Introduction

Bacterial surface glycans as virulence factors

Mechanisms of carbohydrate in pathogen colonization and invasion

Glycans in viral infection

Development of glycan–based therapeutic strategies

Carbohydrate and Bacterial/Viral Infections

Infectious diseases remain a major cause of death, disability, and social and economic disorder

Multiple reasons contribute to the expanding impact of infectious diseases

Development of prevention and treatment strategies requires a well understanding the interaction of viral or bacterial pathogen with human

Glycans are major components of the outermost surface cells, including animal, plant cells, bacteria and viruses

The interactions of microbial pathogens with their hosts are influenced to an important degree by the pattern of glycans and glycan-binding receptors that each expresses-- all stages of infection: initial colonization, spreading, induction of immunological response and/or host-cell injury.
Virulence Factor: Polysaccharide Capsule

- **Virulence factor**: the microbial molecules responsible for diseases manifestation
- Glycan–receptor interactions play crucial roles in microbial pattern recognition as well as in the regulatory signals that govern the normal activities of immune cells. One important reason why certain microbes cause disease is that they have evolved to display their own sugars and receptors in a fashion that mimics or interferes with host glycan-based immune functions
- The disease-causing bacteria share in common is the presence of a **polysaccharide capsule** that covers the bacterial surface.
- Capsule expression by the bacteria poses a particular challenge to immune clearance.
  - Escape recognition and killing by phagocytes
  - Directly bind host cell regulatory protein factor H, attenuating immune response by hosts
  - Cloak protein structures on their surfaces, escaping antibody-mediated killing
  - Infants and elders generate poor antibody responses against bacterial polysaccharide capsules, therefore, are particular prone to invasive infection of the capsulated pathogen
**Virulence Factor: Polysaccharide Capsule**

- **Molecular Mimicry**: Strategy some microbial pathogens use to evade immune recognition by decorating themselves with molecules structurally similar to those of their hosts.
  - Molecular mimicry of common host glycan structures, masking as “self” to avoid immune recognition
    - *Group A Streptococcus* express hyaluronan capsule
    - *Neisseria Meningitidis* (meningococcus) express homopolymeric sialic acid capsule
      - group C meningococcal capsule: 2-9-linked sialic acid polymer/unique bacterial structure/ be a successful vaccine antigen in human
      - group B meningococcal capsule: 2-8-linked sialic acid polymer/found on human neural tissue/nonimmunogenic in human

**Polysaccharide Capsule Structure**

- Different strains of the same bacterial species produce polysaccharide capsules different in compositions and linkages of repeating sugar units.
- These structures are immunologically distinct, allowing classification of different capsule “serotype” strains.
  - *Meningococcus*: five major capsule serotypes (A, B, C, Y, and W-135)
  - *Haemophilus influenzae*: six serotypes (A–F)
  - *Streptococcus pneumoniae* (pneumococcus): > 90 serotypes
- Antibodies generated by the host against the capsule of one serotype strain typically do not provide cross-protective immunity-a strategy to present a moving target to the host immune system (keep escaping)
- Genetic exchange of capsule biosynthetic genes among serotype strains of an individual species - capsule switching *in vivo*, escaping from protective immunity.
Virulence Factor: Lipopolysaccharide (LPS)

- LPS = endotoxin
- Major component of the outer membrane of Gram-negative bacteria.
- LPS is a pathogen-associated molecular pattern (PAMP) that is recognized by the innate immune system and stimulates inflammatory responses to clear bacteria.
- LPS interacts with the opsonic receptor CD14 and the membrane protein Toll-like receptor 4 (TLR4) to initiate the immune signaling process.

Activation of immune signaling by LPS
**Virulence Factor: Lipopolysaccharide (LPS)**

- Dysregulated LPS-TLR4 interaction may lead to sepsis
- Gram-negative bacteria vary or modify their LPS to interfere with host immune defense mechanisms
  - Reduce overall negative charge to attenuate function of cationic host antimicrobial peptides (*Salmonella*: addition of 4-aminoarabinose to the phosphate group of the lipid A backbone; *Pseudomonas aeruginosa*: synthesize a unique hexa-acylated lipid A containing palmitate and 4-aminoarabinose)
  - Temperature-modulated LPS structure change (e.g. *Yersinia pestis*)

**Virulence Factor: Lipooligosaccharide (LOS)**

- Many mucosal pathogens such as *H. influenzae* and *Neisseria gonorrhoeae* lack true O-antigens; instead, they produce lipooligosaccharides (LOSs) that contain a recognizable core structure from which one or more monosaccharide or short oligosaccharide branches extend.
- LOS plays a pivotal role in infection
  - Structurally switch
  - Induce local inflammatory response
  - Resistance to bactericidal activity of serum
  - Molecular mimicry: gonococcal LOS and glycoprophingolipid antigens on human erythrocyte have structural similarity.
Carbohydrate in Pathogen Colonization & Invasion

- **Adhesin**: A protein on the surface of bacteria, viruses, or parasites that binds to a ligand present on the surface of a host cell.
  - most are lectins
  - Interact with cell surface glycoproteins, glycosphingolipids, or glycosaminoglycans
  - Alternatively interact with matrix or mucin
  - The interactions are mediated through terminal sugars or internal carbohydrate motifs

- **Adhesin receptor**: The specific carbohydrate ligands that present on host cell surface and mediate bacterial attachment on the host cell surface.
  - quite diverse in nature
  - The array of available adhesin-receptors determine the tropism of individual bacteria

- **Pili**: Hair-like appendages on the surface of some bacteria that often contain adhesins.
  - *E. coli*: P-blood group-related glycosphingolipids in the bladder epithelium
  - *Salmonella*: adherence to human intestinal cell mucosa

- **Fimbriae**: Proteinaceous fiber-like appendages found in many Gram-negative bacteria

- **Afimbrial adhesin**: a surface-anchored protein expressed by the bacteria.
  - Hemagglutinin: a lectin that recognizes carbohydrates on the surface of red blood cells and causes hemagglutination.
  - *Bordetella pertussis*: Filamentous hemagglutinin (FHA) - strong attachment of the bacteria to the ciliated epithelial cells of the bronchi and trachea, resulting “whooping cough”.
    - FHA is a component of pertussis vaccine.

- **Two step epithelial attachment**: carbohydrate of host is modified by pathogen and generates neostructure which mediates the pathogen adhesion.

Bacterial adherence to host-cell surfaces

a) Pili or Fimbriae

- Major subunit (pili)
- Tip adhesin
- Host glycolipid or glycoprotein

b) Afimbrial Adhesins

- Host extracellular matrix glycoprotein (e.g., fibronectin)
- Host cell-surface glycoprotein or glycan
- Host cell-surface integrin
Carbohydrates & Invasion Factors

- Glycan–lectin interactions play pivotal roles in enabling certain pathogens to penetrate or invade through epithelial barriers.
  - *S. enterica*: outer core oligosaccharide structure of the serovar Typhi LPS mediates the bacteria internalization in epithelial cells.
  - *Streptococcus pyogenes*: attaches to human pharyngeal and skin epithelial cells through specific recognition of its hyaluronan capsular polysaccharide by the hyaluronan-binding protein CD44, resulting cytoskeletal rearrangements and opening of intercellular junction.

Carbohydrates & Biofilm

- **Biofilm**: Community of bacteria that adheres to a moist surface (e.g., surface of ponds or teeth)
- **A mechanism** that promotes bacterial attachment to host surfaces, often in a form of a polymicrobial community.
- **Oral films**: *Streptococcus* species predominate (60–90%).
  - Dental plaque: dense, mushroom-like clumps of bacteria pop up from the surface of the tooth enamel, interspersed with bacteria-free channels filled with **extracellular polysaccharide (EPS)** produced by the bacteria.
- **EPS**: - Serve as diffusion channel—“quorum sensing”
  - Physical barrier for host immune system and antibiotics
  - Bind and inactivate antimicrobial peptides and antibiotics
  - Synthesized by biofilm bacteria vary in composition, and in chemical and physical properties.
    
    Majority of EPS types are polyanionic:
    Contributed by inorganic residues too.
    - serve as the primary carbon reserve for biofilm microorganisms during substrate deprivation
**Heparan Sulfate in Pathogen Infection**

- Heparan sulfate proteoglycan as attachment factors for host epithelia, such as *Chlamydia trachomatis* and *N. gonorrhoeae*

- Heparan sulfate proteoglycan shedding from host cell surface, such as *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The negatively charged heparan sulfate side chains of the shed ectodomain bind tightly to cationic molecules such as antimicrobial defensin peptides and lysozyme, neutralizing their antibacterial activities.
Carbohydrate & Viral Infection

- The specific binding of a virus particle to a target receptor on the host-cell surface is a prerequisite for viral infection.
- Most viral receptors are mapped to the glycan components of cell-surface glycoproteins, glycolipids, or proteoglycans.
- The virus–glycan interactions are responsible for species and tissue tropism.

Influenza Virus

- Common pathogens of the upper respiratory tract.
- Seasonal epidemics affect 10–20% of the general population.
- Can be deadly.
- Influenza virus subtype is named based on their surface glycoprotein - hemagglutinin (H) and neuraminidase (N).
  - H1N1 - 1930s
  - H2N2 - 1958
  - H3N2 - 1968 - recent years
- Typically, human and avian influenza viruses are different and are not infectious for both species.
Carbohydrate & Influenza Virus

- Interactions between influenza virus hemagglutinin and sialic acid determine the tissue and species tropism of the virus.
  - Human influenza viruses: terminal sialic acids containing 2-6 linkages
  - Bird influenza viruses: terminal sialic acids containing 2-3 linkages
  - Pig: 2-3- and 2-6-linked sialic acids occur in the trachea of swine

- The cell-surface receptor(s) for influenza viruses is widely considered to be sialic acid linked to either glycoprotein or glycosphingolipid.

- Influenza virus enters cells may also depend on protein–glycan interactions
  - Host mannose receptors of macrophages

- The final release of influenza virus particles from an infected cell surface relies on the action of the viral neuraminidase

- Neuraminidase may also assist pathogen in evading host mucosa

Mechanisms of viral entry into host cells

a) Influenza Viruses

- Neuraminidase (NA)
- Hemagglutinin (HA)

HA promotes binding & entry

Sialic acid

NA allows budding & release
Herpes Simplex Virus (HSV)

- HSV1 & HSV2.
- Latent, recurrent infections
- HSV1 - recurrent cold sores of the mouth and lips
- HSV2 - sexually transmitted genital ulcer disease
- HSV infection is mediated by a family of ten viral envelope glycoproteins.
  - Initial attachment: HSV glycoproteins B (gB) and C (gC) interact with heparan sulfate
  - High-affinity attachment: glycoprotein D (gD) interacts with a member of the tumor necrosis factor-nerve growth factor receptor family

Mechanisms of viral entry into host cells
Human Immunodeficiency Virus (HIV)

- A retrovirus.
- The etiologic agent of the acquired immunodeficiency syndrome (AIDS)
- The outer envelope glycoprotein gp120 is responsible for the cellular tropism of the virus
- The transmembrane glycoprotein gp41 catalyzes fusion of HIV to the target cell’s membrane
- The very heavy N-glycosylation of gp120/41 blocks immune response
- Heparan sulfate proteoglycans protect HIV virion degradation in blood stream, function as an attachment receptor for macrophage entry, and transmit the viral particle in trans to CD4+ T cells.

Mechanisms of viral entry into host cells
Therapeutic Strategies Based on Microbial Protein-glycan Interaction

- Influenza: Neuraminidase - sialic acid analogs (Zanamivir and Oseltamivir)
- HIV: gp120 - high-mannose-type N-glycans attached (inhibitor of N-glycosidation: N-buty-deoxyno-jirimycin).
- Cyanovirin-N: bind to the unusual oligomannose-type N-glycans presents on gp120. Also against herpesviruses.
- Heparin and other polysulfated molecules.
- Carrageena: a polysulfated glycosaminoglycan-like extract from red seaweed
- Lectins and other carbohydrate-binding agents
- Anti-adhesion therapies: E. coli adhesion in urinary tract - ! - methylmannoside or free mannose

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