

**Indiana University School of Medicine, Department of Microbiology & Immunology**  
**IMMUNOLOGY CORE CURRICULUM**  
**2008**

1. Innate immunity and non-specific antimicrobial mechanisms

- a. Antimicrobial factors on skin and mucous membranes and in the blood and tissues
  - (1) Mechanical antimicrobial factors (mechanical barrier of skin and mucous membranes, ciliated epithelium, normal flora, mucous secretions)
  - (2) Chemical antimicrobial factors (lysozyme, muramidase, spermine, mucin, peroxides, acids, complement, cytokines)
- b. Phagocytic cells and the process of phagocytosis
  - (1) Polymorphonuclear leukocytes, monocytes and macrophages (blood and tissue types; role of macrophage as antigen presenting cell and mediator of inflammation)
  - (2) Phagocyte cell surface receptors and markers: complement, Fc, Class II, Toll-like
  - (3) Receptor-mediated chemotaxis (chemotactic agents: C5a, LTB<sub>4</sub>, chemokines [IL-8, MIP, MCP])
  - (4) Adherence to endothelium (margination and emigration: ICAMs and selectins on endothelium, integrins and selectins on phagocytes)
  - (5) Particle attachment, phagocytosis, formation of phagolysosomes and role of degranulation in tissue damage
  - (6) Metabolic burst and mechanisms of bacterial killing and digestion
    - (a) Oxygen-dependent antibacterial factors: Superoxide generated by NADPH oxidase; hydrogen peroxide generated by superoxide dismutase; hypochlorite and hypiodite generated by myeloperoxidase
    - (b) Oxygen-independent antimicrobial proteins: acid hydrolases, muramidase, lysozyme, lactoferrin, defensins.
- c. Inflammation
  - (1) Definition and overall results: (acute versus chronic) cell activation, cell migration, increased vascular permeability, pain, tissue damage, tissue repair
  - (2) Cells involved: endothelial, PMN, mast, basophils, platelets
  - (3) Inflammatory mediators, their origin and actions:
    - (a) Cytokines: IL-1, IL-6, IL-8, TNF $\alpha$ , IFN- $\gamma$
    - (b) Vasoactive molecules: histamine (H1, 2, & 3 receptors), prostaglandins, leukotrienes, serotonin, nitric oxide
    - (c) Plasma enzyme systems: clotting, fibrinolytic (fibrinopeptides), kinin (bradykinin), and complement (C3a, C5a)
    - (d) Clinical evaluation: fever, leukocytosis (band forms)
- d. Complement
  - (1) Components, sources, and activators
  - (2) Classic pathway, lectin pathway, and alternative pathway: activation, components, and biologically active end products
  - (3) C3 tick-over
  - (4) Membrane attack complex
  - (5) Complement receptors (opsonization, cell activation)
  - (6) Regulation of complement activation (CR1, DAF, MCP, Factor I, Factor H, Factor P, CD59)
  - (7) Complement fixation assay: technique and clinical uses

2. Introduction to acquired immunity
  - a. Detection of microbial antigens (role of Toll-like receptors)
  - b. Specificity: reaction to extracellular versus intracellular pathogens
  - c. Heterogeneity
  - d. Memory
  - e. Discrimination between self and non-self
  - f. Active and passive immunity
  - g. Natural (infection) versus artificial (principles of vaccination)
  
3. Antigens
  - a. Types of antigens (T-dependent, T-independent, antigen processing and presentation via Class I or Class II MHC molecules)
  - b. Antigenic determinants (epitopes) recognized by B and T cells: immunodominance; conformational versus sequence recognition
  - c. Haptens and carriers (application to vaccinology)
  
4. Antibodies
  - a. Immunoglobulin structure and basis for classification: heavy chains ( $\gamma$ ,  $\mu$ ,  $\epsilon$ ,  $\alpha$ ,  $\delta$ ), light chains ( $\kappa$ ,  $\lambda$ ), variable, hypervariable, and constant regions, loop domains, hinge region, J-chains, disulfide bridges, enzyme fragmentation (Fab, Fc,  $F(ab')_2$ ), effector regions (Fc receptor site, complement binding site); definition of isotypes, allotypes and idiotypes.
  - b. Functions (neutralization, opsonization, complement activation, anti-tumor activity), harmful activities (allergies and autoimmune diseases), the receptors for the various isotypes and the cells where they occur, distributions, approximate concentrations and half-lives of the different immunoglobulin isotypes: IgG (IgG<sub>1,2,3,4</sub>), IgM, IgA (IgA<sub>1,2</sub>), sIgA, IgE, IgD
  - c. Hybridoma technology and the generation and clinical uses of monoclonal antibodies
  
5. Antigen-antibody interactions:
  - a. Definitions of specificity, cross-reactivity, affinity, and avidity
  - b. Forces binding antigens and antibodies: specificity for three-dimensional conformation of the epitope and the formation of multiple non-covalent bonds
  - c. Antibody interactions with soluble antigen (the precipitin reaction): lattice formation, antigen excess, zone of equivalence, antibody excess, and soluble immune complexes
  - d. Classic immunologic methods to detect soluble antigen-antibody reactions (principles, methods and uses)
    - (1) Precipitation in solution: tube precipitin reaction (titer)
    - (2) Precipitation in solid medium (principles, methods and uses): radial immunodiffusion, immunofixation electrophoresis,
  - e. Classic immunologic methods to detect insoluble antigen-antibody reactions (principles, methods and uses): agglutination, hemagglutination, Coombs test
  - f. Modern immunologic techniques (principles, methods and uses): Immunoblotting (Western blotting), Enzyme-linked immunosorbent assay, Immunofluorescence (direct and indirect), fluorescence activated flow cytometry
  
6. Cells involved with the immune response, hematopoiesis, and lymphatic organs
  - a. Cells (description, function, important mediators they release, half-lives, relative concentrations in blood)
    - (1) Non-antigen specific: neutrophils, basophils, eosinophil, mast cell, macrophage, natural killer cells (NK; large granular lymphocyte morphology)
    - (2) Antigen-specific: T lymphocytes ( $T_C$ ,  $T_H1$ ,  $T_H2$ ,  $T_H3$ ,  $T_H17$ , NKT), B lymphocytes
    - (3) Antigen presenting cells (function, location, and important cell surface markers): dendritic

- cells, macrophages (Kupffer, intraglomerular, alveolar, serosal, brain [microglia], spleen sinus, lymph node sinus); central role of macrophages in immunity and inflammation (cytokines secreted, vasoactive molecules, tissue damage and healing), and B-cells
- b. Hematopoiesis
    - (1) Stem cells and cytokines (Stem cell factor, IL-1, 3, 6, 7, GM-CSF, G-CSF, M-CSF)
    - (2) Use of stem cells and cytokines to reconstitute or augment the immune system (e.g. recombinant GM-CSF for neutropenia)
  - c. Primary (central) and secondary (peripheral) lymphatic tissue (functions and histologic organization as it pertains to immune function)
    - (1) Primary: bone marrow and fetal liver (B cells); thymus (T cells)
    - (2) Secondary: spleen, lymph nodes, mucosa-associated lymphoid tissue (MALT); MALT: tonsils, adenoids; bronchial, gastrointestinal (including the appendix), and genitourinary tract lymphoid tissues, Peyer's patches (function of M cells)
  - d. Lymphocyte (lymph) traffic: high endothelial venules, efferent lymphatic vessels, thoracic duct, blood lymphocyte pool, and afferent lymphatic vessels
7. Cytokines and chemokines (sources, principal targets, major activities)
- a. IL-1 ( $\alpha$  and  $\beta$ ): from phagocytes, lymphocytes, endothelial cells, etc.; targets lymphocytes, macrophages, endothelium, etc.; activates cells, increases adhesion molecule expression, induces fever, induces release of acute phase proteins.
  - b. IL-2: source and target are T cells; induces T cell proliferation, activates TC cells, stimulates B cells.
  - c. IL-3: from T cells; targets hematopoietic stem cells; induces growth and differentiation.
  - d. IL-4: from  $T_H2$  cells, mast cells, macrophages; targets T and B cells, eosinophils, mast cells; induces  $T_H2$  conversion from  $T_H0$ , B cell growth, IgE and IgG<sub>1</sub> isotype selection, eosinophil and mast cell activation.
  - e. IL-5: from  $T_H2$  cells, mast cells; targets B cells and eosinophils; induces B cell growth and differentiation, IgA isotype selection, eosinophil maturation and activation.
  - f. IL-6: from T cells ( $T_H2$ ), B cells, macrophages, fibroblasts, endothelial cells; targets B cells, hepatocytes; induces B cell differentiation, production of acute phase proteins, fever.
  - g. IL-7: from bone marrow stromal cells; targets B and T precursors; induces proliferation.
  - h. IL-8 (chemokine designation: CXCL8): from macrophages, endothelial cells, fibroblasts; targets neutrophils, basophils, T cells; chemotactic and angiogenic. Note that there are 4 chemokine families (CCL, CXCL, XCL, and CX3CL) based upon the configurations of their cysteine residues at their amino terminus. Another chemokine example is MCP-1 (CCL2): CCL chemokine from macrophages, fibroblasts, epithelial and endothelial cells; targets monocytes, basophils, mast cells; chemotactic agent for monocytes, induces histamine release by mast cells and basophils.
  - i. IL-10: from activated T cells, macrophages; targets  $T_H1$  cells; inhibits cytokine synthesis, promotes B cell proliferation, suppresses cell mediated immunity (CMI).
  - j. IL-12: from B cells, macrophages; targets T cells, NK cells; induces  $T_H1$  cell conversion from  $T_H0$ , suppresses  $T_H2$  activities, activates  $T_C$  and NK cells, promotes CMI.
  - k. IL-13: from  $T_H2$  cells; targets macrophages, B cells; induces B cell growth and differentiation, inhibits production of pro-inflammatory cytokines.
  - l. IL-15: from macrophages, T cells; targets T cells, activated B cells; actions similar to IL-2.
  - m. IL-17: from  $T_H17$  cells; targets neutrophils, monocytes, and fibroblasts; induces the production of IL-6 and 8; important in certain autoimmune diseases and inflammation, especially involving the skin.
  - n. IL-18: (formerly known as IFN $\gamma$ - inducing factor) from phagocytes; targets T cells; induces production of IFN $\gamma$ , IL2, and GM-CSF.
  - o. IL-22: from  $T_H17$  cells; activates keratinocytes; importance like IL-17

- p.  $\text{TNF}\alpha$ : from macrophages, mast cells, lymphocytes, endothelial cells; targets and effects similar to IL-1 plus induces MHC class I protein expression, tumor necrosis, and stimulates angiogenesis.
  - q.  $\text{TNF}\beta$  (lymphotoxin): from lymphocytes; targets and effects similar to  $\text{TNF}\alpha$  except not associated with inducing shock.
  - r.  $\text{IFN}\alpha$ : from lymphocytes, macrophages, epithelial cells; targets tissue cells (e.g. epithelial cells); induces antiviral state, MHC class I expression, stimulates NK cells, production of IL-12, and  $\text{T}_{\text{H}1}$  cells.
  - s.  $\text{IFN}\beta$ : from fibroblasts, epithelial cells; targets tissue cells, leukocytes; induces anti-viral state, MHC class I expression.
  - t.  $\text{IFN}\gamma$ : from T cells, NK cells; targets leukocytes, tissue cells,  $\text{T}_{\text{H}2}$  cells; induces expression of MHC class I and II, activates phagocytes, inhibits  $\text{T}_{\text{H}2}$  cells, enhances leukocyte-endothelial adherence.
  - u. M-CSF: from macrophages, fibroblasts, endothelial cells; targets monocytes and monocyte precursors; induces proliferation of monocyte precursors.
  - v. G-CSF: from macrophages, fibroblasts, endothelial cells; target granulocyte precursors; induces proliferation of precursors to mature granulocytes.
  - w. GM-CSF: from T cells, macrophages, fibroblasts, endothelial cells; targets granulocyte precursors and a number of other cells; promotes the differentiation of precursors, stimulates neutrophils, eosinophils, and macrophages.
  - x.  $\text{TGF}\alpha$ : from macrophages and other cells; targets fibroblasts, epithelial and endothelial cells; stimulates fibroblast growth and angiogenesis.
  - y.  $\text{TGF}\beta$ : from activated T cells, macrophages, and other cell types; targets T cells, macrophages, and other cells; inhibits immune and inflammatory responses, stimulates IgA production.
8. B lymphocytes:
- a. Origin (bone marrow) and events associated with maturation (cytokines: IL-3, 4, 7)
  - b. Maturation:
    - (1) Pre-B cell (expresses cytoplasmic  $\mu$  chains; surface class II MHC)
    - (2) V, D, and J gene rearrangement: generation of diverse antigen receptors (somatic mutation and recombination of H and L chains hypervariable regions)
    - (3) Immature B cell (expresses surface IgM, class II MHC)
    - (4) Positive/negative selection, apoptosis of self-reactive cells
    - (5) Co-expression of  $\mu$  and  $\delta$
    - (6) Mature B cell (expresses surface IgM and IgD, class II MHC, CR1 and 2, Fc receptor, CD19)
    - (7) Collection of B cells in secondary lymphatic tissues
  - c. B cell antigen receptor (structure, accessory proteins and signal transduction)
  - d. Antigen-induced B cell activation and differentiation: Clonal selection, isotype switching (maturation of immune response), V-region hypermutation, differentiation into plasma cells (Ig secretion) and B memory cells (surface IgG, IgA, or IgE); cytokine influence on isotype
9. T lymphocytes, NK, and NKT cells
- a. T cell origin (bone marrow) and events associated with maturation in the thymus (cytokines: IL1, 2, 3, 6, 7, GM-CSF; thymic hormones)
    - (1) Stem cells (multipotent) collect in thymus and move from cortex to medulla while interacting with MHC Class II-bearing nurse, epithelial and interdigitating cells.
    - (2) Maturation progression:
      - (a) Early thymocyte ( $\text{CD}4^+8^-$ , T cell receptor (TCR) gene rearrangements)
      - (b) Common thymocyte ( $\text{CD}4^+8^+$ , T cell receptor gene rearrangements; low TCR and

- CD3 surface expression)
      - (c) Mature thymocyte (CD4<sup>+</sup> or CD8<sup>+</sup> subsets; high TCR and CD3 surface expression; somatic recombination of TCR genes)
      - (d) Positive and negative selection occurs and most self-reactive T cells eliminated.
      - (e) All T cells positive for TCR, CD2, 3, and 28
    - b. T cell antigen receptor (genetics, structure, accessory proteins and signal transduction)
      - (1) Antigen-specific TCR dimers:  $\alpha\beta$  (90-95% of all T cells) or  $\gamma\delta$ ; V, D, J, and C (constant) genes for  $\beta$  and  $\delta$  chains; V, J, and C genes for  $\alpha$  and  $\gamma$  chains.
      - (2) CD3 complex ( $\epsilon_2\zeta_2\gamma\delta$ ) associated with TCR has signal transduction role.
      - (3) TCR/CD3 overall stoichiometry:  $(\alpha\beta)_2\epsilon_2\zeta_2\gamma\delta$
      - (4) Activation of T cells by mitogens and superantigens
    - c. Functional subsets of CD4 cells based on cytokine profile.
      - (1) T<sub>naive</sub> cell = Low levels of IL-2, 4, 5, 6, 10, 13, TNF $\beta$ , IFN $\gamma$  (these may not be detectable; all activated T cells produce IL-2)
      - (2) T<sub>H1</sub> = IFN $\gamma$ , IL-2 (Type 1 cytokines promote cell mediated immunity)
      - (3) T<sub>H2</sub> = IL- 2, 3, 4, 5, 9, 13 (Type 2 cytokines promote humoral immunity)
      - (4) T<sub>H17</sub> = IL-17, 22 (promote cutaneous inflammation)
      - (5) Subset control:
        - T<sub>naive</sub> > T<sub>H1</sub> promoted by IL-12 and IFN $\gamma$  (example: IFN $\alpha$  produced during viral infection stimulates macrophages to produce IL-12); IL-4 & 10 inhibit.
        - T<sub>naive</sub> > T<sub>H2</sub> promoted by IL-4; IFN $\gamma$  inhibits
        - T<sub>naive</sub> > T<sub>H17</sub> promoted by IL-1 and IL-23; IL-4 and IFN $\gamma$  inhibit
    - d. Functional subsets of CD8 cells (Cytotoxic T cells, T<sub>C</sub> or CTL)
      - (1) Pre-CTL > cytolytic CTL promoted by IL-2 & IFN $\gamma$  (IL-6 & 12 also have role)  
Active CTLs lyse targets with TNF $\beta$ , degranulation (perforin, granzymes), and/or Fas-induced apoptosis.
    - e. Regulatory T cells: T cells that can have regulatory activity based on their cytokine profile (inhibitory subsets include CD4<sup>+</sup>CD25<sup>+</sup> & Th3; all produce TGF- $\beta$  and/or IL-10)
    - f. NK cells
      - (1) Distribution: 10-15% of circulating lymphocytes; lack typical T cell markers (i.e. TCR, CD3, CD4, CD8, etc.).
      - (2) Markers: CD16 (IgG Fc receptor), CD28, CD56 (CD16 & 56 used to identify NK cells)
      - (3) Cytokines secreted: IFN $\gamma$ , IL-1, GM-CSF, TNF $\alpha$  ; activated by IFN $\gamma$ , IL-2, IL-12 and IL-18
      - (4) Cytotoxic mechanisms: Attack cells with reduced MHC class I expression. Release perforin.
      - (5) CD-28/B7-1 co-stimulating signal important
      - (6) Can also kill by antibody-dependent cell mediated cytotoxicity (ADCC; Killer cell [K cell] activity)
    - g. NKT cells
      - Have T and NK markers; recognize Ag (lipids, glycolipids) presented by MHC-like CD1d; produce IFN-gamma, IL4, and IL13
10. Major Histocompatibility Complex (MHC; Human Leukocyte antigens [HLA])
- a. Class I, II and III MHC genetic loci (short arm of chromosome 6)
    - Major Class I genes: *HLA-A, B, C*
    - Major Class II genes: *HLA-D* region  
*DP* (A1, A2, B1, B2), *DQ* (A1, A2, B1, B2, B3), *DR* (A, B1, B2, B3)
  - b. Inheritance: Definition of haplotype, example of inheritance pattern, pseudogenes, and cell

surface expression (what cells express Class I, Class II). Define polymorphic nature of the MHC proteins and allotypes and gene polymorphism.

- c. Structure of Class I MHC proteins
  - (1)  $\alpha 1$ ,  $\alpha 2$  and  $\alpha 3$  domains of heavy chain ( $\alpha 1$  &  $\alpha 2$  form peptide binding site [groove]; amino acid differences account for polymorphism and antigen specificity)
  - (2)  $\beta_2$  - Microglobulin (invariant but essential)
- d. Structure of Class II MHC proteins
  - (1) Composed of one  $\alpha$  and one  $\beta$  chain
  - (2)  $\alpha 1$  and  $\beta 1$  domains comprise the peptide binding site (groove). Again, amino acid differences account for polymorphism and antigen specificity.  $\alpha 2$  and  $\beta 2$  domains constant.
- e. Role of MHC proteins in antigen processing and presentation; general aspects of HLA-restriction.
  - (1) Processing and presentation of exogenous antigen via the MHC class II pathway (exogenous pathway):
    - (a) Professional antigen processing cell internalizes antigen.
    - (b) Antigen internalized into endosome & degraded into peptide fragments.
    - (c) MHC class II proteins synthesized in ER and peptide binding site protected by Ii (invariant chaperone; CLIP region of Ii mediates protection of site).
    - (d) Exocytic vesicle (from Golgi) containing MHC II proteins fuses with endosome containing peptides.
    - (e) Ii degraded, CLIP removed by HLA-DM protein.
    - (f) MHC II bind peptides, vesicle fuses with plasma membrane, MHC II/peptides expressed on cell surface, and presented to CD4 cells.
    - (g) Interactions between CD4 and MHC II-expressing antigen presenting cells: (T cell left, APC right)
      - (i) LFA-1  $\rightarrow$  ICAM-1 (adhesion)
      - (ii) CD2  $\rightarrow$  LFA-3 (adhesion)
      - (iii) CD4 (Lck)  $\rightarrow$  MHC II (co-activation with TCR)
      - (iv) TCR/CD3  $\rightarrow$  MHC II/peptide (activation of T cell through PIP<sub>2</sub>/DAG, IP<sub>3</sub> pathway)
      - (v) CD28  $\rightarrow$  B7-1/B7-2 (co-activation with TCR)
      - (vi) T cell CD45 (tyr phosphatase; RO and RA isoforms)
    - (h) End result: activation of transcription factors.
  - (2) Processing and presentation of endogenous antigen *via* the MHC class I pathway (endogenous pathway):
    - (a) Cytoplasmic protein (e.g. viral) ubiquitinated, hydrolyzed to peptide fragments in the proteasome, and peptides transported into the ER via TAP.
    - (b) MHC I proteins synthesized and assembled in ER and association with TAP mediated by calnexin chaperone.
    - (c) MHC I bind peptides, vesicle fuses with plasma membrane, MHC I/peptides expressed on cell surface, and presented to CD8 cells.
    - (d) Interactions between CD8 and MHC I-expressing antigen presenting cells: (T cell left, APC right)
      - (i) LFA-1  $\rightarrow$  ICAM-1 (adhesion)
      - (ii) CD2  $\rightarrow$  LFA-3 (adhesion)
      - (iii) CD8 (Lck)  $\rightarrow$  MHC I (co-activation with TCR)
      - (iv) TCR/CD3  $\rightarrow$  MHC I / peptide (activation of T cell through PIP<sub>2</sub>/DAG, IP<sub>3</sub> pathway)
      - (v) CD28  $\rightarrow$  B7-1/B7-2 (co-activation with TCR)

(vi) T cell CD45 (tyr phosphatase; RO and RA isoforms)

- f. Clinical importance of HLA antigens
  - (1) Transplantation and organ rejection:
    - (a) HLA typing:
      - (i) flow cytometry for HLA typing.
      - (ii) RFLP analysis of class II genes.
      - (iii) PCR/sequence-specific oligonucleotide probes for HLA typing.
  - (2) Diseases associated with specific HLA antigens (see specific disease also):
    - (a) DR3/DR2 > Systemic Lupus Erythematosus
    - (b) DR4 > Rheumatoid Arthritis
    - (c) B7 and DR2 > Multiple Sclerosis
    - (d) B8, DR3/DR4 > Type 1 diabetes
    - (e) B27 > Ankylosing Spondylitis

#### 11. Antibody-mediated immune (humoral) response

- a. B cell differentiation into IgM-secreting plasma cells induced by T-independent antigen.
  - (1) Types of T-independent antigens.
  - (2) Signal transduction mediated by B cell antigen receptor and accessory molecules.
- b. Primary and secondary responses to T-dependent antigen (kinetics of antibody production).
- c. Effects of T<sub>H2</sub> cytokines on B cell differentiation, isotype switching, and memory cells:
  - (1) Signals that generate a T<sub>H2</sub> response: IL-10 from macrophages and IL-4 from Ag-activated T cells and other sources
  - (2) IL-5 → promotes IgA-producing cells (plasma and memory; role in mucosal immunity)
  - (3) IL-4 → promotes IgG<sub>4</sub> and IgE-producing cells (plasma and memory; role in hypersensitive reactions)
- d. Cell surface molecules involved in T/B cell interactions (B cell left/T cell right)
  - (1) MHC II/Ag → TCR/CD3 (signals generated for both cells)
  - (2) CD40 → CD154 (CD40L) (potent B & T cell signal; important for memory)
  - (3) B7-1/2 (CD80/86) → CD28 (potent T cell signal) associated adhesion molecules
  - (4) B7-1/2 (CD80/86) → CTLA-4 (CD152) (ligation down-regulates T cell activity)
- e. Hypermutation and Ig affinity maturation
  - (1) Effects of route and antigen dose on response.
  - (2) Selective expansion of high-affinity clones.
  - (3) Role of anatomical site on response.
- f. FcRn (neonatal Fc receptor) and transplacental transport of IgG
- g. T cell memory; Ag deposition

#### 12. Cell mediated immune (CMI) responses

- a. Function:
  - (1) Control intracellular pathogens, tumors
  - (2) Mediate transplant rejection
  - (3) Mediate Type IV hypersensitivity
  - (4) Contribute to granuloma formation
  - (5) Contribute to chronic inflammation
- b. Predominant cells and mediators (see above sections for details on activation and cell-surface interactions)
  - (1) T<sub>H1</sub> lymphocytes (activated by Ag, IL-12, and IFN $\gamma$ ) release IL-2 and IFN $\gamma$ : induce T cell proliferation, T<sub>C</sub>, NK, and macrophage activation
  - (2) CD8<sup>+</sup> T<sub>C1</sub> and T<sub>C2</sub> (activated by Ag/MHC I [endogenous pathway] and T<sub>H1</sub> cytokines) have

cytokine profile similar to  $T_{H1}$  and  $T_{H2}$ , respectively; both are cytotoxic via  $TNF\beta$ , perforin, granzymes, and Fas-induced apoptosis

- (3) NK cells (see above for activation, etc.) release  $IFN\gamma$  which activates macrophages and stimulates  $T_{H1}$  activity; also release GM-CSF that stimulates PMNs; lyse targets predominantly via granule components (e.g. perforin)
- (4) Monocytes/macrophages [mononuclear phagocytes] (activated by  $IFN\gamma$  and usually a second signal from an Ag [e.g. LPS] or another cytokine [e.g. GM-CSF,  $TNF\alpha$ ])
  - (a) Tissue types: Kupffer, alveolar, etc.
  - (b) Surface markers: C3b receptor (CR-1), LFA-1, Fc receptor, MHC II
  - (c) Macrophages play a central role in CMI and inflammation:
    - (i) Antigen presenting cell important in killing bacteria and lysing tumor cells
    - (ii) Effector/mediator of delayed type hypersensitivity (DTH, see Type IV hypersensitivity)
    - (iii) Release IL-12  $\rightarrow$  induces  $T_{H1}$  response ( $IFN\alpha$  induces)
    - (iv) Release IL-10  $\rightarrow$  induces  $T_{H2}$  response by down-regulating cytokine production
    - (v) Release IL-1, 6,  $TNF\alpha$  and  $\beta$ ,  $INF\alpha$  and  $\beta$
    - (vi) Release enzymes, coagulation factors, complement components, superoxide (etc.), leukotrienes, and prostaglandins
- c. Role of endothelium
  - (1) Differential expression of adhesion molecules and their role in CMI (ICAM-1/VCAM, P- and E-selectin)
- d. Detection of cell-mediated immunity with cytolytic assays ( $^{51}Cr$  release assay)

### 13. Regulation and termination of immune responses

- a. Regulation by the nature of the antigen (e.g. polysaccharides  $>$  IgM)
- b. Regulation by individual history (and genetic background), antigen dose and route of administration
- c. Regulation by antigen presenting cell (presence of co-stimulatory signals)
- d. Modulation by antibody
  - (1) Immune complexes, receptor cross-linking
- e. Regulation by T cells (regulatory T cells and other cells)
  - (1)  $T_H$  and  $T_C$  subsets and cytokine control
  - (2) Soluble forms of TCRs and cytokine receptors
  - (3) Fas/Fas ligand-induced apoptosis
- f. Neuroendocrine controls
  - (1) Hormonal controls (e.g. cortisone, serotonin, epinephrine, norepinephrine)
- g. Tolerance (effects of age and antigen dose)
  - (1) Central tolerance (positive and negative selection)
  - (2) Peripheral tolerance (clonal deletion, apoptosis and anergy)

### 14. Cutaneous and Mucosal Immune systems

- a. Cutaneous (cells, DTH)
- b. Mucosal (cells, tissue, IgA)
  - (1) M cells
  - (2) Polymeric Ig receptor and transepithelial IgA transport

### 15. Immunomodulation

- a. Vaccines
  - (1) Active immunization (principles, complications, and examples; childhood immunizations; antigens [live, dead, toxoids]; adjuvants)

- (2) Passive immunization (principles, complications, and examples)
  - (3) DNA vaccines and recombinant vaccines
  - b. Immunosuppressive drugs (mechanisms of action and examples of their uses)
    - (1) Cycle-nonspecific (radiation, corticosteroids, nonsteroidal anti-inflammatory agents, cyclosporin)
    - (2) Cycle-specific (cyclophosphamide, chlorambucil, azathioprine, methotrexate)
    - (3) Monoclonal antibodies and receptor antagonists (e.g. anti-TNF $\alpha$  Mab (Infliximab, Adalimumab; anti-TNF $\alpha$  receptor antagonist (Etanercept)
  - c. Pathologically induced immunosuppression (immune deficiency diseases, disease-induced anergy)
16. Type I (Immediate) Hypersensitivity
- a. Genetics (atopy, allergen-specific, IgE levels, etc.)
  - b. Cells and allergens (F<sub>C $\epsilon$</sub>  receptors, mast cells, basophils, eosinophils, APC, T<sub>H2</sub>, pollen, etc.)
  - c. Cytokine control by T<sub>H2</sub> cells (IL-4, 13)
  - d. Measurement of IgE (RAST)
  - e. Mechanism of IgE-mediated mast cell activation and degranulation
    - (1) receptor cross-linking
    - (2) kinase cascade
    - (3) calcium mobilization, activation of arachidonic acid pathway, and myosin phosphorylation
  - f. IgE-independent effects on degranulation: C3a, C5a; role of autonomic nervous system (effects of epinephrine, acetylcholine, theophylline)
  - g. Primary mediators: histamine (effects on H1 and H2 receptors), eosinophil and neutrophil chemotactic factors, TNF $\alpha$ , IL-6, heparin
  - h. Secondary mediators: leukotrienes (LTB<sub>4</sub>, LTD<sub>4</sub>, LTE<sub>4</sub>, LTC<sub>4</sub>), prostaglandins (PGD<sub>2</sub>), PAF, IL-1, 3, 4, 5, 6, TNF $\alpha$ , GM-CSF
  - i. Clinical manifestations (incidence, symptoms, pathologic findings, examples of therapy and role of hypo- or desensitization; diagnostic techniques used [e.g. skin test])
    - (1) Generalized anaphylaxis (bee sting, drug allergy)
    - (2) Cutaneous reactions: urticaria, angioedema
    - (3) Atopic (immediate and late responses): allergic rhinitis, asthma (extrinsic, intrinsic), atopic dermatitis, food allergy
17. Type II Hypersensitivity (IgG or IgM-mediated cytotoxicity)
- a. Mechanisms and results of Ig-mediated cytotoxicity
    - (1) activation of complement
    - (2) Antibody-Dependent Cellular Cytotoxicity (ADCC)
    - (3) major effector cells (macrophages, NK cells, neutrophils)
  - b. Examples and clinical manifestations (incidence, symptoms, mechanisms of tissue destruction, pathologic findings, and examples of therapy [e.g. supportive, steroids, plasmapheresis])
    - (1) Hemolytic anemias
      - (a) lab findings indicative of hemolysis (elevated LDH, elevated hemoglobin, elevated bilirubin, hypocomplementemia)
      - (b) warm and cold (autoimmune, drug-induced, secondary), including erythroblastosis fetalis (Coomb's test)
    - (2) Thrombocytopenias (autoimmune, drug-induced, secondary)
    - (3) Nephrotoxic serum nephritis and Goodpasture's syndrome
    - (4) Pemphigus vulgaris
    - (5) Myasthenia gravis
    - (6) Pernicious anemia
    - (7) Acute rheumatic fever

18. Type III (Immune complex) Hypersensitivity
- a. Mechanisms and results of immune complex-mediated reactions (Arthus reaction as the experimental model)
    - (1) Formation of antigen-antibody complexes; complexes form in or deposit in tissue
    - (2) Activation of complement (effects of C3a, C5a)
    - (3) Release of PAF; neutrophil and platelet aggregation and degranulation; increased vascular permeability and necrosis; end result = vasculitis
    - (4) Role of macrophages (release TNF $\alpha$  and IL-1; release of enzymes)
  - b. Examples of acute and chronic clinical manifestations (incidence, symptoms, mechanisms of tissue destruction, pathologic findings, and examples of therapy [e.g. steroids, supportive])
    - (1) Serum sickness
    - (2) Immune complex induced glomerulonephritis
19. Type IV (Delayed-type or Cell-mediated) Hypersensitivity
- a. Classification of Type IV reactions: Contact hypersensitivity, tuberculin, NK and T-cytotoxicity, granulomatous
  - b. Contact hypersensitivity (allergic contact dermatitis)
    - (1) Immunologic mechanisms  
Types of allergens; sensitization and elicitation phases; role of Langerhans cells and keratinocytes; role of antigen-specific T<sub>H1</sub> cells and their cytokines; mechanisms of down regulation.
    - (2) Signs, symptoms, incidence and treatment
  - c. Tuberculin-type hypersensitivity (recall response to antigen encountered during infection)
    - (1) Immunologic mechanisms  
Infections associated with this response; role of antigen-specific T<sub>H1</sub> cells and their cytokines; role of macrophages; role of endothelium [adhesion molecule expression and regulation of the influx of cells: PMN first, followed by monocytes and T cells]; macrophages the main cell type; mechanisms of down regulation (e.g. IL10 from macrophages, limited presence of antigen).
  - d. T<sub>C</sub> and NK cell reactions: see previous section on T cell activation and cell mediated immunity
  - e. Granulomatous hypersensitivity (associated with many pathologic effects seen in T cell-mediated immune reactions)
    - (1) Immunologic mechanisms  
Cells: (antigen-specific T<sub>H1</sub> cells, macrophages [epithelioid cells, giant cells])  
Cytokines: IFN $\gamma$ , TNF $\alpha$ , IL-3, IL-12, GM-CSF
    - (2) Diseases associated with granulomatous hypersensitivity (brief descriptions)
      - (a) Leprosy
      - (b) Tuberculosis
      - (c) Schistosomiasis
      - (d) Sarcoidosis
      - (e) Crohn's disease
      - (f) Hypersensitivity pneumonitis (early form involves Type III)
  - f. Evaluation of DTH
    - (1) Patch test
    - (2) Skin test to evaluate CMI
20. Autoimmunity and autoimmune diseases
- a. Origins of autoimmune diseases
    - (1) Genetic factors: familial incidence; association with specific HLA haplotypes
    - (2) Failure to maintain self-tolerance (either central or peripheral)

- (3) Loss of regulatory T cells (dysregulation of the cytokine network)
- (4) Expression of cryptic self epitopes
- (5) Inappropriate expression of MHC II molecules or co-receptors on specific tissue
- (6) Cross-reacting antigens and antigenic mimicry
- (7) Polyclonal B cell activation
- (8) Association of certain infectious diseases with the onset of autoimmunity
- (9) Hormonal influences
- b. Pathogenic mechanisms of autoimmune diseases (see individual diseases)
- c. Examples of autoimmune diseases (prevalence, signs, symptoms, pathologic consequences of autoimmune mechanisms, examples of treatments [some key features of the diseases are given below])
  - (1) Autoimmune Rheumatic Diseases
    - (a) Rheumatoid Arthritis (Type III and IV hypersensitive reactions; rheumatoid factors; vasculitis, synovitis; HLA-DR4; cellular and chemical effectors [PMN, T<sub>H</sub>1, T<sub>C</sub>, IL-1, TNF $\beta$ , IL-8, PGE<sub>2</sub>, LTB<sub>4</sub>])
    - (b) Systemic Lupus Erythematosus (Type II and III; multiple auto-antibodies; HLA-DR2, HLA-DR3; multiple organ involvement)
    - (c) Sjogren's syndrome (chronic inflammatory condition involving auto-reactive antibodies and T-cells directed at lacrimal and salivary gland antigens)
  - (2) Autoimmune Endocrine Diseases
    - (a) Insulin-Dependent Diabetes Mellitus (Type II and IV; auto-antibodies to beta cell surface antigen, cytoplasmic antigen, and glutamic acid decarboxylase [associated with GABA synthesis]; HLA-DR proteins expressed on beta cells) HLA-DR3, HLA-DR4)
    - (b) Chronic Thyroiditis (Hashimoto's Disease; Type II and IV; goiter; antithyroglobulin and antithyroid peroxidase are prevalent)
    - (c) Graves' disease (hyperthyroidism; anti-TSH receptor autoantibodies; MHC II [HLA-DR] expression on thyroid cells; HLA-DR3)
  - (3) Autoimmune Liver and Gastrointestinal Diseases
    - (a) Inflammatory Bowel Diseases: Crohn's Disease (abnormality of mucosal T cell regulation; granulomatous reaction characteristic; skip-lesions in any part of gastrointestinal tract); Ulcerative colitis (Possibly Type II; continuous mucosal inflammation common)
    - (b) Pernicious Anemia (antiparietal cell antibodies and anti-intrinsic factor antibodies)
  - (4) Autoimmune Neurologic Diseases
    - (a) Multiple Sclerosis (abnormal T cell regulation; Type II and IV; inflammatory demyelination in CNS white matter resulting in "plaques"; auto-antibodies to MBP and PLP of myelin)
    - (b) Acute Disseminated Encephalomyelitis (follows infection or vaccination; Type IV directed at MBP and other myelin antigens)
    - (c) Acute Inflammatory Polyneuropathy (Guillain-Barre' Syndrome; follows viral or *Campylobacter* infection; Type II and IV reaction to peripheral nerve antigens)
    - (d) Myasthenia gravis (Type II autoimmune; Abs directed at acetylcholine receptors)
  - (5) Autoimmune skin diseases (e.g. psoriasis)

## 21. Human Blood Group Antigens

- a. ABO blood groups (structure, inheritance, and naturally occurring antibodies)
- b. Rh blood groups (e.g. erythroblastosis fetalis)

## 22. Transplantation immunology

- a. Definitions of autografts, syngrafts, allografts, and xenografts

- b. The role of MHC antigens (alloantigens), minor histocompatibility antigens and ABO blood type in graft acceptance and rejection
- c. Mechanisms of graft rejection
  - (1) Hyperacute rejection: recipient has pre-existing antibodies to ABO antigens or HLA antigens; occurs within minutes to hours; mechanism by Types II and III reactions.
  - (2) Acute: occurs within a few days to a couple weeks; mediated by antibody (Acute humoral rejection) and/or sensitized T cells (Acute cellular rejection)
  - (3) Chronic: occurs months to years after the transplant; multiple immunologic mechanisms; chronic inflammation, pro-inflammatory cytokines, and increased adhesion molecules on endothelium important
- d. Graft-versus-host reaction (acute and chronic)
  - (1) Immunologic mechanisms and types of transplants involved
- e. Tissue typing, preventing rejection, and inducing recipient unresponsiveness
  - (1) ABO compatibility
  - (2) Crossmatching
  - (3) HLA typing
  - (4) Inducing unresponsiveness
    - (a) Azathioprine
    - (b) Cyclosporin
    - (c) Corticosteroids
    - (d) Anti-lymphocyte antibodies (polyclonal anti-T, monoclonal anti-CD3, 4, 8)
- f. Clinical transplantation
  - (1) Solid tissue (e.g. kidney)
  - (2) Bone marrow and stem cells

### 23. Tumor Immunology

- a. Properties of tumor (transformed) cells
  - (1) Abnormalities in growth and responses to regulation and apoptosis
  - (2) Induction by carcinogens, viruses; spontaneous
  - (3) Role of oncogenes (e.g. growth-promoting oncogenes and oncoproteins)
  - (4) Role of tumor-suppressor genes and genes that control DNA repair
- b. Tumor antigens
  - (1) Tumor-associated antigens (Ags found on certain tumor cells and some normal cells)
  - (2) Tumor-specific antigens (Ags characteristic of certain tumor cells; not found on normal cells)
  - (3) Antigens resulting from mutation (such as the mutation of an oncogene or proteins produced through translocations)
  - (4) Over expressed antigens; antigens expressed at abnormal stage of development
  - (5) Viral antigens
  - (6) Non-immunogenic
    - (a) Oncofetal antigens: carcinoembryonic antigen (CEA),  $\alpha$ -fetoprotein
    - (b) Differentiation antigens: common acute lymphoblastic leukemia antigen (CALLA, CD10) found on cells of acute lymphoblastic leukemia and PSA on prostate
- c. Anti-tumor immunity
  - (1) CMI ( $T_C$ ,  $T_{H1}$  cytokines, NK, macrophage)
  - (2) Humoral (ADCC and activation of complement)
  - (3) "Immunosurveillance": There is an increased frequency of cancers in immunocompromised individuals, however, most cancers occur in individuals with no overt immunodeficiency. More likely that tumors develop ways of evading the immune system.
  - (4) Examples of tumor evasion:

- (a) out-growth of antigen-negative variants
  - (b) loss or reduced expression of MHC proteins
  - (c) secretion of immunosuppressive cytokines (e.g. TGF $\beta$ )
  - (d) inducing T<sub>C</sub> apoptosis
  - (e) lack of co-stimulatory signals
  - (f) soluble tumor antigens
- d. Immunotherapy
- (1) Non-specific stimulation (adjuvant therapy)
  - (2) Active immunization with tumor antigens; role of co-stimulatory molecules
  - (3) Cytokine therapy
  - (4) Anti-tumor antibodies (coupled to cytotoxins or radioisotopes)
- e. Examples of neoplastic diseases of the immune system
- (1) Multiple myeloma
  - (2) Leukemia
  - (3) Lymphoma

## 24. Immunodeficiency Diseases

- a. General signs and symptoms of immunodeficiency diseases
- b. Laboratory tests to assess immune function
- (1) T cell: Enumeration (flow cytometry), functional assays (mitogen response, MLR, DTH skin tests)
  - (2) B cell: Enumeration, circulating antibody levels
  - (3) Macrophage: Enumeration, functional assays (nitroblue tetrazolium)
  - (4) Complement: Direct measurement of complement components, complement hemolysis assay
- c. Primary B cell immunodeficiencies (symptoms, description of defect, current therapy)
- (1) X-linked Agammaglobulinemia (Bruton's syndrome); btk deficiency
  - (2) Common Variable Immunodeficiency (acquired hypogammaglobulinemia)
  - (3) Selective IgA deficiency (most common immunodeficiency disorder)
  - (4) Other (minor):
    - (a) Transient hypogammaglobulinemia of infancy
    - (b) Selective deficiency of IgG subclasses
- d. Primary T cell immunodeficiencies (symptoms, description of defect, current therapy)
- (1) Congenital thymic aplasia (DiGeorge's Syndrome or Third and Fourth Pharyngeal Arch Syndrome)
  - (2) Chronic mucocutaneous candidiasis (uncontrolled CMI to *Candida albicans*)
- e. Combined B and T cell immunodeficiencies (symptoms, description of defect, current therapy)
- (1) Severe Combined Immunodeficiency (SCID; a group of genetically determined diseases)
    - (a) X-Linked combined immunodeficiency (accounts for 50-60% of all SCID; defect in IL-2 receptor)
    - (b) Adenosine deaminase deficiency (an autosomal recessive SCID; accounts for ~20% of all SCID)
    - (c) Other mechanisms of SCIDs: Purine nucleoside phosphorylase deficiency, TCR immunodeficiency, MHC class I or II deficiency (Bare Lymphocyte Syndrome), Defective IL-2 production
- f. Primary phagocyte deficiencies (symptoms, description of defect, current therapy)
- (1) Neutropenia
  - (2) Chronic Granulomatous Disease
  - (3) Leukocyte Adhesion Deficiency
- g. Primary complement deficiencies (symptoms, description of defect, current therapy)
- (1) Deficiency of Complement Components

- (a) Classic pathway: C1, C4, C2, C3
  - (b) Alternative pathway: Factor D, Properdin
  - (c) MAC: C5, C6, C7, C8, C9
  - (e) Regulator proteins: Factors H, I, C1 inhibitor  
Hereditary Angioedema (C1INH deficiency)
- h. Secondary immunodeficiencies
- (1) Drug or radiation-induced (steroids, other cytotoxic drugs)
  - (2) AIDS (HIV target cells and immune dysfunction [see Virology for other aspects the viral infection])
  - (3) Nutritional deficiency (reduced protein, calorie, biotin, B<sub>12</sub>, Iron, Vit. A, Zinc; thymic atrophy pathologic result)
  - (4) Autoimmune Disease
  - (5) Other (postviral, chronic infection, neoplastic diseases)
25. Immunology of Pregnancy
- a. Mucosal immunity of the female genital tract
  - b. Trophoblast invasion (theories on why the fetus is not rejected as an allograft: reduced immunogenicity of fetal cells; local altered immunity [altered MHC expression, secretion of immunosuppressive factors such as  $\alpha$ -fetoprotein, IL-10, TGF $\beta$ ], inhibition by T-regulatory cells)
26. Immunity in the Elderly
- a. Effects of aging on the immune system; thymic atrophy