Basic immunology for the non-immunologist

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The following relationships exist related to this presentation:

None.
Outline

• **Immune system – development**
• **Innate immune system**
  – Pattern recognition receptors
  – Dendritic cells
  – NK cells
• **Adaptive immune system**
  – T cell development/maturation
  – T cell subsets
  – T cell activation/differentiation
  – Regulatory T cells
  – Homeostatic T cell cytokines
• **Cancer immunology – brief introduction**
Immunology – basic principles

• Attributed to Edward Jenner (late 1700s)
  – Found that inoculation with cowpox virus conferred protection against smallpox
    • Coined the term “vaccination”
• The immune system evolved to provide protection against invasive pathogens
• Consists of a wide variety of cells and proteins whose purpose is to generate immune responses against micro-organisms
• Whether the immune system provides active surveillance of malignant cells is debatable
Immune system - development

- All immune cells are produced in the bone marrow
  - **T cells** mature in the thymus
  - **B cells** mature in the marrow

- **Primary lymphoid organs** (bone marrow, thymus) – where immune cells are produced/matured

- **Secondary lymphoid tissues** (lymph nodes, spleen, mucosal lymphoid tissues) – where immune responses are initiated

Figure 1-3 Immunobiology, 7ed. © Garland Science 2008
Immune system – a division of labor

- Immune system is comprised of:
  - Innate immune system
  - Adaptive immune system

- Innate immune system
  - Provides initial recognition of self vs non-self
  - Comprised of *cells* (granulocytes, monocytes, dendritic cells and NK cells) and *proteins* (complement)
  - Recognize non-self via pathogen-associated molecular patterns (PAMPs)
    - conserved structures (i.e. LPS) in microbes
  - Pattern recognition receptors (PRRs) expressed on innate immune cells recognize PAMPs
  - Necessary for priming adaptive immune responses
  - Does not provide immunological memory
Innate immunity – on the front line of host defense

- Classes of PRRs
  - Toll-like receptors
  - NOD proteins
  - C-type lectin receptors

- Differential expression of PRRs on innate immune cells determines “functionality”

<table>
<thead>
<tr>
<th>Receptor characteristic</th>
<th>Innate immunity</th>
<th>Adaptive immunity</th>
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<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 (e.g. 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 pathogens</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>
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<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>
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Figure 2-13 Immunobiology, 7ed. (© Garland Science 2008)
Innate immunity – the Toll-like Receptors

- TLRs originally described in Drosophila
  - Bruce Beutler received Nobel prize in 2011 for discovering that LPS bound TLR4
- 10 expressed TLR genes in humans
- Present on extracellular or intracellular membranes
- Binding of TLR by ligand induces signalling through MyD88 adaptor protein
  - leads to NF-kB activation
  - upregulation of MHC molecules
  - costimulatory molecules
  - cytokines (TNF-α, IFN-β, IL-12) and chemokines
Innate immunity – dendritic cells

- Ralph Steinman (1970s) hematopoietic cells which excelled at antigen presentation and T cell activation
  - Nobel prize in 2011 for discovery of DC
- DC classified functionally in 2 groups
  - Conventional DC
    - Antigen presentation
    - T cell activation
  - Plasmacytoid DC
    - Type I IFN production
    - Important for immune responses against viruses

Colin et al. Nat Rev Immunol 2011
Innate immunity – dendritic cells

- DC receive signals through PRRs and other receptors (i.e. CD40) to become activated
  - Activation/licensing of DC results in:
    - MHC upregulation
    - Upregulation of costimulatory and cell adhesion molecules
    - Production of pro-inflammatory cytokines (IL-12, TNF-α, type I IFNs)
    - Alteration of chemokine receptor expression
    - Migration (to site of inflammation)
  - Only licensed DC will activate naïve T cells
  - Non-licensed DC can induce peripheral tolerance (T cell deletion or anergy)
Innate immunity – NK cells

- Natural killer cells (NK cells – CD3⁻CD56⁺CD16⁺/⁻ lymphocytes)
  - Develop in bone marrow from CLP
  - Circulate in blood
  - Able to kill lymphoid tumor cell lines in vitro without prior activation
  - Mechanism of killing – secretion of cytotoxic granules containing perforin and granzymes
    - Also express Fc receptors - effectors of ADCC
  - Important for early host recognition of infected host cells
    - HSV and Leishmania
  - NK cells are “activated” in response to Type I IFNs, TNF-α and IL-12
    - killing capacity and production of IFN-γ

Cooper et al. Trends Imunol 2004
Innate immunity – NK cell receptors

- 2 families of NK receptors
  - Killer lectin-like receptors (KLRs)
  - Killer cell Ig-like receptors (KIRs)
- Both KLRs and KIRs can act as activating or inhibitory receptors
  - Makes the study of NK cell activation complicated
  - Further complicated by the fact that KIR genes are also polymorphic
- Missing self hypothesis:
  - NK cells do not kill self cells due to MHC class I expression (MHC = major histocompatibility complex)
  - NK cell do kill target cells which lack MHC class I

Lanier L. Ann Rev Immunol 2005
Innate immunity – NK cells and cancer

- **NKG2D** – Activating C-type lectin receptor on NK cells
  - Recognizes RAE proteins and MICA and MICB
    - RAE and MICA/B - MHC class I-like molecules expressed on virally-infected cells and some malignant cells
    - Recognition by NKG2D is a “danger” signal, resulting in “costimulation” of NK cells
    - Leads to lysis of targets and production of IFN-γ

- **KIRs and graft-versus-leukemia effect following allogeneic SCT**
  - Donor vs recipient KIR “incompatibility” provides GVL effect
  - Similar analyses have confirmed that KIR mismatched allo-grafts led to decreased risk of AML relapse following alloSCT
  - Ongoing studies are evaluating the efficacy of adoptive KIR-mismatched NK cell therapy in myeloid leukemias
Adaptive immunity – lymphocytes

- Adaptive immune system (vertebrates only) evolved to provide a nearly unlimited diversity of antigen receptors to protect the host from infection
- Comprised of B and T lymphocytes
- B and T cells express unique antigen receptors generated following random recombination of variable and constant region gene segments
  - Diversity (in part) and antigen specificity conferred by Complimentary Determining Regions (CDR) of the BCR and TCR
    - CDR regions are located at the joining segments of the BCR or TCR
    - $10^8$ unique lymphocyte receptors present in humans!
- B cell receptor = antibody – recognizes intact extracellular antigens
  - Proteins/glycoproteins
- T cell receptor – recognizes peptides in the context of MHC molecules

- Our discussion will focus on T cell development, homeostasis and activation
Adaptive immunity – T cell development and maturation

- T cells develop in the bone marrow and mature in the thymus
- T cell receptor gene rearrangement occurs in the thymus
- The TCR is comprised 2 chains: TCR-α and TCR-β
  - TCR-α = VαJαCα
  - TCR-β = VβDβJβCβ
- Successful rearrangement of TCR-α and β chains necessary for further development and “selection” of thymocytes
Adaptive immunity – Thymic selection of T cells

- Thymic T cells (rearranged TCR) undergo both **positive** and **negative** selection
- Positive selection – T cell must bind host MHC (determined by CDR1 and CDR2 regions of the TCR)
  - If thymocyte binds MHC class I it becomes a CD8⁺ T cell, etc.
  - If no MHC binding affinity, T cell is deleted
- Negative selection – T cell must not recognize MHC:peptide with strong affinity
  - If yes, then T cell is deleted
  - Mechanism of central (thymic) tolerance – ensures that auto-reactive T cells do not escape the thymus
- Developing T cells exposed to tissue-specific proteins in the thymus via AIRE (autoimmune regulator)
  - TF expressed in thymic medullary stromal cells – induced expression of tissue-specific proteins
  - AIRE mutations lead to autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy syndrome (APECED)
Adaptive immunity – CD4 and CD8 T cell subsets

- 2 main “flavors” of mature T cells
  - CD8\(^+\) T cells
  - CD4\(^+\) T cells

- CD8\(^+\) T cells recognize peptides (7-9aa) presented by MHC class I
  - Cytosolic antigens (intracellular pathogens and self peptides)

- CD4\(^+\) T cells recognize peptides (20aa) presented by MHC class II
  - Exogenous antigens

Gascoigne et al. Nat Rev Immunology 2008
Adaptive immunity – Activation of naïve T cells

- Naïve T cells can survive long-term without encountering cognate antigen

- Activation of naïve T cells requires (at least) 2 signals
  - MHC/peptide:TCR (signal 1)
  - B7:CD28 (signal 2)
  - Cytokines (IL-12) (signal 3)

- Activated T cells proliferate and differentiate into effectors that do not require costimulation to act
Adaptive immunity – Activation of naïve T cells

- If a naive T cell receives signal 1 in absence of signal 2:
  - Clonal deletion
  - Anergy
  - Mechanisms of peripheral tolerance
- The maturation state of APC is important
  - Quiescent APC – poor costimulation → tolerance
  - Activated APC – strong costimulation → T cell activation

Figure B-23 Immunobiology, 11e (11) Garfield Science 2008
Adaptive immunity – Positive and negative costimulatory receptors

- Modulate magnitude of T cell activation and effector function

- Positive costimulatory receptors:
  - CD28 (classical)
  - ICOS (inducible costimulator)
  - CD27 (TNF family receptor)

- Negative costimulatory receptors:
  - CTLA-4 (cytotoxic lymphocyte antigen – 4)
  - PD-1 (programmed death -1)
  - TIM-3 (T cell immunoglobulin mucin -3)

- CTLA-4 and PD-1 blocking mAbs are effective in preventing tumor-induced T cell dysfunction melanoma).
Adaptive immunity – CD8$^+$ T cell differentiation and effector function

- Following activation, CD8$^+$ T cells differentiate into cytotoxic lymphocytes (CTL)
  - Functions
    - 1) killing via release of cytoplasmic granules containing granzymes and perforin which induce target cell apoptosis
    - 2) release of effector cytokines (IFN-γ, LT-α, TNF-α)
Adaptive immunity – CD4\(^+\) T cells are “helpers” in the immune response

- Similar to CD8\(^+\) T cells, activated CD4\(^+\) T cells proliferate and acquire effector functions
- Classical functions of CD4\(^+\) T cells:
  - Production of IL-2 to promote proliferation of activated CD8\(^+\) T cells
  - Licensing of dendritic cells through CD40-CD40L interactions
  - Production of effector cytokines (T\(_H\) subtype-dependent)
  - Lysis of target cells
Adaptive immunity – CD4\(^+\) T cell differentiation and effector function

- Differentiation pathways for CD4\(^+\) T cells are more complicated than for CD8\(^+\) T cells
- 4 subsets of CD4\(^+\) T cells (a.k.a. T\(_H\) cells)
  - T\(_H1\) – Typical bacterial infection, viral infection, tumor immunity
  - T\(_H2\) - allergy
  - T\(_H17\) – gut homeostasis, autoimmunity
  - **Regulatory T cells (Tregs)** – suppress conventional T cells, peripheral tolerance
- Which of these pathways a CD4\(^+\) T cell follows depends on
  - Antigen specificity
  - Local environment - signal 3 received (IL-12, TGF-\(\beta\), IL-6, IL-4)
- Each CD4\(^+\) T cell subset acquires a unique effector program (cytokine production) and drives a different type of immune response
Adaptive immunity – CD4+ T cell differentiation

Hooper et al. Nat Rev Immunol 2010
Adaptive immunity – Regulatory T cells

- Subset of CD4⁺ T cells with suppressive function
  - Definitively described in 1998 by Sakaguchi and colleagues
  - Immunophenotype: CD4⁺CD25⁺FoxP3⁺
  - ~ 5-10% of circulating CD4⁺ T cells
  - Treg development & function controlled by the FoxP3 transcription factor
    - Necessary for Treg development and maintenance of functional properties
    - Mutations of FoxP3 locus lead to severe autoimmunity (IPEX syndrome)
    - Treg are critical to maintain peripheral tolerance
Adaptive immunity – Regulatory T cell development

**Treg subsets**

1. **Natural Treg (nTreg)**
   - Develop in thymus
   - Recognize self-Ag

2. **Induced Treg (iTreg)**
   - Exit thymus as CD4⁺FoxP3⁻ naïve CD4 T cells
   - In the presence of TGF-β, induced to express FoxP3
   - Both nTreg and iTreg have potent suppressive capability in vivo
Adaptive immunity – Regulatory T cells – suppressive mechanisms

- Treg suppress conventional T cell function via multiple mechanisms:
  1. Secretion of suppressive cytokines (TGF-B, IL-10, IL-35)
  2. Act as cytokine sinks (Binding of local IL-2)
  3. Secrete granzymes to kill effector T cells and DC
  4. Block costimulatory ligands on DC
Adaptive Immunity – Regulatory T cells and cancer

- Treg expand in patients with a variety of malignancies
  - Inverse correlation between Treg numbers and cancer survival
- iTreg may also suppress anti-tumor immune responses
- Treg depletion leads to enhanced anti-tumor immunity
- Strategies to deplete or inhibit Treg:
  - Denileukin Diftitox (IL-2 immunotoxin)
  - Daclizumab (anti-CD25 mAb)
  - CTLA-4 blockade
  - Cyclophosphamide
Adaptive immunity – Naïve and memory T cell homeostasis

- The size and composition of the peripheral T cell pool is constant
- Naïve and memory T cells survive long-term
  - Proliferation balanced by death
- T cell homeostasis is dependent on:
  - Interaction with self-MHC:peptide
  - Cytokine signals (IL-7, IL-15)
    - Upregulate pro-survival and cell cycle-dependent genes
Adaptive immunity – Cytokines required for T cell survival and proliferation

Baccala R et al. Trends Immunol 2006
Adaptive immunity – Receptors for homeostatic cytokines

- Homeostatic cytokines (IL-2, IL-7 and IL-15) signal through a family of common receptor subunits:
  - Common γ-chain – CD132
  - IL-2Rα – CD25
  - IL-2Rβ – CD122
  - IL-7Rα – CD127
  - IL-15Rα – CD215
Cancer and Immunity

- William Coley, MD (1862-1936) established link between infection and cancer
- Administered steptococcus and serratia (Coley’s toxin) to patients with bone sarcomas
- Several had objective tumor responses
  - Many died of infection
- “Father” of immunotherapy??
Cancer and Immunity

• 50-60 years ago, observation: rejection of transplanted tumor cells in syngeneic mice
• 20 years ago, tumor antigens recognized by T cells began to be identified
• More recently, components of the immune system which are necessary for rejection of transplanted tumors have been clarified
  – For most tumor cell lines, both innate and adaptive immunity must be functional for tumor rejection to occur
• The concept of immune surveillance of cancer has been developed (Bob Schreiber)
  – Based on clinical observation that immunosuppressed individuals have a higher cancer risk
  – 3 phases of immune surveillance
    • Elimination
    • Equilibrium
    • Escape

![Diagram of immune surveillance phases](image-url)

Figure 15-13 Immunobiology, 7ed. (© Garland Science 2008)
Cancer and Immunity – Immune evasion

• Putative immune evasion mechanisms
  – Tumor-induced T cell anergy
  – Expression of negative costimulatory receptors on T cells (PD-1, TIM-3, CTLA-4)
  – Tregs
  – Suppressive myeloid-derived cells (MDSC, TAM)
  – Secretion of inhibitory cytokines (IL-10, TGF-β)
  – Antigen-loss variants (loss of MHC)
  – Production of enzymes which deplete essential amino acids (IDO, arginase)
  – Others

• Overcoming negative regulation in the tumor environment will be necessary to harness effective anti-tumor immunity
Cancer and Immunity – Immunotherapy: current approaches

- Cancer vaccines
  - Peptide-based
  - Cellular-based (i.e. DC vaccines)
- Adoptive T cell therapy
  - Ex vivo expansion of tumor-infiltrating T cells and infusion into cancer-bearing hosts
  - Tumor Ag-specific TCR transduced T cell therapy
  - Chimeric antigen receptor (CAR) adoptive therapy (CD19)
- Immune checkpoint blockade
  - CTLA-4 blockade
  - PD-1 blockade
- Reversal of immune evasion
  - Treg depletion
  - IDO inhibition (1-MT and derivatives)
  - Prevention of tumor-induced T cell anergy (lymphodepleted host and adoptive T cell therapy)