Cells of the Innate Immune System

- **Neutrophils (PMNs):** Produced in bone marrow, increase 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** and mast cells: 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).

- **Macrophage** and dendritic cell: Phagocytic and antigen-presenting. Possess Fc and C3b receptors which facilitates phagocytosis of opsonized targets. Dendritic cells transport antigen from epithelial sites (such as gut, skin) to lymph nodes.

- **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 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 Fc-mediated killing of cells coated with antibodies.
  - 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 a 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 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 chemotactic, 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 antigens 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 antigens to T-helper (CD4+) cells
    - Two polypeptides, a tetramer 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 chemotraction 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 erythematosus, 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 fluorosence.
- 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 gladin (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 CD11 a,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 g/dL, whereas levels of 6-7 g/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.