

Innate Defense Against Infection
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I. Innate versus adaptive immune response

- A. The host has two inter-related systems, the “innate” and “adaptive” immune response that cooperate in the eradication or control of an infection with a pathogen. The innate immune response occurs within hours, whereas the adaptive immune response occurs within days following an infection. These two responses have very distinct characteristics:

<u>Characteristics</u>	<u>Innate</u>	<u>Adaptive</u>
Specificity	For structures shared by groups of related microbes	For antigens of microbes and non-microbial antigens
Diversity	<u>Limited</u>	Very large
Memory	None	Yes
Non-reactivity to self	Yes	Yes
<u>Components</u>		
Physical/chemical barriers	Skin, mucosal epithelia, anti-microbial chemicals	Lymphocytes in epithelia, antibodies at epithelial surface
Blood proteins	Complement	Antibodies
Cells	Phagocytes (macrophage, neutrophil), natural killer cells	Lymphocytes (B and T cells)

- B. The “quality” of the initial innate immune response can influence the “quality” of the subsequent adaptive immune response-e.g. early release of IL-12 by dendritic cells can induce subsequent CD4+ T helper type 1 (IFN- γ)-dominated adaptive response.

Products of the adaptive response can synergize with, and thus augment the function of, components of the innate response-e.g. antibody-dependent complement activation and T cell-derived, IFN- γ -mediated activation of macrophages.

- C. Within the innate immune system itself there are two major subdivisions: **constitutive** barriers to infection, and processes that are **induced** once the pathogen has invaded.

II. Constitutive Innate Barriers to Infection

A. Lung

1. Normal lung is free from bacteria
2. Particles >10 μ M-nose upper airways; 3-10 μ M-trachea and bronchi; 1-5 μ M (size of most bacteria)-terminal airways and alveoli; <1 μ M-suspended and exhaled.
3. nasal clearance-non-ciliated, remove by sneezing and blowing.
4. tracheobronchial clearance-swept to nasopharynx by mucociliary escalator and swallowed or expectorated.
5. alveolar clearance-phagocytosis by macrophages; macrophage propelled to oropharynx and swallowed or move through interstitium to re-enter bronchiole or go to lymphatics; if macrophages overwhelmed-particles go to lymph node and/or bloodstream directly.

6. natural IgA-blocking pathogen entry into epithelia, antibody-dependent cellular cytotoxicity (ADCC).
7. bronchial secretions-lysozyme, NAMLAA (N-acetyl muramyl-L-alanine amidase), β -defensin (e.g. hBD-1, hBD-2), macrophages (with opsonization by collectins [e.g. SP-A, SP-D-gram-negative bacteria, fungi, and *Pneumocystis carinii*]), lactoferrin, nitric oxide; mucins have no direct anti-microbial effect but act as barrier and segregator of particles (mucins are upregulated by TNF- α).

B. Gastrointestinal Tract

1. acid pH of stomach (lethal for some enteric pathogens [e.g. *Vibrio cholerae*], but not others [e.g. *Shigella* and *Giardia*]), pancreatic enzymes, bile (chemical destruction of pathogens); Paneth cells of the small intestine secrete defensins (hD-5 and hD-6), lysozyme, and type II phospholipase A, peristalsis, turnover of epithelial cells, normal bowel flora.
2. Microflora-1) competition for nutrients, 2) competition for same host cell receptors, 3) bacteriocins (antibiotics), 4) volatile fatty acids or other metabolites toxic to competing microbes, 5) immune system stimulation with class II expression on APCs, 6) natural antibodies; two types of microflora-regular residents and transient colonizers.

C. Skin

1. prevent microbial penetration
2. dry
3. mildly acidic (pH 5-6)
4. normal skin flora
5. desquamation.
6. Skin infections in normal persons-breaks in skin or burns (respiratory, GI, or GU require virulent organisms).

D. Genitourinary

1. urine is sterile (e.g. Tamm-Horsfall protein made in large amounts by kidneys avidly bind to certain bacteria), flushing of urine, length of male urethra (urinary infections 14x more common in females than males), hypotonic state of kidney medulla (hyperglycemia leads to pyelonephritis),
2. vagina-estrogen stimulates vaginal cells to produce glycogen; gram-positive rods (esp. lactobacilli) break down glycogen into lactic acid.

E. Mucous membrane

1. moist environment ordinarily would support growth of microorganisms.
2. secretions like cervical mucous, tears, and prostatic fluid contain lysozyme and NAMLAA-both hydrolyze amino acid backbone of peptidoglycan of gram positives
3. defensins
4. natural Ig (agglutination and/or blocking of host cell receptor attachment)
5. iron-binding proteins (deprive microbes of needed iron).
6. Eye-flushing by tears which contain lysozyme (breaks down peptidoglycan) and other anti-microbials.

F. Circulating natural antibodies

1. some induced by colonizers in oropharynx, gut, and other sites and some arise independent of antigen stimulation
2. mostly IgM, low affinity, non-mutated, cross-reactive
3. Fc and complement-mediated antigen localization in lymphoid organs and opsonization by phagocytic cells.

III. Induction of Innate Defense

A. Toll-like receptors (TLRs)

TLRs are expressed by multiple immune cell types, are specific for conserved pathogen structures, and mediate signals that initiate the innate immune response.

1. TLRs 1-10-different TLRs involved in recognizing distinct, conserved microbial structures, as well some host products:
 - a. TLR2-lipoproteins, lipoteichoic acid, peptidoglycan, lipoarabinomannan (*M. tuberculosis*), LPS (*Leptospira*), LPS (*P. gingivalis*), zymosan (yeast), GPI anchor (*T. cruzi*).
 - b. TLR4-LPS (from most Gram-negative bacteria), taxol (plant), F protein (respiratory syncytial virus [RSV]), hsp60 (host), fibronectin (host); fibrinogen (host)
 - c. TLR5-flagellin
 - d. TLR9-bacterial DNA
2. cytoplasmic domain homologous with IL-1R (called Toll-ILR or TIR domain) binds to adaptor protein called MyD88 which links to signaling cascade resulting in NF- κ B and AP-1 (jun/fos) translocation to the nucleus with resultant gene activation (i.e. genes involved in inflammation and early immunity).
3. expressed by multiple cells involved in the innate response (e.g. macrophages and dendritic cells) and mediates initial cytokine/chemokine response to pathogen.
4. necrotic, but not apoptotic, cells release heat shock protein which can trigger inflammatory response through TLR4.

B. Acute Phase Proteins

These proteins both participate in the elimination of the pathogen and also appear in part to counteract the deleterious effects of the pro-inflammatory cytokines that induce them.

1. Definition of acute phase response-plasma concentration increase or decrease by at least 25% during inflammatory disorders; eg 50% for ceruloplasmin; 1000-fold for C-reactive protein and serum amyloid A.
2. discordance in plasma concentrations indicate individual regulation of different proteins during different disease states.
3. regulated by cytokines (e.g. IL-1 β , IL-6, TNF- α , IFN- γ , TGF- β , ?IL-8); mostly from macrophages and monocytes at inflammatory sites; IL-6 stimulate most, whereas others stimulate subgroups of acute phase proteins; cytokines interact with each other in additive, inhibitory, or synergistic ways depending upon the acute phase protein in question.
4. Function of acute phase proteins
 - a. C-reactive protein-act as opsonin-bind to phosphocholine on pathogens and to phospholipid constituents of damaged cells; activate complement (classical pathway) and also directly bind to phagocytic cells; net effect in CRP-transgenic mice is anti-inflammatory.
 - b. haptoglobin-protect against reactive oxygen species; aids in wound repair by stimulating angiogenesis.
 - c. α 1-antichymotrypsin-inhibit proteolytic enzyme; inhibit generation of superoxide ion.
 - d. fibrinogen-promote tissue repair (stimulate endothelial cell adhesion, spreading, and proliferation).
 - e. transthyretin-*decreases*; normally inhibits IL-1 but if it goes down may then be pro-inflammatory.

- f. Lipoproteins-bind to and inhibit LPS
 - g. Tissue inhibitor of metalloproteinase-1.
 - h. Heme oxygenase and manganese superoxide dismutase limit oxidant-mediated tissue injury
 - i. lipids-nutrients for host cells and regeneration of damaged membranes.
5. Deleterious effects of acute phase proteins-anemia, impaired growth, septic shock, cachexia, secondary amyloidosis (serum amyloid A).

C. Complement

The complement system comprises a series of interacting soluble proteins, whose synthesis is upregulated during infection. Infection with pathogen leads to proteolytic processing of these proteins (complement activation) that generates various components that play multiple roles in host defense.

1. induced as part of the acute phase response.
2. Three pathways converge at point of cleavage of C3
 - a. **classical**-antibody bound to antigen on surface of pathogen, also C-reactive protein
 - b. **mannose-binding lectin pathway**-lectin (member of collectin family; i.e. collagenous lectin), which is associated with the serine proteases MASP1 and MASP2, binds to arrays of terminal mannose groups on the surface of a variety of bacteria, and mediates complement cleavage
 - c. **alternative**-covalent binding of small amount of C3b to hydroxyl groups on pathogen cell surface carbohydrates and proteins (low-grade cleavage of C3 in plasma).
3. Regulatory mechanisms-complement fragments focused on pathogens, but limited on normal cells.
4. Pyogenic infections
 - a. opsonic activity (C3b, iC3b)-operates for most bacteria,
 - b. lytic activity (membrane attack complex)-Neisseria meningitides,
 - c. mannose-binding lectin (opsonic)
5. Bacteria and viruses have a variety of strategies to use the complement system to their advantage (e.g. EBV virus gp350 binds to complement receptor type 2 on B cells for cell entry).
6. Patients with various complement system deficiencies have increased susceptibility to infections.
7. Natural IgM-activate classical complement pathway during an innate response.
8. C5a and C3a-anaphylotoxins (increased vascular permeability).
9. C5a-chemotaxis and leukocyte activation.
10. Complement binds to apoptotic cells and promotes their clearance [note complement deficiency and SLE] (also clears immune complexes, including antibody-coated bacteria).
11. Tissue ischemia and reperfusion also activate complement through exposure of phospholipids and mitochondrial proteins (e.g. activation of mannose-binding lectin); complement may promote necrosis in myocardial infarction and stroke.

D. Defensins

1. cationic peptides; kill gram-positive and negative bacteria, fungi, mycobacteria, and viruses; highly abundant in phagocytic cells; oxygen-independent killing; also in epithelial cells for luminal defense
2. production by epithelial cells is upregulated by cytokines and microbial activators (e.g. IL-1 β , TNF- α , IFN- γ , muramyl dipeptide, LTA);

3. β -defensins (HBD-1 and HBD-2) chemotactic for DCs and memory T cells (CCR6-mediated)-potential to connect innate with adaptive immunity.

E. Nitric Oxide (NO)

1. iNOS (inducible nitric oxide synthase) and NO synthesis by macrophages in response to pathogen, IFN- γ , and/or IFN- α/β .
2. NO mediates direct intracellular killing in macrophages
3. NO required for NK cell production of IFN- γ and cytotoxicity in response to IL-12 or IFN- α/β through activation of Tyk2 kinase (JAK-STAT pathway)
4. IFN- γ downregulates TGF- β production in macrophages through NO; NOS2-/- mice enhanced expression of TGF- β (TGF- β promotes development of DCs but inhibits a number of macrophage functions).

F. Collectins

1. C-type lectins; interact with wide range of microorganisms: bacteria, viruses, fungi, mycobacteria; also interact with various pollens and dust-mite allergen.
2. Opsonic-agglutination and bridging between pathogen carbohydrate and collectin receptors on phagocytes
3. Chemotactic for neutrophils and macrophages
4. promote macrophage synthesis of TNF- α , other cytokines, NO.
5. Surfactant-associated proteins (SP-A, SP-D) important in lung; secreted by lung epithelial cells; production rapidly increased in response to LPS.

G. Phagocytosis (mechanisms to enhance clearance by macrophages)

1. Scavenger receptor family-SRAI, SRAII, MACRO, CD36, CD68, CLA-1-detect phospholipid structures on microbes.
2. Mannose receptor-detect mannose-containing structures on microbes
3. Integrins (CD11b/CD18, CD11c/CD18, α_v/β_1 , α_v/β_3)-bind to complement components, vitronectin, and fibronectin which then bind to damaged or altered cells, collagen debris, or microbes.
4. C1qR on macrophages-recognize collectins (mannose-binding protein, C1q, and SP-A) which can bind to microbes.
5. CD14/LPS-binding protein/TLR4
6. resting macrophages versus induction of neutrophils and activation of macrophages (resting macrophages are less efficient killers); resting macrophage kills ingested microbes by: toxic oxygen metabolites (hydrogen peroxide and superoxide), acidification of phagosomes, acid hydrolase (and other lysosomal enzymes) in phagosome, production of granule-associated anti-microbial molecules.

IV. Infection

In light of the elaborate systems in place to protect the host from infectious disease, the host may still suffer morbidity and mortality in response to particular infectious agents. Many times this relates to the intrinsic virulence of the organism. Other times, it relates to the quality or behavior of the host. The following are examples of the latter, host-related factors:

A. Decrease in resistance of host

1. chronic disease
2. immunodeficiency
3. immunosuppressive agents
4. leukopenia
5. unusually virulent infection.

B. Impaired defense mechanisms

1. loss or suppression of cough reflex (may also lead to aspiration of gastric contents)-coma, anesthesia, neuromuscular disorders, drugs, chest pain.
2. injury to mucociliary escalator-tobacco, hot or corrosive gases, viruses (e.g. influenza), genetic (e.g. immotile cilia syndrome).
3. macrophage dysfunction-alcohol, tobacco, anoxia, or oxygen intoxication.
4. pulmonary congestion and edema.
5. accumulation of secretions-e.g. cystic fibrosis, bronchial obstruction.

C. Heritable/Congenital differences in response to infecting agents

1. Pathogen binding to host cell receptors- E.g.-Plasmodium vivax (malaria)- infects RBCs using Duffy blood group determinants; not expressed in blacks, hence P. vivax absent from much of Africa; CCR5 and HIV-1.
2. Immunologic response-E.g.-Coccidioides immitis (fungus)-Caucasians control by cell-mediated immunity (CMI); 14x more common in blacks and 175x more frequent in Filipinos due to poor CMI response to this particular pathogen.
3. Congenital complement deficiency-C5, C6, C7, C8-prevent formation of membrane attack complex (e.g. disseminated Neisseria).

D. Effect of behavior on infection

1. sexually transmitted diseases
2. occupation-brucellosis and Q fever-contact with infected farm animals or their secretions; schistosomiasis-farmers who work in fields irrigated by infected water (also swimmers).
3. people who walk barefoot-hookworm and Strongyloides stercoralis-humid soil-penetrate skin.
4. improperly cooked or canned food-anisakiasis and diphyllbothriasis (helminths)-poorly cooked fish; toxoplasma-poorly cooked meat (or exposure to cat feces); botulism (Clostridial toxin) from improperly canned food.
5. hyperabsorbent tampons-toxic shock syndrome (Staphylococcus aureus).
6. aerosols from cooling plants, faucets, and humidifiers-Legionnaires disease