What you need to know about innate immunity

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Innate Immunity

- First line of defense, immediate defense
  - Day to day protection
  - Only when innate defense bypassed, evaded or overwhelmed is adaptive immunity required

- Non-specific

- Recognize pathogens in a generic way

- Does not confer long lasting or protective immunity to host

- Evolutionarily older, found in primitive organisms
<table>
<thead>
<tr>
<th>Receptor characteristic</th>
<th>Innate immunity</th>
<th>Adaptive immunity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specificity inherited in the genome</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Expressed by all cells of a particular type (eg, macrophages)</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Triggers immediate response</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Recognizes broad classes of pathogen</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Interacts with a range of molecular structures of a given type</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Encoded in multiple gene segments</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Requires gene rearrangement</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Clonal distribution</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Able to discriminate between even closely related molecular structures</td>
<td>No</td>
<td>Yes</td>
</tr>
</tbody>
</table>
Innate Immunity and Inflammation

1) Respond rapidly to tissue damage
   - physical and chemical barrier
   - recruitment of immune cells to site of injury

2) Limit spread of infection
   - identification and removal of foreign substances
   - activation of the complement cascade
   - activation of coagulation cascade

3) Initiate adaptive immune response
   - antigen presentation and cytokine production

4) Initiate tissue repair
# Innate Immunity – Physical/Chemical Barriers

<table>
<thead>
<tr>
<th></th>
<th>Skin</th>
<th>Gut</th>
<th>Lungs</th>
<th>Eyes/nose</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mechanical</strong></td>
<td>Epithelial cells joined by tight junctions</td>
<td>Longitudinal flow of air or fluid</td>
<td>Movement of mucus by cilia</td>
<td></td>
</tr>
<tr>
<td><strong>Chemical</strong></td>
<td>Fatty acids</td>
<td>Low pH</td>
<td>Enzymes (pepsin)</td>
<td>Salivary enzymes (lysozyme)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Antibacterial peptides</td>
<td></td>
</tr>
<tr>
<td><strong>Microbiological</strong></td>
<td>Normal flora</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Figure 2-4 part 2 of 2 Immunobiology, 6/e. (© Garland Science 2005)*
Breach of physical barrier →
-“resting” innate immune cells become activated to kill microbes, secrete cytokines to recruit and activate additional leukocytes, and to promote systemic killing and removal of microbes.
Innate Immunity - 1st responders

Multipotential hematopoietic stem cell (Hemocytoblast)

Common myeloid progenitor
- Erythrocyte
- Mast cell
- Myeloblast
- Megakaryocyte
- Basophil
- Neutrophil
- Eosinophil
- Thrombocytes
- Monocyte
- Macrophage

Common lymphoid progenitor
- Natural killer cell (Large granular lymphocyte)
- Small lymphocyte
  - T lymphocyte
  - B lymphocyte
- Plasma cell
Innate Immunity - Monocytes/Macrophages

- Monocyte-derived macrophages “large eaters” or histiocytes, are present in all tissues
- “Sentinels” of immune system - survey for “foreign” invaders
- Foreign microbes are recognized via various cell surface and intracellular receptors
- Receptor ligation and cytokines causes macrophage activation
- Activated macrophages
  - Digest and present antigens from microbes
  - Produce chemokines, cytokines, other molecules to recruit other immune cells
Macrophage Phagocytosis

Microbe killing = Phagosome + lysosome = phagolysosome

- Nitric oxide (nitric oxide synthase, iNOS2)
- Superoxide anion (NADPH oxidase, respiratory burst)
- Hydrogen peroxide (superoxide dismutase)

-Macrophages kill internalized microbes via reactive oxygen and nitrogen species
Phagocyte Toll receptors are stimulated by Pathogen Associated Molecular Patterns (PAMPS)
NOD-like receptor (NLR) proteins are intracellular pattern recognition receptors (PRRs)
Induced Innate Responses mediated by cytokines secreted by stimulated sentinel cells
Chemokines secreted by stimulated sentinel cells recruit additional immune cells

<table>
<thead>
<tr>
<th>Class</th>
<th>Chemokine</th>
<th>Produced by</th>
<th>Receptors</th>
<th>Cells attracted</th>
<th>Major effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>CXC</td>
<td>CXCL8 (IL-8)</td>
<td>Monocytes, Macrophages, Fibroblasts, Keratinocytes, Endothelial cells</td>
<td>CXCR1, CXCR2</td>
<td>Neutrophils, Naive T cells</td>
<td>Mobilizes, activates and degranulates neutrophils. Angiogenesis.</td>
</tr>
<tr>
<td></td>
<td>CXCL7 (PBP, β-TG, NAP-2)</td>
<td>Platelets</td>
<td>CXCR2</td>
<td>Neutrophils</td>
<td>Activates neutrophils. Clot resorption. Angiogenesis</td>
</tr>
<tr>
<td></td>
<td>CXCL10 (IP-10)</td>
<td>Keratinocytes, Monocytes, T cells, Fibroblasts, Endothelium</td>
<td>CXCR3</td>
<td>Resting T cells, NK cells, Monocytes</td>
<td>Immunosuppressive. Anti-angiogenic. Promotes T, H1 immunity.</td>
</tr>
<tr>
<td></td>
<td>CXCL12 (SDF-1)</td>
<td>Stromal cells</td>
<td>CXCR4</td>
<td>Naive T cells, Progenitor (CD34+) B cells</td>
<td>B-cell development. Lymphocyte homing.</td>
</tr>
<tr>
<td></td>
<td>CXCL13 (BLC)</td>
<td>Stromal cells</td>
<td>CXCR5</td>
<td>B cells</td>
<td>Lymphocyte homing.</td>
</tr>
</tbody>
</table>

**CC**

- **CCL3 (MIP-1α)**
  - Produced by: Monocytes, Macrophages, Fibroblasts, Keratinocytes, Endothelium
  - Receptors: CCR1, CCR3, CCR5
  - Cells attracted: T cells, Mast cells, Fibroblasts, Basophils, Dendritic cells
  - Major effects: Competes with HIV-1. Antiviral defense. Promotes T,H1 immunity.

- **CCL4 (MIP-1β)**
  - Produced by: Monocytes, Macrophages, Fibroblasts, Keratinocytes, Endothelium
  - Receptors: CCR1, CCR3, CCR5
  - Cells attracted: Monocytes, NK and T cells, Dendritic cells
  - Major effects: Competes with HIV-1.

- **CCL2 (MCP-1)**
  - Produced by: Monocytes, Macrophages, Fibroblasts, Keratinocytes
  - Receptors: CCR1, CCR2B
  - Cells attracted: Monocytes, NK and T cells, Basophils, Dendritic cells

- **CCL5 (RANTES)**
  - Produced by: Monocytes, Macrophages, Epithelial cells, T cells
  - Receptors: CCR1, CCR3, CCR5
  - Cells attracted: T cells, Endothelial cells, Platelets
  - Major effects: Monocytes, NK and T cells, Basophils, Eosinophils, Dendritic cells
  - Chronic inflammation.

- **CCL11 (Eotaxin)**
  - Produced by: Endothelium, Monocytes, Epithelial cells
  - Receptors: CCR3
  - Cells attracted: Eosinophils, Monocytes, T cells
  - Major effects: Role in allergy.

- **CCL18 (DCCK)**
  - Produced by: Dendritic cells
  - Receptors: CCR2
  - Cells attracted: Naive T cells
  - Major effects: Role in activating naive T cells.

**C**

- **XCL1 (Lymphoattractin)**
  - Produced by: CD8+CD4+ T cells
  - Receptors: CXCR1
  - Cells attracted: Thymocytes, Dendritic cells, NK cells
  - Major effects: Lymphocyte trafficking and development.

- **CXXC (CX3C)**
  - Produced by: Monocytes, Endothelium, Microglial cells
  - Receptors: CX3CR1
  - Cells attracted: Monocytes, T cells

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(Figure 2-41 part 1 of 2 Immunobiology, 6/e, ©Garland Science 2005)
Innate Immunity-Macrophage

Systemic Inflammatory Response

- Toll-like receptors
  - Macrophage mannose receptor
  - IL-8
  - TNFα

- Pattern Recognition Receptors
  - NLRs
    - Macrophage mannose receptor
    - Scavenger receptors

- Macrophage chemokines/cytokines
  - Co-stimulatory molecules
    - CD80/86

- MBL

Complement Cascade

- TNFα
- IL-6
- IL-1

Recruitment of other cells

Phagocytosis (clearance of microbes)

- Neutrophils
Cytokine mediated vascular dilation and vascular permeability facilitate neutrophil extravasation into infected tissues

-Macrophage activation causes degradation of membrane phospholipids and rapid production of prostaglandins, leukotrienes and platelet-activating factor which act with cytokines directly on smooth muscle and endothelial cells
Cytokine induced adhesion → cytokines and chemokines mediate neutrophil weak and firm adhesion to vascular endothelium
Innate Immunity - 1st responders

Multipotent hematopoietic stem cell (Hemocytoblast)

- Common myeloid progenitor
  - Erythrocyte
  - Mast cell
  - Myeloblast
  - Megakaryocyte
  - Thrombocytes
  - Basophil
  - Neutrophil
  - Eosinophil
  - Monocyte
  - Macrophage

- Common lymphoid progenitor
  - Natural killer cell (Large granular lymphocyte)
  - Small lymphocyte
    - T lymphocyte
    - B lymphocyte
  - Plasma cell
Neutrophils

- Essential to innate immunity, hallmark of acute inflammation
- Most prevalent WBC in blood with 50-100 billion produced per day
  - 55% bone marrow weight dedicated
- Migrate in response to IL-8, C5a, leukotrienes, fMLP via chemotaxis
- Circulate 5.4 days, live in tissue 1-2 days
  - Limit propagation of certain pathogens
  - Limit host damage due to inflammation
  - Phagocytosed by macrophages after pathogen digestion
Neutrophil NET formation

Neutrophil activation

- LPS
- IL-8
- PMA
- IFNα/γ + C5a
- GM-CSF + C5a
- TLR-4
- platelet

Microbial Pathogens

NADPH oxidases

- NADPH + 2 O₂ → NADP⁺ + H⁺ + 2O₂⁻ → H₂O₂

“ETosis” cell death pathway

PAD4-mediated citrullination of histones promotes chromatin decondensation

Microbial entrapment

- Microbial killing
- Proinflammatory effects (e.g., contact system activation)

Extracellular trap formation

Granule Proteases

Antimicrobial Peptides

Histones
Innate Immunity-Macrophage

Systemic Inflammatory Response

- Toll-like receptors
- NLRs
- Macrophage mannose receptor
- Scavenger receptors
- Macrophage chemokines/cytokines
- Co-stimulatory molecules
  - CD80/86

Phagocytosis (clearance of microbes)

- Neutrophils

Recruitment of other cells

- IL-8
- TNFα
- IL-6
- IL-1

Complement Cascade

- MBL

Pattern Recognition Receptors

- Toll-like receptors
- NLRs
Cytokines from PAMP-stimulated sentinel cells stimulate the production of acute phase proteins, which opsonize a large spectrum of pathogens bearing common pathogen associated molecular patterns.
There are two innate mechanisms by which complement can be activated: the lectin pathway and the alternative pathway.
Complement feeds forward to activate and increase macrophage and neutrophil phagocytosis.

**Diagram Description:**
- **Bacterium is coated with complement by the alternative and MBL pathways.**
- **When only C3b binds to CR1, bacteria are not phagocytosed.**
- **C5a can activate macrophages to phagocytose via CR1.**

*Figure 2-32 Immunobiology, 6/e. (© Garland Science 2005)*
Complement can form the membrane attack complex, which leads to the lysis of target pathogens.
The spread of viruses is limited by the interferon response.
Viral nucleic acids stimulate the production of interferon by the infected cell, which mediates both autocrine and paracrine protective responses.
**NK cells**

- Overlap innate and adaptive immunity
- Stimulated by Type I interferons
- Kill cells with down-regulated MHC I expression
  - Down-regulated by viruses and tumors trying to avoid CD8+ T-cell killing
- Kill “non-self” via mostly shared mechanisms with cytotoxic T-cells
  - TRAIL
  - GranzymeB
  - Perforin

**MHC class I on normal cells is recognized by killer cell immunoglobulin-like receptors (KIRs) or by lectin-like CD94:NKG2**

**NK cell does not kill the normal cell**

**‘Altered’ or absent MHC class I cannot stimulate a negative signal. The NK cell is triggered by signals from activating**

**Activated NK cell releases granule contents, inducing apoptosis in target cell**
1) Respond rapidly to tissue damage
   - physical and chemical barrier
   - recruitment of immune cells to site of injury
2) Limit spread of infection
   - identification and removal of foreign substances
   - activation of the complement cascade
   - activation of coagulation cascade
3) Initiate adaptive immune response
   - antigen presentation and cytokine production
4) Initiate tissue repair
Adaptive response initiation: Antigen presentation to T cells
The context in which macrophage-derived dendritic cells present antigen to T-cells determines the type of adaptive T cell response that follows.
Hallmarks of Cancer: The Next Generation

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DOI 10.1016/j.cell.2011.02.013

An Emerging Hallmark: Evading Immune Destruction

An Enabling Characteristic: Tumor-Promoting Inflammation
Immune surveillance in cancer

- Carcinogen-induced tumors arise more frequently and quickly in immunodeficient mice
- Cancer cells that arise in immunodeficient mice are inefficient at initiating secondary tumors in syngeneic immunocompetent mice
Immune surveillance in cancer

- Heavy CTL and NK cell infiltration predicts improved outcome in several human tumors
- Immunosuppressed organ transplant recipients develop donor-derived cancer from ostensibly tumor-free donors
Immune escape in cancer

- Clinically apparent tumors variably suppress the anti-tumor immune response
- Tumor-associated inflammation can enhance tumor progression
Immunoediting

Normal Cells

Transformed Cells

1) Elimination/Immunosurveillance

- IFN-γ and IFN-α/β
- Perforin
- Trail
- Th1 and Tc T cells, NKT, NK, γδ T cells

2) Equilibrium/Tumor Persistence

- Genetic instability and immune selection
- Tumor variants
- Immune exhaustion/inhibition

3) Escape/Tumor Progression

- T regulatory cells – IL-10 and TGF-β
- Tumor-derived cytokines, growth factors, chemokines
- Myeloid-derived suppressor cells (MDSC)

Protection

• Inflammation/radiation/carcinogens/viruses

• Tumor antigens/peptide-MHC I/ MHCII cross-presentation/NKG2D

Chronic Inflammation has a known role in cancer initiation

- Tobacco, asbestos → Bronchial CA
- Alcohol → Hepatocellular CA, Gastric CA, Pancreatic CA
- *Helicobacter pylori* → MALT lymphoma
- Shistosoma → bladder CA
- HCV → Hepatocellular CA
- HPV → Cervical CA
- Endogenous inflammation in inflammatory bowel disease → Colon CA
- Barrett's esophagus → Esophageal CA
-Scale largely tipped to pro-tumor effect of macrophages in established cancers
Macrophage and chemotherapy

- TAM modulate responses to chemotherapy
- Macrophages and DCs are known to mediate “immunogenic cell death” (ICD) which some chemotherapies induce in some tumor models
  - release of “eat-me” signals (e.g. ATP and high-mobility group B1 [HMGB1]) from dying tumor cells enhanced by some chemotherapies
  - monocyte activation and enhancement of their APC capacity and promotion of T cell responses against immunogenic tumors
- Antitumor activity of some cytotoxic agents may depend on their ability to reprogram pro-tumoral macrophages
Macrophage and chemotherapy

- TAM depletion (anti-CSF1 antibodies) enhances the efficacy of some combination chemotherapy
  - cyclophosphamide, methotrexate, and 5-fluorouracil in chemoresistant, human breast cancer xenografts in immunodeficient mice
  - Paclitaxel in immunocompetent mice via increased anti-tumor CD8+ T-cell responses when macrophages were depleted

- Macrophage-derived cathepsins protect cancer cells from the direct cytotoxic effects of several chemotherapeutics
  - CathepsinB $\rightarrow$ inflammasome $\rightarrow$ IL-1b $\rightarrow$ IL-17 $\rightarrow$ blunted chemo effect

- Macrophage-derived factors activate STAT3 in cancer stem cells to promote chemoresistance

- Ultimate effect may depend on tumor immunogenicity, sensitivity of macrophages to drug, and the inherent state of the macrophages in the particular tumor
Macrophages initiate a wound reparative program that enhances tumor regrowth.
<table>
<thead>
<tr>
<th>Potential</th>
<th>Reality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor cell killing through reactive oxygen species??</td>
<td>Genotoxicity associated with neutrophil ROS may initiate cancer</td>
</tr>
<tr>
<td></td>
<td>Neutrophilia, and high neutrophil:lymphocyte (N:L) ratio associated with poor outcome in multiple tumor types</td>
</tr>
<tr>
<td></td>
<td>Promote angiogenesis</td>
</tr>
<tr>
<td></td>
<td>Anti-tumor T-cell suppression</td>
</tr>
<tr>
<td></td>
<td>Tumor cell migration, invasion and metastasis</td>
</tr>
</tbody>
</table>
NK cells

- Prevent tumor progression
  - Immunosurveillance $\rightarrow$ Elimination phase
  - Cell stress
  - Non-self (low or absent MHC I)
- Prevent tumor metastasis
  - Attack tumor emboli in the lungs
- NK cell tumor targeting can be inhibited by platelets
  - “Pseudo-self”
(A) Recognition of tumor MHC class I
→ T cell activation
→ NK cell inhibition

(B) Missing self
→ Evasion of T cell immunity
→ NK activation

MHC class I down-regulation
Platelet coating

(C) Transfer of platelet-derived MHC class I
→ No T cell activation
→ NK inhibitory "pseudo-self"

- tumor cell
- immune effector cell
- activating/costimulating receptors/ligands
- inhibitory NK receptor for MHC class I
- T cell receptor
- MHC class I presenting tumor antigens
- MHC class I presenting platelet ligandome
- cytolysis
- platelet
- inhibition
- activation/costimulation
- effector function

Summary

- **Function of Innate Immunity**
  - 1) Respond rapidly to tissue damage
  - 2) Limit spread of infection
  - 3) Initiate adaptive immune response
  - 4) Initiate tissue repair

- **Innate Immunity in Cancer**
  - **Neutrophils**
    - Promote tumor initiation
    - Promote tumor spread
  - **Macrophages**
    - Pro and anti-tumor effects in tumor initiation
    - Promote tumor spread
  - **NK cells**
    - Inhibit tumor initiation
    - Inhibit tumor spread
Questions?
1) What is the importance of innate immunity in cancer?

- A) Innate immunity initiates malignant transformation via neutrophil-derived genotoxic stress
- B) Innate immunity eradicates transformed tumor cells via NK recognition of non-self or cell stress
- C) Innate immunity promotes tumor spread via myeloid cell mediated CD8+ T cell inhibition
- D) Innate immunity promotes tumor spread via angiogenesis upregulation
- E) All of the above
2) Which of the following is not a function of Macrophages?

- A) Phagocytosis and presentation of microbes or tumor-associated antigens to T-cells
- B) Upregulation of acute phase protein production and systemic inflammatory response
- C) Extrude a web of fibers composed of chromatin and serine proteases that trap and kill microbes extracellularly
- D) Promote wound healing through increased angiogenesis
- E) All of the above are functions
3) Complement cascade can be activated by the lectin pathway and the alternative pathway?

- A) True
- B) False
4) Tumor associated macrophages are mostly M2-polarized, which is desirable for tumor eradication?

- A) True
- B) False
5) Tumor infiltrating neutrophils promote tumor cell invasion and metastasis via matrix metalloproteinases?

- A) True
- B) False