Pharmacrystal

Immunology

Immunoglobulins I, Structure and Function

I. Innate vs. Adaptive Immunity
- A. *Innate* immunity: quick response to general invariant microbe features
- B. *Adaptive* immunity: delayed response to diverse antigens, highly specific

II. Features of Adaptive Immunity
- A. B and T lymphocytes mediated
- B. B cells produce antibody, recognize Ag outside of cells
  - 1. Soluble antibody serves…
    - To neutralize viruses and toxins
    - To facilitate phagocytosis (opsonization)
    - To activate complement
  - 2. Membrane bound antibody
    - activates lymphocytes
    - mediates Ag uptake for presentation by T cells
- C. T cells have TCR, can recognized Ag expressed inside cells
- D. Clonal selection theory: random Ab diversity and clonal expansion
- E. Memory causes secondary response to be faster and more specific

III. Antibody Structure
- A. Heavy chains
  - 50 kDa
  - variable (V_H) and constant (C_H 1-3) regions
  - μ, δ, γ, α, ε chains corresponding to Ab class
- B. Light chains
  - 25 kDa
  - variable (V_L) and constant (C_L) regions
  - κ (major), λ chains
- C. Other
  - Ab are glycosylated at C_H2
  - Papain digestion yields F_{ab} and F_c fragments
  - Hybridomas provide monoclonal antibodies
  - IgG is flexible: hinge and elbow
  - Isotype vs. allotype vs. idiotype

IgG Monovalent
- Major Ab in serum
- Four heavy chain isotypes with different functions

IgM 5-valent, joined by J chain
- First to appear in immune response
- Hinge region replaced by C_H domain

IgA Monovalent to trivalent
- Extravascular secretion (glands, mucous membranes)

J chain plus secretory component (artifact of epithelial transport)

IgD Monovalent
Major surface bound Ab on mature B cells

IgE Monovalent
Responsible for immediate hypersensitivity
Hinge region replaced by C_H domain

V. Measurement of Ag-Ab interactions
   A. Immunogenicity determined by: foreignness, size, complexity, genetic factors, mode of Ag administration
   B. Antigens can have multiple determinants (epitopes)
   C. Scatchard plot of bound/free vs. bound gives K_A (slope) and valency (x-icept)
   D. Nonlinearity due to heterogeneous mixture of Ab
   E. ELISA, RIA, and competitive bindings measure Ag-Ab binding

Genetic Diversity

I. Structural basis of Ag binding
    A. Hypervariable regions are CDR1-3, corresponds to loops that bind Ag
    B. Binding pocket made of CDRs from both heavy and light chains, mostly hydrophobic interactions
    C. Ab's made of linked globular domains, each with the immunoglobulin fold

II. V(D)J recombination
    A. Genome structure:
        - κ (light): V-J joining, followed by single C exon
        - λ (light): V joins JC
        - Heavy: DJ joining then VDJ joining, C exon chosen by RNA splicing
    B. Gene rearrangements detectable by southern blot
    C. Molecular mechanism
        - RSS sequences (7/12/9 or 7/23/9 structure) are joined, 12-23 rule
        - RAG-1 and RAG-2 are transposases that cut RSSs
        - Recombination mediated by general DNA repair mechanism
        - DNA junction can have N region (TdT mediated) or P regions, adds diversity to CDR3

III. Class switching
    A. Class switching occurs without change in binding specificity
    B. IgM and IgD expressed together (differential polyadenylation and splicing)
    C. IgG, IgA, IgE expressed by switch recombination (loss of intervening genomic DNA)
    D. Switch recombination is stimulated by specific external stimuli

IV. Somatic hypermutation
    A. Accounts for variability at CDR1 and CDR2 and affinity maturation
    B. Requires presence of enhancer sequences

V. Membrane bound versus secreted
    A. Mediated by differential polyadenylation and splicing in the heavy chain
    B. Membrane bound has slightly longer heavy chain, for transmembrane segment

VI. Gene rearrangement diseases
    A. agammaglobluinemia: no antibody at all
    B. hyper IgM syndrome: no switch recombination
    C. scid mutation: prevents V(D)J recombination
    D. cancer: aberrant rearrangements, e.g. translocations

Functions of Antibody

Remember: Ab effector functions are mediated by the heavy chain constant region (Fc)!!!

I. Transport across mucous membranes

A. Mucosal surfaces (e.g. GI and respiratory tract) present special immunologic challenges

B. The mucosal immune system is essentially independent from systemic immune system

C. IgA is used:

1) Prevents attachment of pathogens to mucosa
2) Is a barrier to environmental antigens (hence prevents allergies)
3) Most abundantly secreted Ig (3g/day into intestines!)
4) Is a dimer held together by J chain

D. Secretory component

Produced by epithelia, acts as IgA receptor, transcytoses it

SC1 domain non-covalently interacts with IgA/J, SC5 covalently links to $C_{\alpha}2$

Prevents proteolysis of IgA by self and pathogens

E. *Across placenta*: IgG is actively transported across placenta to fetus via Fc receptors

II. Non-Inflammatory Activities

A. Review of influenza virus

HA and NA coat glycoproteins are responsible for attachment and infection

Viral strains can be distinguished by antigenically different HA and NA

B. Mechanisms of neutralization by Ab

1) Inhibit binding of virus to target cells (sterically block NA)
2) Inhibit virus from entering cell
3) Inhibit endocytic vessel fusion necessary for infection

III. Inflammatory Activities (4 items)

1) Opsonization

Mediated by IgG and neutrophils/macrophages

Ag binds bacteria; Fc receptors by Ab; particle is phagocytosed in zipper fashion

2) Complement

IgM or crosslinked IgG activate C1q, which starts complement cascade

Complement forms a porin in bacterial cell wall causing lysis

C3b enhances opsonization

3) Activation of mast cells

Mediated by IgE bound to Fc receptors on mast cells

Crosslinking IgE causes degranulation and phospholipase A_2 activation
Results in inflammatory response (e.g. against parasites or allergens)

4) Antibody-dependent cell-mediated cytotoxicity (ADCC)
NK cells have IgG Fc receptor; crosslinking activates cytotoxic killing
Applications in engineered antibodies

Phagocytic Cells, Innate Immunity, and Host Defense

Innate Immunity: Definition, Activation, Receptors

I. Innate versus Adaptive Immunity
Rehash from previous lectures

II. Activation of Innate Immune Response: PAMPs and PRRs
- General invariant features of pathogen surfaces are recognized (Pathogen Assoc. Molecular Patterns, PAMPs) by a limited set of fixed receptors (Pattern Recognition Receptors, PRRs)

III. Toll Like Receptors
- TLRs are PRRs, originally discovered as dToll and 18-Wheeler in Drosophila
- In humans, 9 types of TLRs recognize PAMPs and activate innate immune response

Cells of Innate Immunity: Differentiation, Activation, Migration

IV. There are 3 Effector Cells of the Innate Immune System
1) Neutrophils: short lived phagocytes, kill
2) Macrophages: long lived phagocytes, kill, involved in inflammation, remodeling
3) Natural Killer (NK): Kill viral infected cells that lack MHC I
- Metchnikoff first observed phagocytic cells in late 1800's

V. Differentation of Neutrophils and Macrophages
A) Pluripotent hematopoietic stem cell gives rise to: 1) neurons, 2) endothelium, 3) erythroids, 4) megakaryocytes, 5) myeloids, 6) lymphoids
B) Neutrophils
PHSC → myeloblast → promyelocyte → myelocyte → metamyelocyte → neutrophil
Two types of granules: primary/azurophil and secondary/specific
Signals (IL-1, TNF-α, G-CSF, GM-CSF) stimulate neutrophil production
Increased immature neutrophils in circulation is sign of infection/inflammation
C) Macrophages
Promonocytes → monocytes → macrophages

Signals (IL-3, M-CSF, GM-CSF) stimulate monocyte production
Monocytes in circulation enter tissues and differentation into macrophages

VI. Macrophage Activation

A) Macrophages may be activated through PRRs; activated cells phagocytose pathogens and actively kill them (e.g. ROS, NO)

B) Adaptive and innate immune system interact

- macrophages are APCs that can activate T cells
- interferons produced by T cells can activate macrophages
- together this raises level of macrophage activation to fight pathogen

VII. Neutrophil accumulation at sites of infection

1) Rolling adhesion: by E-, L-, P-selectins

2) Tight binding: mediated by LFA-1 and ICAM's; activation by cytokines increases levels of these mediators and promotes attachment

3) Diapedesis: migration through endothelial cells

4) Migration: via chemotaxis in response to C5a, N-fMet (a PAMP), chemokines, lipid-derived factors

- Macrophages arrive later

VIII. Phagocytosis, Especially As Mediated by FcγR on Neutrophils and Macrophages

- FcγR (three classes) on neutrophils and macrophages; FcεR on mast cells and basophils
- Receptors have varying affinity for IgG, a result of structural differences
- Phagocytosis involves Ab bound to leukocyte, which binds antigenic pathogen; or, direct binding of Ab-coated pathogen with displacement of Ab bound to leukocyte
- Attachment can be opsonin dependent or independent; internalization then happens through a zipper mechanism
- Signal transduction and effector function depends on differences in cytoplasmic portion of FcγR
- Activating FcγR are cytoplasmically associated with ITAM; inactivating are not
- Complement receptors also trigger phagocytosis in an activated state
- Fc activated phagocytosis leads to oxidative killing, but complement does not

IX. Intracellular Killing by Reactive Oxygen Species (ROS)

- NADPH oxidase, a special electron transport chain containing flavocytochromes, produces ROS
- Production of superoxide, hydrogen peroxide, and HOCl (via MPO) are toxic to bacteria
- The process in obviously aerobic; O2 consumption by neutrophils leaps
- Macrophages can also kill with NO (produced by NOS) when activated by IFN-γ or LPS

X. Cytokine production of macrophages

- Important cytokines produced by macrophages: IL-1, IL-6, TNF-α
- Results in acute response, e.g. inflammation, fever, activation of endothelial cells

Complement

I. Overall principles of complement
1) Two overall phases: enzymatic and lytic (pore forming)
2) Split products are formed during complement cascade, both products are biologically active
3) There are three ways to activate C', and all converge at C3

II. There are three ways to activate C'
1) Classical Pathway
Activated by C1 binding to IgM or IgG which are bound to Ag
Specifically, C1q portion binds two Fc regions, then C1r/C1s cleaves C4
C4b reacts fast, e.g. covalently binds bacterial cell surface whereas C4a remains soluble
C4b binds C2, C1s cleaves C2, results in C4b2a complex (the C3 convertase)
2) Mannose Binding Lectin
MBL is homologous to C1, but does not require antibody to activate
Like C1, MBL cleaves C2 and C4 to create the C3 convertase
3) Alternative Pathway
C3 can "tickover", i.e. spontaneously covalently bind surfaces
In appropriate environment, C3 complexes with B; B is cleaved by D
C3bBb complex recruits properdin (P); this is a C3 convertase

III. Further down the complement cascade
A) What C3 does
C3a is anaphylactic: causes mast cells and basophils to release histamine
C3b collects around the C3 convertase (ground zero) and opsonizes target (phagocytes have complement receptor, CR1)
Phagocytosis greatly enhanced by C3 and bound Ab
C3b when bound to either C3 convertase becomes a C5 convertase
B) What C5 does
C5a is anaphylactic and a chemoattractant for granulocytes
C5b recruits C6 and C7, which inserts into target cell membrane
C8 and lots of C9 join, forming membrane attack complex (MAC)
MAC is a membrane pore, causes lysis of target cell

IV. Regulation
A) C1
- C1-inhibitor is a suicide substrate for C1
B) Classical C3 convertase
- DAF and CR1 promote dissociation of C4b2a
- Factor I inactivates C4b by cleavage
C) Alternative C3 convertase
- Factor H binds C3b instead of factor B on cells with sialic acid

- DAF and CR1 also inhibits
- Factor I inactivates C3b by cleavage

- Note that Factor I cleavage products (iC3b, C3dg) are still biologically active

D) MAC
- CD59 and vitronectin blocks MAC formation

IV. Other
A) Complement components are highly homologous
B) Interactions between CD19-CR2 on B cells with C3dg can activate B cells
C) Some pathogens have evolved specific ways to inhibit complement