

THE JOURNAL OF
ALLERGY
AND
CLINICAL IMMUNOLOGY

VOLUME 94

NUMBER 6, PART 1

Committee report

Core content outline for clinical and laboratory immunology

AAAI Training Program Directors' Committee: William T. Shearer, MD, PhD, Chair, Daniel C. Adelman, MD, Co-Chair, David P. Huston, MD, Renata J. Engler, MD, Sudhir Gupta, MD, PhD, Dennis K. Ledford, MD, Manuel Lopez, MD, and Lawrence M. Du Buske, MD, PhD

In January 1993, the Allergy and Immunology Training Program Directors (TPD) requested that a subcommittee develop a core content outline for clinical and laboratory immunology (CLI). The CLI Subgroup of the TPD Core Curriculum Subcommittee was formed and began a year-long effort in producing this document, which was approved by the TPD 1 year later at the TPD Retreat in Dallas, January 1994. The authors of this Core Content Outline for CLI present a comprehensive and diverse outline of clinical and laboratory topics that are important to the quickly expanding field of CLI. Although not intended as a study guide for candidates taking the certification examination in CLI, the outline by its extensive nature, will touch on areas suitable for examination. This outline is primarily intended as a listing of clinical and laboratory areas of expertise that are and will be important for subspecialists in CLI. Readers will note that the list of topics goes well beyond what has characteristically been considered the domain of the allergist/immunologist. For example, extensive

Abbreviations used

CLI: Clinical and laboratory immunology
MHC: Major histocompatibility complex
PCR: Polymerase chain reaction
RA: Rheumatoid arthritis
SLE: Systemic lupus erythematosus
TPD: Training Program Directors

listings are made of primary immunodeficiency diseases; multisystem and organ-specific autoimmune diseases; bone marrow and organ transplantation; tumor immunology; quantitation of immunoglobulins, antibodies, complement components, and function; cellular phenotyping and functional assays; nucleic acid amplification and binding assays; genetic testing, prenatal diagnosis, histocompatibility typing, quality control testing, and regulatory agency interactions; and data management and statistical analysis. These nontraditional areas of expertise have been added to an expanded listing of immediate hypersensitivity diseases. The authors of the CLI Core Content Outline hope that this document captures the essence of the present thrust of development of the field of CLI. Although revisions will be quickly necessary, this outline will serve as an initial focus of preparation of the TPD in defining areas of expertise of the new clinical and laboratory immunologist.

From the American Academy of Allergy and Immunology.
Received for publication May 19, 1994; accepted June 6, 1994.
Reprint requests: AAAI Executive Office, 611 E. Wells St.,
Milwaukee, WI 53202-3889.

J ALLERGY CLIN IMMUNOL 1994;94:933-41.
Copyright © 1994 by Mosby-Year Book, Inc.
0091-6749/94 \$3.00 - 0 1/1/58345

CLINICAL CONDITIONS**I. Hypersensitivity diseases****A. Basic science**

1. Humoral immune responses in hypersensitivity disease
 - a. IgE-mediated immediate and late-phase reactions
 - (1) Regulation and expression
 - (2) Biologic effects mediated by high- and low-affinity Fc_ϵ receptors
 - (3) Effector cell populations
 - (4) Role of IgE and effector cells in allergic inflammation
 - b. IgG-, IgA-, IgM-mediated reactions
 - (1) Regulation and expression of IgG, IgA, IgM
 - (2) $Fc\gamma$ receptors, opsonization
 - (3) Antibody-mediated cytotoxicity
 - (4) Immune complex disease
 - (5) Complement activation
2. Cellular immune responses in hypersensitivity disease
 - a. Regulation of immune response
 - b. Immune modulation and modulators
 - (1) Cytokines
 - (2) Adhesion proteins in hypersensitivity diseases
3. Inflammatory mediators of hypersensitivity diseases
 - a. Lipid mediators: origins, structure, effects, sites of action, regulation
 - (1) Arachidonic acid metabolites
 - (2) Platelet activating factor
 - b. Cytokines and accessory cell growth support
 - c. Kinins
 - d. Enzymes/proteins
4. Diagnostic measures
 - a. Immediate hypersensitivity skin tests
 - b. RAST, ELISA
 - c. Histamine release assays

B. Clinical diseases

1. Upper airway diseases
 - a. Clinical disease: rhinitis, polyposis, otitis, laryngeal dysfunction
 - b. Interpretive capabilities: sinusitis, allergen skin testing, nasal secretion analysis
2. Lower airway diseases
 - a. Clinical disease: asthma, allergic bronchopulmonary aspergillosis, occupational asthma, hypersensitivity pneumonitis, bronchitis, bronchiectasis, cystic fibrosis (children and adults), ciliary dysfunction, sarcoidosis

- b. Interpretive capabilities: pulmonary function tests/spirometry, sputum analysis, bronchoalveolar lavage cell phenotype analysis, sweat chloride test, ciliary structure/function

3. Ocular disease

- a. Clinical disease: conjunctivitis (atopic, vernal, giant papillary), blepharitis, uveitis, keratoconjunctivitis sicca
- b. Interpretation of conjunctival secretion analysis

4. Dermatologic disease

- a. Clinical disease: urticaria/angioedema, atopic dermatitis, contact dermatitis, urticaria pigmentosa, bullous diseases, erythema multiforme, erythema nodosum, drug rash
- b. Interpretive capabilities: dermatopathology/microscopy, immunofluorescence, interpretation of patch testing results

5. Systemic mast cell- and eosinophil-mediated diseases

- a. Clinical disease: anaphylaxis, anaphylactoid reactions, systemic mastocytosis, hypereosinophilic syndromes
- b. Interpretive capabilities: histamine/metabolite assays, tryptase assay, eosinophil activation marker assays, major basic protein, prostaglandin D_2 assays

6. Adverse reactions to ingestants

- a. Clinical disease: food allergy versus intolerance, celiac disease
- b. Interpretive capabilities: allergy food testing, double-blind, placebo-controlled challenges

7. Insect hypersensitivity

- a. Clinical disease: Hymenoptera, Triatoma
- b. Interpretation of testing techniques

II. Primary and secondary immunodeficiency diseases**A. Primary immunodeficiencies**

1. Antibody deficiencies: X-linked agammaglobulinemia (Bruton's tyrosine kinase deficiency), X-linked agammaglobulinemia with growth hormone deficiency, selective IgA deficiency, selective IgM deficiency, selective IgG subclass deficiency, common variable immunodeficiency, transient hypogammaglobulinemia of infancy, X-linked lymphoproliferative (Duncan) syndrome, antibody deficiency with normal or near-normal levels of immunoglobulins

2. Cellular (T cell) deficiencies: DiGeorge syndrome, Wiskott-Aldrich syndrome, ataxia telangiectasia, chronic mucocutaneous candidiasis, cellular immunodeficiency immunoglobulins
 3. Combined (T and B cell) immunodeficiencies: severe combined immunodeficiency, adenosine deaminase deficiency, nucleoside phosphorylase deficiency, major histocompatibility complex (MHC) class I deficiency (bare lymphocyte syndrome), MHC class II deficiency, activation T-cell receptor deficiencies, cartilage-hair hypoplasia, short-limbed dwarfism with immunodeficiency, Omenn's syndrome, immunodeficiency with hyper-IgM (CD40 ligand deficiency)
 4. Complement deficiencies: classic pathway deficiency (C1q, C1r, C4, C2), alternate pathway deficiency (factor I, H, P, D, C3), membrane attack complex deficiency (C5, C6, C7, C8, C9), inhibitor deficiency (hereditary, acquired)
 5. Phagocytic cell defects: chronic granulomatous disease, Chédiak-Higashi syndrome, leukocyte adhesion deficiency, congenital neutropenia, cyclic neutropenia, hyperimmunoglobulinemia E syndrome
 - B. Secondary immunodeficiency diseases
 1. Prematurity/newborn
 2. Acquired immunodeficiency syndrome (AIDS)
 3. Hereditary and metabolic diseases: chromosome abnormalities, diabetes mellitus, exudative enteropathy, intestinal lymphangiectasia, iron deficiency, malnutrition, myotonic dystrophy, nephrotic syndrome, sickle cell disease, uremia, antibody deficiency with growth hormone deficiency, acrodermatitis enteropathica (zinc deficiency)
 4. Immunosuppressive agents
 5. Infectious diseases
 6. Infiltrative and hematologic diseases
 7. Surgery and trauma
- III. Multisystem autoimmune diseases**
- A. Rheumatoid arthritis (RA)/Felty's syndrome
 - B. Juvenile RA
 - C. Postinfection arthropathies
 1. Viral infections: Epstein-Barr virus, parvovirus arthropathy, hepatitis B, rubella virus
 2. Nonviral infections: Lyme disease, bacterial, chlamydial
 - D. Systemic lupus erythematosus (SLE)/subsets
 1. Immunopathogenesis
 2. Immunogenetics
 3. Autoantibodies
 4. Interpretation of immunodiagnostic tests (methods and performance): antinuclear antibodies, anti-cardiolipin antibodies and lupus anticoagulant studies, anticellular antibodies, other autoantibodies, complement profiles, immune complexes, skin biopsy, renal biopsy, acute phase reactants
 - E. Mixed connective tissue disease
 - F. Antiphospholipid antibody syndrome
 - G. Sjögren's syndrome
 - H. Progressive systemic sclerosis (scleroderma)
 - I. Polymyositis/dermatomyositis
 - J. Spondyloarthropathies
 1. Ankylosing spondylitis
 2. Reactive arthropathies
 - K. Systemic vasculitic syndromes
 1. Primary vasculitis
 - a. Large and medium sized blood vessels
 - b. Medium and small sized blood vessels
 - c. Small blood vessels: microscopic polyarteritis (hypersensitivity vasculitis, leukocytoclastic vasculitis), Wegener's granulomatosis, Schönlein-Henoch purpura
 2. Secondary vasculitis
 - a. Infectious causes
 - b. Noninfectious causes: sarcoidosis, thromboangiitis obliterans
 - c. Vasculitis associated with other connective tissue diseases: RA, SLE, mixed connective tissue disease
 - d. Other vasculitic syndromes: hypersensitivity vasculitis, malignancy-associated vasculitis, lymphocytic vasculitis, hypocomplementemic urticarial/vasculitis, essential mixed cryoglobulinemia, inflammatory bowel disease, retroperitoneal fibrosis, primary biliary cirrhosis, Goodpasture's syndrome, transplantation vasculitis
- IV. Organ-specific autoimmune diseases**
- A. Autoimmune thyroid disease
 1. Hypothyroidism
 2. Hyperthyroidism: Graves' disease
 - B. Autoimmune pancreatic disease diabetes mellitus (type I)
 1. HLA associations
 2. Cell-mediated immune abnormalities
 3. Autoantibodies: anti-islet cell antibodies, insulin autoantibodies, proinsulin autoantibodies, antibodies to glucose transporters, islet cell surface antibodies

- C. Autoimmune adrenal atrophy: Addison's disease
 - D. Autoimmune pituitary disease
 - 1. Lymphocytic adenohypophysitis
 - 2. Anterior pituitary antibody syndromes
 - E. Autoimmune parathyroid disease: idiopathic hypoparathyroidism
 - F. Autoimmune polyendocrine disorders
 - 1. Type I polyglandular syndrome
 - 2. Type II polyglandular syndrome
 - G. Autoimmune gastrointestinal diseases
 - 1. Idiopathic inflammatory bowel disease
 - 2. Celiac sprue (gluten-sensitive enteropathy)
 - 3. Chronic gastritis-pernicious anemia
 - H. Autoimmune hepatobiliary diseases
 - 1. Autoimmune aspects of infectious hepatitis
 - 2. Autoimmune chronic active hepatitis
 - 3. Primary biliary cirrhosis
 - 4. Primary sclerosing cholangitis
 - I. Autoimmune hematologic diseases
 - 1. Thrombocytopenia
 - 2. Lymphopenia
 - 3. Neutropenia
 - 4. Anemia
 - J. Autoimmune diseases of the eye
 - 1. Uveitis
 - 2. Scleritis
 - 3. Episcleritis
 - 4. Granulomatous diseases
 - 5. Sympathetic ophthalmitis
 - 6. Manifestations of other ocular autoimmune diseases
 - K. Autoimmune lung diseases
 - 1. Goodpasture's syndrome
 - 2. Vasculitis
 - 3. Polymyositis and dermatomyositis
 - 4. Pneumonitis/fibrosing alveolitis/pleuritis
 - 5. RA
 - 6. Sarcoidosis
 - L. Autoimmune dermatologic diseases
 - 1. Vesiculobullous diseases: pemphigoid, bullous pemphigoid, cicatricial pemphigoid, herpes gestationis, epidermolysis bullosa acquisita, bullous SLE, dermatitis herpetiformis
 - 2. Psoriasis
 - M. Autoimmune renal diseases
 - 1. Goodpasture's syndrome
 - 2. Wegener's granulomatosis
 - 3. Cryoglobulinemia
 - 4. Amyloidosis
 - 5. IgA nephropathy
 - 6. Autoimmune tubulointerstitial nephritis
 - 7. Membranous nephropathy
 - 8. Mesangiocapillary glomerulonephritis
 - 9. Poststreptococcal glomerulonephritis
 - 10. Minimal change nephropathy
 - 11. Focal necrotizing glomerulonephritis
 - N. Autoimmune neurologic diseases
 - 1. Idiopathic inflammatory polyneuropathies
 - 2. Myasthenia gravis
 - 3. Multiple sclerosis
 - 4. Chronic inflammatory demyelinating polyneuropathy
 - O. Autoimmune cardiovascular diseases
 - 1. Carditis
 - 2. Rheumatic heart disease
 - P. Autoimmune reproductive diseases
 - 1. Reproductive autoimmune failure syndrome-female: autoimmune infertility, endometriosis, anti-sperm antibodies, premature ovarian failure, pregnancy wasting
 - 2. Autoimmune reproductive diseases-male
- V. Immunomodulators as therapy for autoimmune diseases**
- A. Immunoglobulin
 - B. Anti-lymphocyte antibodies
 - C. Pharmacologic immunosuppressive agents
 - D. Thymectomy
 - E. Splenectomy
 - F. Total lymphoid irradiation
 - G. Plasmapheresis
 - H. Immunoabsorbents
- VI. Immunologic aspects of bone marrow and organ transplantation**
- A. Donor-recipient matching
 - 1. MHC
 - 2. Blood group antigens
 - 3. Selection of donors: serologic testing, cellular assays, DNA techniques, cross-matching, cadaveric versus living donor
 - 4. Graft versus host disease
 - B. Preparation of recipient in organ tissue transplantation
 - 1. Immunosuppression
 - 2. Radiation ablation
 - 3. Multiple transfusions for kidney transplants
 - C. Preparation of donor bone marrow in bone marrow transplantation
 - 1. HLA identical
 - 2. HLA haplo-identical (T-cell depletion)
 - 3. Harvesting of stem cells
 - 4. Augmentation of stem cell population
 - D. Indications for type of bone marrow transplantation
 - 1. Allogeneic

2. Autologous
3. Gene therapy
- E. Use of peripheral blood stem cells for immune reconstitution
- F. Posttransplantation complications and treatment
 1. Graft rejection in organ transplantation
 - a. Monitoring for immunologic rejection: hyperacute, acute, chronic
 - b. Treatment of immunologic rejection
 - (1) Corticosteroids
 - (2) Cytotoxic agents
 - (3) Immunophyllin antagonists
 - (4) Monoclonal antibodies: anti-T cell, anti-intercellular adhesion molecule-1, anti-interleukin-2, designer antibodies (chimeric humanized)
 - (5) Soluble receptor: IL-2R
 2. Venooclusive disease of the liver after bone marrow transplantation
 3. Opportunistic infections
- G. Monitoring after bone marrow transplantation
 1. Assessment of engraftment
 2. Lymphocyte phenotypic subsets
 3. Lymphocyte proliferation to mitogens and antigens
 4. Delayed skin test response to antigens
 5. Specific antibody formation

VII. Tumor immunology

- A. Mechanisms of carcinogenesis
 1. Oncogenes
 2. Tumor suppressor genes: p53
- B. Host immunologic defenses
 1. Cellular: cytotoxic T cells, natural killer cells, lymphokine-activated killer cells, tumor-infiltrating lymphocytes, macrophages
 2. Antibodies
 3. Cytokines
 4. Immune complexes
- C. Factors important in immunologic escape of tumor cells
 1. Antigenic modulation
 2. Antigen masking and shedding
 3. Lymphocyte trapping
 4. Growth factor
 5. Soluble cytokine receptors
- D. Tumor markers and antigens
 1. Tumor-associated and specific antigens
 2. Viral induced tumor antigens, host-derived antigens
 3. Differentiation antigens
 4. Embryonic antigens

- E. Immunotherapy
 1. Active immunotherapy: vaccine, bacterial products, synthetic molecules, biologic response modifiers
 2. Adoptive immunotherapy: lymphokine-activated killer cells, tumor-infiltrating lymphocytes, bone marrow transplantation
 3. Passive immunotherapy: monoclonal antibodies, designer monoclonal antibody
 4. Gene therapy
- F. Immunoprophylaxis: hepatitis B vaccine

IMMUNOLOGY LABORATORY

I. Quantitation of immunoglobulins

- A. Zone electrophoresis
- B. Immunoprecipitation
- C. Immunodiffusion
- D. Immunoelectrophoresis
- E. Solid-phase assays

II. Measurement of specific antibodies

- A. Hemagglutination
- B. Precipitation or aggregation
- C. Radioimmunoassay and radioabsorbent assays
- D. ELISA
- E. Western blot
- F. Complement fixation
- G. Immune complex assays
 1. Solubility-dependent assays: cryoglobulins, polyethylene glycol precipitation, ultracentrifugation
 2. Complement activation-dependent assays, Raji cell assay, radiolabeled C1q, binding, conglutinin binding, anticomplementary activity or complement consumption
 3. FcR-dependent assays, monoclonal rheumatoid factor binding, macrophage phagocytosis inhibition, platelet aggregation

III. Complement testing: quantitative and functional

- A. Proteins of complement system
 1. Classic pathway: C1q, C1r, C1s, C4, C2
 2. Alternate activation pathway: properdin D, B, C3
 3. Terminal sequence-membrane attack complex: C5, C6, C7, C8, C9
 4. Plasma control proteins: C1 inhibitor, C4 binding protein, factor H, factor I, anaphylatoxin inactivator, S protein, vitronectin
 5. Membrane control proteins: CR-1 (CD35) membrane cofactor protein, decay-accelerating factor, homologous restriction factor, membrane inhibitor of reactive lysis (CD59)

- B. Hemolytic assays (functionally based)
 - 1. Total hemolytic complement activation: CH50
 - 2. Alternate pathway hemolytic complement: APH50 (AP50)
 - 3. Hemolytic assays of specific components
 - 4. Hemolytic assays of inhibitory proteins
 - C. Antigenic assays of specific components
 - 1. Single radial immunodiffusion
 - 2. Nephelometry
 - 3. Functional assays
 - D. Assays for complement activation and inhibition
 - 1. Immunoelectrophoresis or rocket immunoelectrophoresis: C3a
 - 2. Two-dimensional immunoelectrophoresis: C3-NeF
 - 3. Immunoassays (radioimmunoassay, ELISA)
 - a. Classical: C4a, C4d, C4/C4d
 - b. Alternate: Ba, Bb
 - c. Terminal: C3a, C3d, iC3b, C5a, SC5b-9
 - d. Inhibitors: C1-inhibitor
 - 4. Functional assays: C1-inhibitor
 - E. Clinical applications of complement assays
 - 1. Increased consumption
 - 2. Decreased production
 - 3. Evaluation of angioedema
 - a. Heterogeneity of C1-inhibitor-deficient angioedema
 - b. Functional versus antigenic assays of C1-inhibitor
 - c. Discriminating between hereditary and acquired angioedema
 - 4. Spurious results obtained by improper collection or handling
 - a. Hemolysis of collected specimen
 - b. Plasma instead of serum
 - c. Warming of serum, freezing or thawing of serum
 - 5. Spurious results caused by assay variability
 - a. Titration of hemolysin
 - b. Normal range variability
 - F. Allotyping complement proteins
 - 1. Genetic restriction fragment length polymorphism
 - 2. MHC markers of certain diseases
 - 3. Confirmation of inherited disease states of complement proteins
 - 4. Adjunct to HLA typing in the identification of family donors for organ or tissue transplantation
- IV. Cellular assays of quantification and function**
- A. Lymphocytes: quantitative and functional assays
 - 1. In vivo T-cell function testing: absolute lymphocyte count, peripheral smear morphology, delayed-type hypersensitivity testing with recall antigens
 - 2. In vitro T-cell function testing
 - a. Immunophenotyping: flow cytometry
 - b. Lymphocyte proliferation: mitogens/monoclonal antibodies, alloantigens, specific antigens
 - c. Cytokine production
 - d. Cytotoxicity assays: allogeneic cell-mediated lympholysis, modified self-cell-mediated lympholysis
 - 3. In vivo B-cell function
 - a. Quantitative immunoglobulins
 - b. Specific antibody testing
 - (1) Protein antigens: tetanus and diphtheria toxoid
 - (2) Polysaccharide/carbohydrate antigens: pneumococcal old *Hemophilus influenza* type B vaccine
 - (3) Neoantigens: bacteriophage ϕ X-174, keyhole limpet hemocyanin
 - 4. In vitro B-cell function
 - a. B-cell phenotyping
 - b. Polyclonal immunoglobulin production to mitogen, superantigen
 - c. Antigen-specific immunoglobulin production
 - d. Isotype switching with specific signals, cytokines
 - e. Assessment of T-cell help/suppression
 - f. Assessment of isotype precursor frequency
 - 5. Natural immunity testing: natural killer cell phenotyping, natural killer cell function, lymphokine-activated killer cell function, antibody-dependent cellular cytotoxicity
 - B. Neutrophil function
 - 1. In vivo assays: absolute neutrophil counts, peripheral smear examination of neutrophils
 - 2. In vitro assays
 - a. Peripheral blood leukocyte phenotyping: CD11a,b,c and CD18
 - b. Chemotaxis: random versus directed
 - c. Oxidative burst: nitroblue tetrazolium dye reduction, flow cytometric nitroblue tetrazolium dye reduction, ferricytochrome C reduction, chemiluminescence
 - d. Microbial killing
 - C. Monocyte/macrophage function
 - 1. In vivo assays: monocyte counts, delayed cutaneous hypersensitivity, adhesive gly-

coprotein expression, HLA antigen expression

2. In vitro assays: antigen presentation, assay, monocyte-mediated cytotoxicity, chemotaxis (directed or random), cytokine production, microbial killing

D. Cell activation signaling components

1. Early signal transduction events: membrane activation events, intracellular second messenger generation, activation of protein kinases, synthesis of or phosphorylation of nuclear factors, functional activation of nuclear factors
2. Transcriptional activation of a variety of genes
3. Expression of new cell surface molecules
4. Secretion of cytokines
5. Induction of mitotic activity

V. Autoantibody assessment

A. Hemagglutination, precipitation, and aggregation reactions with beads and particles

1. Rheumatoid factor by latex agglutination
2. Indirect Coombs test
3. Anti-thyroglobulin antibody by hemagglutination
4. Anti-microsomal antibody by hemagglutination

B. Immunofluorescence

C. Radioimmunoassay

D. Immunoprecipitation

E. Immunoabsorbent assays

F. Nephelometry

G. Cytotoxicity and other bioassays

VI. Rapid tests for infectious disease

A. Isotype of antibody response

1. IgM antibody assays
2. Agglutination assays
3. Complement fixation assays

B. Factors influencing application of specific assays

1. Prozone phenomenon
2. Serum rheumatoid factor
3. Competing antigens

VII. Nucleic acid amplification and binding assays

A. Collection and extraction of DNA and RNA from biologic material

B. Theory of nucleic acid-based detection methods

1. Principles of nucleic acid hybridization
2. Characteristics of probe and target nucleic acids: probe form, probe size, specificity of the probe, labeling probes, choice of target nucleic acids

C. Hybridization strategies

1. Filter hybridization: dot or colony blots
2. Solution or liquid hybridizations

D. Amplification of DNA and RNA

1. Polymerase chain reaction (PCR): PCR with nesting primers, inverse PCR, anchored PCR, amplification refractory mutation system, sequence-specific oligonucleotide probes (dot blots, reverse dot blot), sequence-specific primers, PCR-restriction fragment length polymorphism sequencing
2. DNA ligase-chain amplification (oligonucleotide ligation assay)
3. Transcription-based amplification system: RNA-based
4. Cycling probe reaction
5. Q β (Q β bacteriophage) replicase amplification
6. Strand displacement amplification
7. Signal amplification and detection methods

E. Analysis of DNA and RNA

1. Gel electrophoresis
2. Southern blotting: DNA
3. Northern blotting: RNA
4. In situ hybridization

F. Enzymatic manipulation of DNA and RNA

1. Digestion with restriction endonucleases
2. Restriction mapping
3. Chromosome walking, jumping
4. Construction of hybrid DNA

G. Clinical applications of molecular techniques

1. Infectious disease diagnosis
2. DNA typing of HLA class II gene polymorphism
3. Prenatal diagnosis of genetic diseases
4. Forensic analysis
5. Chromosomal rearrangements in malignancy

VIII. Genetic testing

A. Human genome: background

B. Laboratory tools used in testing procedures

1. Chromosome analysis
2. DNA-based testing
3. Analysis of activity or concentration of enzymes, proteins, or metabolites in the serum or urine

C. Presymptomatic genetic testing

1. Accuracy
2. Validity
3. Predictive value

D. Social and bioethical concerns

1. Potential harms: intrusion of privacy and confidentiality, inaccurate predictions, and discrimination in insurance and employment

2. Current disease applications: adenosine deaminase deficiency severe combined immunodeficiency, Huntington's chorea, multiple endocrine neoplasia, cystic fibrosis

IX. Prenatal diagnosis

A. Tools

1. Serologic assays for antigen, enzyme/protein quantity/activity
2. Molecular probes
3. X chromosome inactivation and linkage analysis
4. Restriction fragment length polymorphism

B. Fetal infection: toxoplasmosis, rubella, cytomegalovirus, varicella, parvovirus

C. Maternal X chromosome inactivation and linkage analysis

1. Current and potential practical applications: chronic granulomatous disease, properidin deficiency, severe combined immunodeficiency, X-linked agammaglobulinemia
2. Future application potential: Wiskott-Aldrich syndrome, immunodeficiency with hyper-IgM syndrome, X-linked proliferative syndrome

X. Histocompatibility testing

A. Background

1. MHC

- a. Class I: HLA-A,-B,-C,-E,-F,-G
- b. Class II: HLA-DR,-DQ,-DP
- c. Class III: complement (C2, BF, C4A, C4B), tumor necrosis factor- α , tumor necrosis factor- β , 21-hydroxylase, heat shock protein 70

2. HLA nomenclature

- a. Serologically defined, sequential numbering system
- b. Immunoelectrophoresis defined subtypes
- c. Cellularly defined specificities
- d. DNA sequence-based alleles

3. Basic genetics: linkage, linkage disequilibrium, mendelian inheritance, haplotypes, genotypes, phenotypes, polymorphism

4. Other histocompatibility antigens: ABO blood groups, endothelial and monocyte antigens, minor histocompatibility antigens

B. HLA typing: antigen identification

1. Serology

- a. Sources of typing sera; immune response to HLA antigens, pregnancy, multiparous donors; blood component thromboplastin replacing factor (red

blood cells, whole blood, platelets); solid organ transplant recipients

b. Complement-dependent microlymphocytotoxicity

c. Terminology: epitopes/antigens (cross-reactive, public/private, typical, super-typical, subtypic), monospecific or multi-specific

2. Cellular techniques: mixed lymphocyte culture, primed lymphocyte test, cell-mediated lympholysis

3. DNA-based methodologies: restriction fragment length polymorphism, PCR

C. Antibody identification

1. Microlymphocytotoxicity

- a. National Institutes of Health, Amos-modified, multiple wash techniques
- b. Anti-hemophilic globulin technique
- c. T versus B versus mixed cells
- d. Peripheral blood, lymph nodes, or spleen
- e. Viable cells required

2. Antibody-dependent cellular cytotoxicity

3. Flow cytometry

4. Cellular "cross match" by mixed lymphocyte culture

5. Panel-reactive antibody

- a. Significance in solid organ transplantation
- b. Definition of antibody specificity

D. Quality control

E. Clinical applications of histocompatibility testing

1. Transplantation: solid organs, skin, bone marrow, bone, cornea, cartilage, heart valves

2. Population genetics

3. Paternity testing

4. Forensics

5. Disease associations

6. Blood component therapy

XI. Quality control testing and regulatory agency interactions

A. Principles of quality control

1. Assessment of test precision
2. Parallel testing
3. Data generated by College of American Pathologists

B. Principles of assay development

1. Definition of positive and negative controls
2. Development of a reference standard
3. Critical elements of documenting methodology

4. Defining sensitivity, specificity, accuracy, and precision
 - a. Analytic "quality" and "effectiveness"
 - b. Effectiveness as a dimension of quality—quality and cost trade-offs
 - c. End user dimension—the clinician
5. Critical issues in placing the test in the clinical laboratory
 - a. Clinical question for which test selection is appropriate
 - b. Optimum specimen collection and handling
 - c. Technology and methodology development in a clinical laboratory
 - d. Laboratory processing and analysis
 - e. Validation and reporting of results

- f. Interpretation and evaluation of results
- C. Certification of laboratories
1. Centers for Disease Control
 2. Clinical Laboratory Improvement Amendment of 1988

XII. Data management and statistical analysis

- A. Test utility assessment
1. Sensitivity
 2. Specificity
 3. Predictive value: positive and negative
- B. Statistical management of data
1. Arithmetic versus geometric distribution and statistics
 2. Nonparametric statistics
 3. Regression analysis in comparative studies