Lecture 24

Immune Diseases - Chapter 16
- Allergies
  - Autoimmunity
  - Immunodeficiency

Disease Diagnostics - Chapter 17
- Sample Collections
  - Phenotypic Method
  - Genotypic Method
  - Immunological Method

Autoimmunity

- Antibodies, T cells or both, mount an immune response against self antigens
  - Systemic or organ-specific
  - Type II (fixed Ag) or III (soluble Ag) reactions

An example some major autoimmune diseases.

Table 16.4

<table>
<thead>
<tr>
<th>Disease</th>
<th>Target</th>
<th>Type</th>
<th>Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rheumatic lupus erythematosus</td>
<td>Nucleic acids</td>
<td>III</td>
<td>Systemic disease of nucleic acid antibodies</td>
</tr>
<tr>
<td>Rheumatological arthritis</td>
<td>Nucleic acids</td>
<td>III and IV</td>
<td>Arthritis due to immune complex deposition in joints</td>
</tr>
<tr>
<td>Autoimmune diseases</td>
<td>Nucleic acids</td>
<td>II</td>
<td>Arthritis due to autoantibodies</td>
</tr>
<tr>
<td>Multiple sclerosis</td>
<td>Nucleic acids</td>
<td>II and IV</td>
<td>Arthritis due to autoantibodies</td>
</tr>
</tbody>
</table>

Origins of autoimmunity

- Sequestered antigens
- Clonal selection against self
- Immune deficiency
- Inappropriate expression of MHC II
- Molecular mimicry
- Viral infections

An example of systemic lupus and rheumatoid arthritis.

Table 16.4 Selected autoimmune diseases.

Diseases

- Systemic autoimmunities
  - Systemic lupus erythematosus
  - Rheumatoid arthritis
- Endocrine
  - Graves disease
  - Hashimoto thyroiditis
  - Diabetes mellitus
- Neuromuscular
  - Myasthenia gravis
  - Multiple sclerosis

An example of systemic lupus and rheumatoid arthritis.
Immunodeficiency

- A person can be born with or develop a weakened immune system
  - Primary
  - Secondary

Primary

- Antibody production and phagocytosis
- Inherited abnormality
  - Deficiencies in B-cell or T-cell and development and expression
  - Combined B- and T-cell deficiency

Secondary

- Caused by
  - Infection
  - Chemotherapy
  - Radiation
Summary of the primary and secondary immunodeficiency diseases.

Table 16.5 General categories of immunodeficiency diseases with selected examples

<table>
<thead>
<tr>
<th>Primary Immunodeficiency (Genetic)</th>
<th>Secondary Immunodeficiency (Acquired)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6-cell defects (low levels of B cells and T cells)</td>
<td>Inherited factors (e.g., mutations)</td>
</tr>
<tr>
<td>Agammaglobulinemia (AB) linked, immunodeficiency</td>
<td>Immunodeficiency, immune deficiency</td>
</tr>
<tr>
<td>Severe combined immunodeficiency</td>
<td>Autoimmune diseases</td>
</tr>
<tr>
<td>HIV/AIDS (loss of CD4+ T cells)</td>
<td>Neoplastic diseases</td>
</tr>
<tr>
<td>Chronic neutrophilic leukocytes</td>
<td>ergy</td>
</tr>
<tr>
<td>Combined B cell and T cell deficiencies (usually caused by lack of ceramide deficiency)</td>
<td>Infections (e.g., viral infections)</td>
</tr>
<tr>
<td>Severe combined immunodeficiency (SCID)</td>
<td>Immunodeficiency (e.g., AIDS)</td>
</tr>
<tr>
<td>Destructive disorders (autoimmune deficiency)</td>
<td>Dermatologic diseases</td>
</tr>
<tr>
<td>Acute lymphocytic leukemia</td>
<td>Immunodeficiency (e.g., AIDS)</td>
</tr>
<tr>
<td>Macroglobulinemia</td>
<td>Immunodeficiency (e.g., AIDS)</td>
</tr>
</tbody>
</table>

Sample Collection

- Typical samples
  - Saliva
  - Sputum
  - Blood
  - Urine
  - Fecal
  - Skin
  - Cutaneous membrane samples

Topics

- Sample Collections
- Phenotypic Method
- Genotypic Method
- Immunological Method
Phenotype

• Microscopic investigation
• Cultivation
• Biochemical analysis
• Clinical significance

Microscopic investigation

• Common methods
  – Gram stain
  – Acid-fast stain
  – Direct fluorescence
  – Direct antigen testing

Stains

• Determine cell shape
• Determine cell wall property
  – Gram-positive (thick peptidoglycan layer)
  – Gram-negative (thin peptidoglycan layer)
  – Mycolic acid OM in Gram-positive wall

Cultivation

• Enable growth of specific microbes
  – Specialized media
  – Selective media
  – Defined media
  – Differential media
  – Biochemical media
• Enable pure culture isolation

Biochemical

• Indirect method
• Common tests
• Rapid test
• Schemes
Indirect method of identification

- Identify enzyme and product
  - Express genes related to nutrient use and stress defense

- Common tests
  - Carbohydrate fermentation
  - Hydrolysis of gelatin, starch
  - Catalase
  - Oxidase
  - Coagulase

An example of a rapid test, which includes 23 biochemical tests.

Clinical significance

- Number of microbes present
- Presence of microbes in a sterile site (e.g., cerebrospinal fluid, inner eye)
- Repeated isolation
- Isolation of a true pathogen (e.g., Mycobacterium tuberculosis)

Genotypic Method

- Genetic probes
- rRNA sequencing
- Polymerase Chain Reaction (PCR)
- G + C base composition

Genetic probes

- Specific DNA probes for genes that identify a particular microbe
- Probes bind to complimentary sample DNA (hybridization), enabling positive identification
Restriction digests, followed by hybridization with different probes generates specific patterns that enables identification of a particular microbe.

**rRNA sequencing**
- rRNA and protein specific to a ribosome
- rRNA sequence is stable
- Identification or signature for a particular microbe

Also:
- Establishes evolutionary relationships and distinguishes major taxonomic units

**PCR**
- Amplify specific fragment DNA or gene
- Sensitive – amplify small amounts of sample DNA
- Quick identification – 1 day

**G + C base composition**
- General indicator of relatedness
- Very small rate of change
- Allows taxonomic determination; however, cannot be used to identify specific microbe or virus

**Immunological Methods**
- Basic principle
- Agglutination
- Precipitation
- Western blot
- Complement fixation
- Immunofluorescence
- Common assays

**Basic principle**
- Serology (*In vitro* method)
  - Antibodies have specificity for antigens
    - Detect
    - Identify
    - Quantify
  - Sensitive
Agglutination

- Antigens are whole cells (e.g., RBC or bacteria)
- Antibodies cross-link antigens => Ag-Ab complex
- Allows determination of serum titer

Types of tests
- Rapid plasma test (syphilis)
- Cold agglutinin test (mycoplasma)
- Weil-Felix test (rickettsia)
- Latex agglutination test (pregnancy)

Precipitation

- Soluble antigens
- Binding of antibody to antigen, makes the complex insoluble or visible

Types of test
- Tube precipitation
- Ouchterlony (double diffusion)
- Immunoelectrophoresis
**Western blot**

- Electrophoretic separation of protein (antigen) sample
- Specific antibodies bind to protein sample
- Specific and sensitive
- Counterpart of the Southern blot (DNA test)

**Complement fixation**

- Antibody (lysin), antigen, complement, and sensitized sheep RBCs are required
- Complement is fixed to a Ab-Ag-complex
- Fixed complement cannot participate in rbc lysis = positive reaction or identification

**Immunofluorescence**

- A monoclonal antibody labeled with a fluorescent dye
  - Direct testing
  - Indirect testing
Common assays

- Radioimmunoassay (RIA)
- Enzyme-Linked Immunosorbent Assay (ELISA)
- T and B cell differentiation
- Virus testing

RIA

- Antibody or antigen is labeled
- Detect corresponding antibody or antigen
- Competition between unlabeled and labeled molecule

ELISA

- Horseradish peroxidase or alkaline phosphatase used as indicators
- Enzyme releases dye (chromogen)
- Types of test
  - Indirect
  - Capture or Sandwich

Fig. 17.15 direct and indirect Immunofluorescence testing

An example of the indirect and capture ELISA methods.

Fig. 17.16 Methods of ELISA testing.

The rosette formation method and the fluorescent technique are used to identify lymphocytes (T and B cells), in order to evaluate immune dysfunctions.

Fig. 17.17 Tests for characterizing T cells and B cells.
Fig. 17.18 Summary of methods used to diagnose Viral infections.