-associated molecular patterns), binds complement and acts as an opsonin
  – CRP is used clinically to screen for inflammation/infection, e.g., >0.8 mg/dL is positive
  – “high-sensitivity” CRP (hsCRP) describes an assay with an analytical range that goes at least as low as the normal range; this test can be used as a risk factor for cardiovascular disease, where >0.3 mg/dL would be abnormal
• Haptoglobin, which binds free hemoglobin and prevents its oxidative activity, and fibrinogen and Factor VIII, coagulation factors, are clinically measured to evaluate hemolysis and coagulation, respectively; they happen to also be acute phase proteins
Immunoglobulin-related facts

• Surface membrane immunoglobulins are the specific antigen receptor for B-cells
• Allergen-specific IgE levels that are measurable but below the cut-off for a positive result, are of unknown clinical significance; component panels for allergen-specific IgE, such as for peanut, allow for assessment of risk for anaphylaxis
• The production of mouse monoclonal antibodies involves the technology of cell fusion, specifically between mouse splenocytes (from a mouse immunized with the antigen of interest) and a malignant plasma cell line*, producing a somatic cell hybrid known as a hybridoma; this tumor can be propagated in perpetuity
• Immunoglobulins have the variable region at the N-terminal part of the molecule, and the Fc portion at the C-terminal part of the molecule; receptors for Fc occur on NK cells, allowing for NK cells to participate in ADCC (antibody-dependent cytotoxicity), i.e., NK cells recognize antibody-coated cell targets
• Within the variable region of each heavy and light chain are three CDRs (complementarity-determining regions); these hypervariable sequences, in the folder immunoglobulin molecule, bind the antigen epitope; remember that the epitope is the specific region on the antigen that reacts with the Fab portion of the antibody
• AFTER antigen exposure, the responding B-cells undergo DNA hypermutation in the CDR regions, with those B-cells mutating toward higher and higher affinity for the antigen, successfully competing for antigen binding and with it, survival signals; this process is known as affinity maturation, and it occurs in germinal centers.
• Flow cytometry involves monoclonal antibodies, labeled with fluorochromes
• Immunoglobulins and T-cell receptors are both cell-membrane-bound, but only immunoglobulins are, in addition, secreted by cells
Specific Immunoglobulin characteristics

- **IgG**
  - Only immunoglobulin that crosses the placenta into the fetus, via “neonatal receptor”
  - Longest half-life of immunoglobulins, because of “neonatal” receptor (active throughout life (not just pregnancy)), that recycles IgG
  - Four subtypes, three of which fix complement (IgG1, IgG3 and to some extent IgG2) and one that does not fix complement (IgG4)
  - Participates in opsonization, neutralization, precipitation events

- **IgA**
  - Secreted IgA transported through epithelial cells via binding to Secretory Piece (synthesized by epithelial cell)
  - Important for mucosal immunity (e.g., respiratory tract, gastrointestinal tract), where it exists as a dimer, held together by J chain

- **IgM**
  - First immunoglobulin to be produced in a primary infection; finding IgM in serum that has specificity for a microbe implies active or very recent infection by that microbe
  - Best at fixing complement (pentameric structure allows single molecule to cross-link globular heads of C1q)
  - Secreted as pentamer (valence of 10); thus it tends to have high avidity
  - Co-expressed with IgD on the surface of naïve B-cells

- **IgD**
  - Co-expressed with IgM on naïve B-cells
  - Very small concentration in serum; unknown function, if any, as a secreted immunoglobulin

- **IgE**
  - Binds to basophils and mast cells via Fc receptor
  - Cross-linking by allergen activates cell to release granule contents which include histamine
  - Allergen-specific IgE can be measured to assess allergy; risk of clinical allergy increases with higher values; values below the cutoff for a positive result are of uncertain significance
Complement

- Three pathways – classical, alternate, and lectin
- C5a is a neutrophil chemoattractant, opsonin, and vasodilator, i.e., it is proinflammatory
- Deficiency of late components (C5, C6, C7, C8) associated with susceptibility to Neisseria infections, e.g., Neisseria meningitidis
- Common assays include C3, C4 (most prevalent complement proteins in serum), C1 esterase inhibitor (deficient in hereditary angioedema), and CH50 (a functional test of the entire complement system)
- C4 can be used in addition to C1 esterase levels to screen for hereditary angioedema, because the disease could be associated with a mutant C1 esterase that is present in normal amounts (giving a normal C1 esterase level; such patients could be successfully screened with C4, which should be low because of consumption)
- Low-level hydrolysis of C3 producing C3b is involved in the alternate pathway
- Paroxysmal nocturnal hemoglobinuria is associated with a loss of CD55 (decay accelerating factor) from cells due to a defect in synthesizing the glycosyl phosphatidylinositol (GPI) anchor that attaches CD55 to the cell membrane
MHC

- Codominant expression (both maternal and paternal copies are expressed)
- Highly polymorphic in the human population
- More than one, but not an infinite diversity of peptides may bind in the peptide-binding site of MHC molecules; the exact peptides that can bind depend upon which two alleles the patient has at each MHC gene locus; two different, unrelated patients would typically be able to respond to infection by a particular virus, but the exact viral peptides, that each patient’s T-cells would be presented with, would be expected to be different, from one patient to the next
- The stretch of DNA encoding the MHC is known as a haplotype; a patient needing a bone marrow or kidney transplant has a 1:4 chance of a sibling being a “two-haplotype match”, i.e., a sibling who inherited the same maternal and paternal alleles as the patient

- MHC Class I
  - Expressed on all nucleated cells
  - Presents antigen to cytotoxic (CD8+) T-cells
  - One polypeptide chain noncovalently bound to beta-2-microglobulin
  - Genes are HLA-A, HLA-B, HLA-C

- MHC Class II
  - Expressed on a subset of cells, primarily antigen-presenting cells such as macrophages, monocytes, dendritic cells, and B cells
  - Presents antigen to T-helper (CD4+) cells
  - Two polypeptides, a heterodimer composed of alpha and beta chains
  - Genes are HLA-DR, HLA-DP, HLA-DQ
Selected facts about cells in the innate and adaptive immune system

- B-cells generated in the bone marrow
- B-cells occupy follicles, T-cells occupy the parafollicular (interfollicular) area of lymph nodes
- Antigen-presenting cells include macrophages, dendritic cells, B-cells
- Innate immune system cells express toll-like receptors
- Neutrophils cause much of the tissue damage in type II and type III hypersensitivity diseases, because complement fixation leads to chemoattraction of neutrophils to the area
- Receptors/ligands mediate entry of neutrophils to tissue sites of infections, e.g., endothelial p- and e-selectin/neutrophil selectin receptor to cause rolling of neutrophils; neutrophil integrins/endothelial receptors such as ICAM-1 to cause stable adhesion.
- Chemokine receptor modulation results in movement of B and T cells toward each other in lymph nodes (for T-dependent B-cell reactions, e.g., response to protein)
- CD3 expression is a characteristic of T-cells, CD19 expression of B-cells
- The T-cell receptor complex includes a number of proteins, including the T-cell receptor, zeta-chain, CD4 (for helper T-cells) or CD8 (for cytotoxic T-cells)
- Characteristic cytokines for TH1 response is gamma interferon, for TH2 response, IL-4, IL-5, IL-10
- Eosinophils are characteristic of allergic reactions (eosinophil chemotactic factor is one of the granule contents of basophils and mast cells)
- Costimulation occurs between antigen-presenting cell expressing B7 and T-cell expressing CD28
Interaction of T cells with other cells

- Interaction with B cells and macrophages
  - T cell receptor/peptide presented in the context of MHC
  - T cell CD28/B7 of B cell or macrophage (provides costimulation – without it, T cell may become anergic)
  - T cell CD40L (ligand) with B cell or macrophage CD40

- Naive T cells constantly circulate between the interfollicular (parafollicular) areas in peripheral lymphoid organs and the bloodstream
Hypersensitivity disease examples

• Type I – allergic rhinitis, hay fever, atopic dermatitis, asthma, anaphylaxis, food allergy, etc. Histamine effects include dilating blood vessels and lowering blood pressure, and narrowing airways through bronchoconstriction

• Type II – Rh disease of the newborn, anti-acetylcholine antibodies in myasthenia gravis, Graves Disease

• Type III – systemic lupus erythematous, polyarteritis nodosa

• Type IV – contact dermatitis, e.g., hypersensitivity to cosmetics or metals. Note: Type IV is mediated by T-cells, not antibodies or immune complexes
Selected facts about assays

- T-cell function is more difficult to assess in a clinical lab test (as compared to B-cell function, which involves simply measuring antibodies), because T-cells require MHC-restricted antigen presentation (this means at least a short-term culture in the clinical lab).
- Automated immunoassay analyzers sold currently use chemiluminescence; this principle of detection is more sensitive than fluorescence.
- Heterogeneous assays require separation of bound from unbound antibody, whereas homogenous assays do not.
- Prozone phenomenon occurs in precipitation assays when antibody or antigen excess produces a negative or diminished signal (a falsely low result); can be recognized and overcome by testing diluted sample; this problem has been largely eliminated by the introduction of capture assays.
- Red cell agglutination assays use reagent anti-human globulin to bridge the gap between RBCs coated with IgG, to produce hemagglutination as the read-out.
- Reverse passive agglutination assays, e.g., cryptococcal antigen tests in CSF, use controls for rheumatoid factor (an anti-IgG autoantibody which, if present, could produce a false-positive result).
- Assays that involve antibody-antigen binding typically are carried out within a pH range of 6.5 to 7.5.
Characteristic markers for selected autoimmune diseases

- Sjogren’s syndrome – anti-SSA (Ro) and anti-SSB (La)
- Anti-beta-2-glycoprotein-1 in antiphospholipid antibody syndrome (includes vascular thrombosis and recurrent pregnancy loss)
- TSH-mimicking antibody in Graves disease (hyperthyroidism)*
- Deamidated gliadin (IgG and IgA) and TTG IgA in celiac disease (for the latter, confirm negatives by ruling out IgA deficiency)
- Anti-thyroglobulin and anti-thyroid peroxidase (microsomal) in Hashimoto’s thyroiditis*
- Anti-SCL70 in progressive systemic sclerosis (scleroderma)
- Anti-Jo1 in polymyositis
- Anti-c-ANCA in Wegener’s granulomatosis (distinguish c-ANCA’s antibody distribution throughout the neutrophil cytoplasm from p-ANCA’s perinuclear distribution; p-ANCA does not carry unique specificity for Wegener’s)
- Anti-CCP (cyclic citrullinated peptide) in rheumatoid arthritis
- Anti-acetylcholine antibodies in myasthenia gravis
Follow-up to positive ANA

• Main goal is to identify what the cause of the positive ANA is
• A cost-effective approach uses a panel of common tests, supplemented by additional tests as dictated by the clinical situation
• Example: perform anti-double-stranded DNA (anti-DS DNA), SS-A, SS-B, RNP, Sm; supplement as needed with others, e.g., Scl70, Jo1, phospholipid, etc.
Fungal immunodiffusion – a precipitation assay

- Available for detection of antibody to Aspergillus, Blastomyces dermatitidis, Histoplasma capsulatum, Coccidiodes immitis
- Two examples in the figure
- Note that M bands may be persistent after resolution of acute histoplasmosis, and thus finding them does not ensure that the patient's current disease is histoplasmosis; H bands are much more specific for acute infection than M bands, but there is low sensitivity (many acute patients are negative for H bands)
- Histoplasma antigen, typically measured in urine has proved to be helpful in diagnosing acute histoplasmosis

Immunodiffusion testing for antibodies to Coccidiodes immitis (left) and Histoplasma capsulatum (right); antigen is in the center well, and control antisera are in wells 1 and 4.
Patient three has the F band of Coccidiodes immitis and the M band of Histoplasma capsulatum
Patient two has both the H and the M band of Histoplasma capsulatum
Nephelometry – another precipitation assay

- Reagent antibody added to patient sample
- Measures light scatter as lattice of antigen/antibody complex form
- Most are “kinetic” assays, i.e., they measure light scatter at two closely-spaced timepoints, measure the slope between the two points and relate that slope to the concentration of the analyte in patient serum
- Must be carried out in antibody excess, so that as the patient serum analyte increases, lattice increases
Immunofixation electrophoresis – an example of a precipitation assay

- Typically used to investigate monoclonal antibodies in patient serum
- Can also be used to distinguish CSF from serum, for example in the clinical setting where clear fluid is leaking from the nose or ear of a patient who may be suspected of having a basilar skull fracture; in this situation, IFE analysis of beta-2-transferrin is done because serum typically contains only one isoform while CSF contains two
- Can be used to detect oligoclonal bands, typical of neuroinflammatory disorders such as multiple sclerosis
Fluorescence assays

• Direct fluorescence – antibody reagent is directly labeled with fluorochrome; examples include 1) staining of kidney biopsies to reveal complement or immunoglobulin deposition, and 2) flow cytometry

• Indirect fluorescence – primary antibody is actually patient serum, which means that it is unlabeled; in these types of assays, a secondary, commercially available antibody, e.g., an anti-human globulin labeled with a fluorochrome, is added in order to detect that an antigen/antibody reaction occurred when both antigen and antibody (patient serum) are themselves unlabeled
Selected facts about immunodeficiency

- Selective IgA deficiency is the most common inherited immunodeficiency; note that it is even more common in patients with celiac disease, and thus a total IgA is needed to fully understand the significance of a negative tTG IgA result.

- Chronic granulomatous disease could be caused by several different protein gene defects that affect the phagocyte oxidase system; one of these is X-linked thus producing disease in boys, not girls, but others of these proteins are autosomal, thus affecting boys or girls.

- Severe combined immunodeficiency may be produced by several different gene defects; the common feature of these is deficiency of both T-cells and immunoglobulin levels.

- Leukocyte adhesion (adherence) deficiency is caused by integrin defects, e.g., CD18 or CD11a,b,c.

- Chédiak-Higashi disease is associated with giant cytoplasmic granules in neutrophils.

- Bruton’s agammaglobulinemia presents clinically when maternal antibodies have waned in the serum of infant boys, around 5-8 months of age.
Monoclonal immunoglobulins

• MGUS is the most common clinical diagnosis; features include low level of M-spike at presentation, slow (if any) rise over time, lack of hypercalcemia, anemia, bone pain, little or no Bence Jones protein (free monoclonal light chains in the urine)

• Hyperviscosity syndrome is most likely associated with IgM paraproteins, because IgM is a macromolecule (pentamer); begin to consider hyperviscosity at IgM levels of 4 gm/dL, whereas levels of 6-7 gm/dL or higher are typically required for this syndrome to occur with non-IgM paraproteins

• The bone marrow contains excess plasma cells in multiple myeloma and excess lymphoplasmacytic cells in Waldenstrom’s macroglobulinemia
Selected facts about infectious disease

- Detection of recent Group A strep infection is done with assays for ASO and anti-DNAse B
- Rocky Mountain Spotted Fever is caused by Rickettsia species
- EBV is the cause of infectious mononucleosis
- The RIBA test to confirm Hepatitis C antibody testing is no longer available; an alternative is HCV PCR
- Finding IgM positivity for an infectious organism suggests current or very recent infection
Syphilis serology

• Two categories of tests:

• Nontreponemal tests such as RPR or VDRL are screening tests; false positives (acute or chronic biologic false positives) can be seen; nontreponemal tests can be titered and then followed to assess response to treatment. With successful treatment, titer will drop to zero.

• Treponemal tests such as TP-PA are confirmation tests used to assess a positive non-treponemal test; once positive, they remain positive for life.