Objectives:

- The attendee will be able to discuss at least 3 methodologies used in the microbiology lab for diagnosing infections/infectious diseases.
- The attendee will be able to discuss the role of each of the 5 immunoglobulins on the patient’s response to an infection.
- The attendee will be able to list at least 3 tests, not performed in the microbiology lab, used to aid in the diagnosing of infections/infectious diseases.
- The attendee will be able to discuss the implications of various types of white blood cells (WBCs) on a differential.

Methodologies in Microbiology:

- Microscopy
- Culture
- Antigen Detection
- Molecular Tests

Microbiology/Immunology Laboratory

- Blood tests/Antibody Levels

Chemistry/Hematology

- Cell Counts/Differentials/Chemistries
Microscopy:

- Light
- Fluorescent
- Confocal
- Electron

Light Microscopy

- Bright-field – most common – uses incandescent light sources; stains
- Dark-field – examine fresh material – look for motile organisms not able to be stained, i.e. treponemes (syphilis); objects appear against a dark background for better resolution
- Phase-Contrast – examine fresh material – takes advantage of the different densities of cellular elements and makes them stand out; CSF examination for Naegleria

Fluorescent Microscopy

- Utilizes a UV light source
- Coupled with antibodies
- Ease of interpretation
- Quicker review of slide
- Calcofluor White
- Acridine Orange
- Auramine-Rhodamine
- DFA – Direct Fluorescent Antibody
Confocal Microscopy
- A type of fluorescent microscopy
- Uses a laser to provide the excitation light to get higher intensities
- Light eventually passes through a pinhole and is picked up by photomultiplier tubes
- Builder 3-D images

Electron Microscopy
- Utilizes a beam of electrons produced by an electron-emitting tungsten filament
- Increased resolution
- Special gold or palladium stains used to prepare the specimens
- Images viewed on a computer monitor

Cultures
- Artificial Media – solid, semi-solid, liquid
- Tissue Culture

Evaluate:
- Colonial morphology
- Cellular morphology
- Growth rates
- Growth conditions
- Biochemical reactions
- Susceptibility patterns
Antimicrobial Susceptibility Testing

- Disk Diffusion (Kirby-Bauer)
- Broth Dilution (MICs)
- Disk Diffusion-Broth Dilution – E-test
- Beta-Lactamase
- Disk Approximation Test – D-Test for inducible clindamycin resistance
- Synergy Testing – Hodge Test for ESBL
Antigen Testing

- Molecular structures, proteins or polysaccharides
- Stimulate the immune system to produce antibodies
- Utilize immunologic or serologic procedures
- Early diagnosis before cultures positive or not practical

Antigen Testing Methodologies

- Agglutination Tests (i.e., Hemagglutination, Cold Agglutinin, MHA-TP)
- Precipitin Assays (i.e., Double Diffusion, Countercurrent Immunoelectrophoresis)
- Complement Fixation (Q-Fever)
- Immunofluorescence (i.e., DFA, IFA)
- ELISA (Enzyme-linked Immunosorbant Assay)
- Radioimmunoassay (Hepatitis A)
- Neutralization (C. difficile)
- Immunoelectroblot (Western Blot)
Molecular Techniques

- Gene Probes
- PCR (Polymerase Chain Reactions)
- RT-PCR (Reverse Transcriptase PCR)
- PFGE (Pulsed Field Gel Electrophoresis)
- Molecular Hybridization (Southern [DNA], Northern [RNA], Western Blots [proteins])

Immune System - Immunoglobulins

- A complex of lymphoid organs
- Highly specialized cells
- Circulatory system separate from the blood circulatory system
- Lymph in Greek means a pure, clear stream
- Lymph – a fluid containing WBCs, primarily lymphocytes
Four Primary Functions

- Recognition of self
- Self tolerance
- Immunologic privilege
- Immunosurveillance
- Intracellular Hormones
- Defense against infection

Immune System Components

B-Cells
- Mature in bone marrow
- Produce antibodies
- Part of antibody-mediated (humoral) immunity
- Greeks called blood and lymph the "body’s humors"

T-Cells
- Mature in the thymus
- Attack and destroy
- Responsible for cell-mediated (cellular) immunity
- Regulate and coordinate the overall immune response

Types of Antibodies

- Isotypes:
  - IgM
  - IgG
  - IgA
  - IgE

IgM – there to mop up the infection

- Largest antibody
- Most important for IPs
- Indicates current disease process
- First at the site of initial exposures
**IgG – I got it and its Gone**

- Second at the site of the first exposure
- First at the site of subsequent exposures
- Acute and Convalescent Sera
  1. Acute sera with elevated IgM
  2. Convalescent sera with 4-fold rise in titer
- Single specimen with elevated IgG just means there has been an infection

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**IgA**

- Secreted on the lining of the mucous membranes
- Protection against respiratory and gastrointestinal (GI) infections
- Infants have an immature IgA system, hence infections with agents like RSV and Rotavirus

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**IgE**

- Least abundant immunoglobulin
- Works with basophils and mast cells
- Involved in allergic reactions and immediate hypersensitivity
- Mast cells – reside in connective tissues, release granules rich in histamine and heparin
- Benefit in wounds healing and defense against pathogens
**IgD**
- Secreted in small amounts in serum by the B-lymphocytes’ cell surface, with IgM
- Responsible to signal the B-cells to be activated
- Also binds to basophils and mast cells to activate them
- They produce antimicrobial factors to participate in the respiratory immune defense

**Mechanical Action of Immune System**
- Skin: fatty acids (pH 1), competitive colonization, enzymes (hydrolyze)
- Flushing: tears, saliva, micturation, peristalsis, coughing
- Antimicrobial Agents: enzymes, bile salts, gut pH
- Mucous Membranes
- Inflammatory Response
- Fever

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**Vascular Phase Inflammatory Response**
- Pathogen invades
- Tissue damage results
- Causes degranulation of mast cells
- Release of heparin & histamine into the area
- Increased blood flow to the area
- Results in dilated capillaries
- We see “red skin”

**Cellular Phase Inflammatory Response**
- Phagocytic cells squeeze through dilated capillaries
- Temperature at site increases
- Bradykinin & serotonin release, causing edema
- Fibrin leaks into area; clot formation
- Enzymes and dead WBCs form pus
- Goal to wall off infection and prevent spreading
Staphylococcal organisms cause a rapid inflammatory response resulting in a localized infection

Streptococcus organisms have a slow response, allowing for disseminated infections

### Immune Suppression
- Genetic deficiencies
- Drug-induced
- Cancer-induced – alters electrical charges of the brain (bidirectional effect b/w brain and immune system) – psychoneuroimmunology
- Viral Infections – similar to cancer-induced
- Bacterial Infections
- Malnutrition
- Stress
- Iatrogenic Factors

### Immune System – Friend or Foe?
- Allergy – response to a false alarm
- Auto-immune Diseases
- Antigen-Antibody Complex – adverse effect occurs when the infection is overwhelming
- DIC (Disseminated Intravascular Coagulation) – over activation of complement
- Cytokine Storm – result of persistent fever; releases tumor necrosis factor (TNF) and interleukins resulting in hypoglycemia and shock

### Non-Specific Immunity
- Leukocytes
- Complement:
  - Complex linear protein
  - Called an “effector” molecule
  - It helps to stimulate some immune activities
**Neutrophils (Polys)**

- Most dominant WBC (40-70%)  
- “First Responder”  
- Acts against pyogenic (pus-forming) bacteria  
- Life expectancy ~ 7 hours in circulatory system  
- Large reserve in bone marrow  
- Leukopenia – can be poor prognostic indicator  
- Hypersegmentation – suggest B12 or folate deficiency

**Neutrophil Ratios**

- **Degenerative Left Shift** – increase in bands with no leukocytosis; poor prognosis  
- **Regenerative Left Shift** – increase in bands with leukocytosis; good prognosis  
- **Right Shift** – few bands with increase in segmented neutrophil seen in liver disease, hemolysis, drugs, cancers, allergies, or megaloblastic anemia  
- **Hypersegmentation** – without bands is seen in megaloblastic anemia and chronic morphine addiction  
- **Myeloid Left Shift** – Bands, Metamyelocytes (Metas), Myelocytes, Promyelocytes (Pros), Blasts
Neutropenia

- Acute overwhelming bacterial infections – poor prognosis
- Viral infections
- Rickettsial and some parasitic diseases
- Drugs, chemicals, radiation, toxic chemicals
- Anaphylactic shock
- Severe renal disease
- Sepsis due to E. coli – reduced survival of polys
- Hormonal Disorders

Neutropenia in Neonates

- Maternal neutropenia
- Maternal drug ingestion
- Maternal isoimmunization to fetal WBCs
- Inborn errors of metabolism (i.e., maple syrup urine disease)
- Immune deficits
- Myeloid disorders
- Defective intrinsic factor secretion

WBC Abnormalities...

- **Toxic Granulation** – infections or inflammatory diseases – WBC is active
- **Dohle Bodies** – infections, inflammatory diseases, burns, Myeloproliferative disorders, cyclophosphamide therapy

Lymphocytes

- 25-40% of WBCs
- Fight viral infections
- Pertussis
- Chronic granulomatous diseases, i.e., TB
- Crohn’s disease
- Ulcerative Colitis
- Addison’s Disease
- Brucellosis
### Lymphopenia

- Chemotherapy
- After administration of cortisone
- Obstruction of lymphatic drainage, Whipple’s disease or tumors
- Hodgkin’s disease
- HIV/AIDS
- Trauma

### Monocytes

- Fight severe infection via phagocytosis
- 3-7% of WBCs
- Bacterial infections
- TB
- SBE
- Syphilis
- Parasitic, fungal, rickettsial diseases

### Basophils

- 0-1% of WBCs
- Mast Cells are tissue basophils
- Secrete histamine, serotonin, & prostaglandins – increase blood flow to area
- Hodgkin’s Disease
- Parasitic infections
- Inflammation
- Allergy
- Sinusitis
- After splenectomy
- TB
- Smallpox, Chickenpox
- Influenza

### Eosinophils

- 1-4% of WBCs
- Are cytotoxic
- NAACP….
- Neoplasm
- Asthma/Allergy
- Addison’s Disease
- Collagen/Vascular Disease
- Parasitic Infections
- Chronic Skin Conditions
Absolute WBC Counts

- Relative Number = percentage
- Absolute Count = Percentage X Total WBC Ct.
- Can have normal WBC count yet be neutropenic
- Need to look at WBC count and differential
- Normal WBC ranges:
  1. Adults ~ 3.5-10,000
  2. Newborns ~ 9-30,000
  3. 2 weeks ~ 5-20,000
  4. 1 yr ~ 6-18,000
  5. 4 yr ~ 5500-17,000
  6. 10 yr ~ 4500 – 13,500

Cell Count and Differential

- CSF should be clear & colorless
- Glucose 40-70 mg/dl
- Protein 15-45 mg/dl
- CSF Glucose = ~2/3 serum glucose
- Bacterial Meningitis:
  1. WBC = increased
  2. Diff – neutrophils
  3. Protein = marked increase
  4. Glucose = markedly decreased
- Viral (Aseptic) Meningitis:
  1. WBC = increased
  2. Diff – lymphs
  3. Protein = moderate increase
  4. Glucose = Normal
- TB/Fungal Meningitis:
  1. WBC = increased
  2. Diff – Lymphs and Monos
  3. Protein = moderate to marked increase
  4. Glucose = Normal to decreased

C-Reactive Protein (CRP)

- Protein virtually absent from serum of healthy person
- Seen in inflammatory process
- Classical, most dramatic acute-phase reactant
- Present within 18-24 hours of tissue damage
- Can be used for monitoring wound healing, especially burns and organ transplant
- Normal = ~<0.8 mg/dl

Liver Enzymes

- ALT/SGPT – Alanine Aminotransferase/Serum Glutamic-Pyruvic Transaminase
- AST/SGOT – Aspartate Transaminase/Serum Glutamic-Oxaloacetic Transaminase
- Total Bilirubin
- Direct Bilirubin
- GGT – Gamma-Glutamyl Transpeptidase
- Alkaline Phosphatase
ALT/SGPT
Elevated in:
- Viral/infectious hepatitis
- Infectious Mono
- Severe Shock
- Burns
Decreased in:
- UTI
- Malnutrition

AST/SGOT
Elevated in:
- Acute/chronic hepatitis
- Infectious Mono
- Trauma
- Trichinosis
- Cardiac Cath
- Gangrene
- Mushroom poisoning
Decreased in:
- Chronic Renal Disease

Bilirubin
Total Bili – damage to parenchymal cells; viral hepatitis, Infectious Mono
Indirect Bili (unconjugated) – protein bound bilirubin; pulmonary infarcts, large hematomas
Direct Bili (conjugated) – circulates freely; pancreatic cancer

Gamma GT
Elevated in:
1. Hepatitis
2. Alcoholism
3. Infectious Mono
Normal in:
1. Bone growth
2. Strenuous exercise
3. Renal Failure

Alk Phos
Elevated in:
1. Hepatitis
2. Infectious Mono
3. Diabetes mellitus
4. Ulcerative colitis
5. Bowel perforation
6. Chronic renal failure
Decreased in:
1. Malnutrition

D-Dimer
- Are produced only by the action of plasmin on cross-linked fibrin (clots)
- Presumptive evidence of DIC
- Normal = <250 ng/ml
- Positive after surgery
- Late in pregnancy, postpartum
Fibrinopeptide A (FPA)

• To determine thrombin action
In increased in:
  – DIC
  – Infections
  – Post-op patients

Platelet Counts

Increased in:
  • Splenectomy
  • Acute infections, inflammatory diseases
  • TB
  • Renal Failure
Decreased in:
  • Viral, bacterial, rickettsial infections
  • HIV
  • DIC
  • Hypersplenism

Sepsis

• An exaggeration of the body’s normal response
to infection
• Activate coag cascade
• Acute renal failure/Oliguria
• ARDS
• Oxygen demand can increase 12 fold
• Decreased platelet count
• Increased aPTT & INR
• Decreased pH
• Increased PaCO2 (hypoxemia)

BUN

• The amount of urea excreted varies directly
  with dietary protein intake, increased
  excretion in fever, diabetes and adrenal
  gland activity
• Increased with UTI
• Decreased in hepatitis

Creatinine

• By-product in the breakdown or muscle
  creatine phosphate
• Increased in impaired renal function
• Decreased in severe liver disease, pregnancy
• Decreased BUN:Creatinine ratio in
  renal failure
Lactate/Lactic Acid
- Increased in:
  - Sepsis/Shock
  - Liver disease
  - Diabetes
  - Pulmonary Failure

Cortisol
- Increased in:
  - Pregnancy
  - Stress (trauma, surgery)
  - Obesity
- Decreased in:
  - Hepatitis
  - Cirrhosis
  - Taking steroids

Ammonia
- An end-product in protein metabolism
- Bacteria act on intestinal proteins
- Increased in:
  1. Liver Disease
  2. Sepsis/Shock
  3. Renal Disease
  4. GI tract infection with distention and stasis

Diarrhea
- Metabolic Acidosis:
  1. Decreased pH
  2. Decreased Bicarbonate
  3. Normal PaCO2
  4. Hyponatremia
  5. Hypokalemia (if dehydrated, hyperkalemia)

Vomiting
- Metabolic alkalosis:
  1. Increased pH
  2. Increased Bicarbonate
  3. Normal PaCO2
  4. Hyponatremia
  5. Hypokalemia