Basic immunology for the non-immunologist

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Society for Immunotherapy of Cancer

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 - developement

- All immune cells are produced in the bone marrow
  - **T cells** mature in the thymus
  - **B cells** mature in the marrow
- **Primary lympoid organs** (bone marrow, thymus) – where immune cells are produced/matured
- **Secondary lymphoid tissues** (lymph nodes, spleen, mucosal lymphoid tissues) – where immune responses are initiated

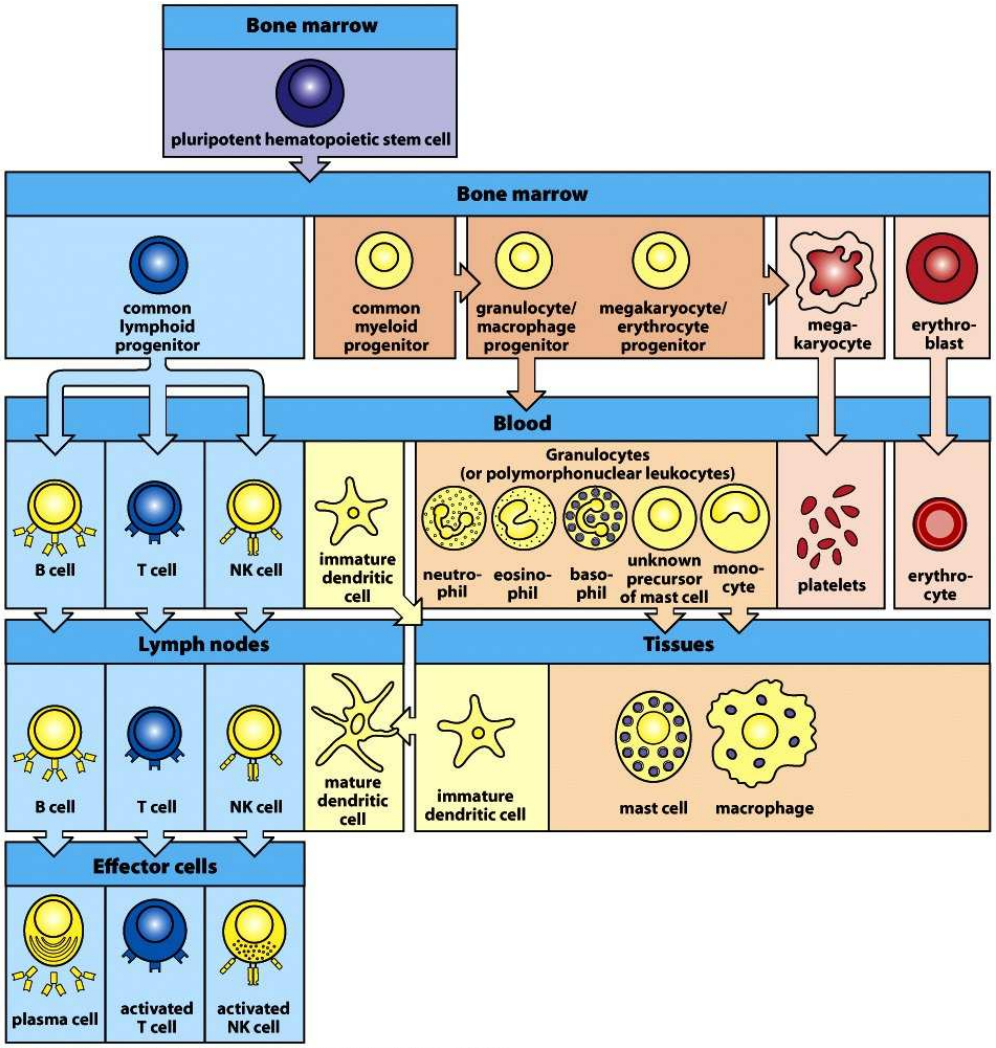


Figure 1-3 Immunobiology, 7ed. (© Garland Science 2008)

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

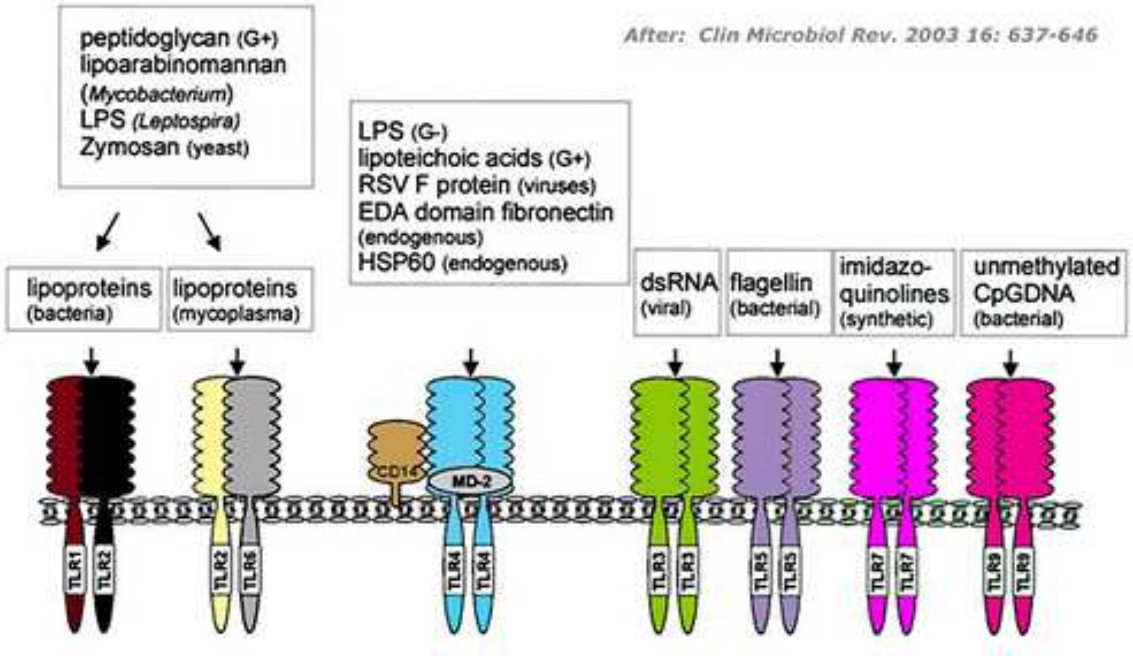
Innate immunity – on the front line of host defense

- Classes of PRRs
  - Toll-like receptors
  - NOD proteins
  - C-type lectin receptors
- Differential expression of PRRs on innate immune cells determines "functionality"

Receptor characteristic	Innate immunity	Adaptive immunity
Specificity inherited in the genome	Yes	No
Expressed by all cells of a particular type (e.g. macrophages)	Yes	No
Triggers immediate response	Yes	No
Recognizes broad classes of pathogens	Yes	No
Interacts with a range of molecular structures of a given type	Yes	No
Encoded in multiple gene segments	No	Yes
Requires gene rearrangement	No	Yes
Clonal distribution	No	Yes
Able to discriminate between even closely related molecular structures	No	Yes

Figure 2-13 Immunobiology, 7ed. (© Garland Science 2008)

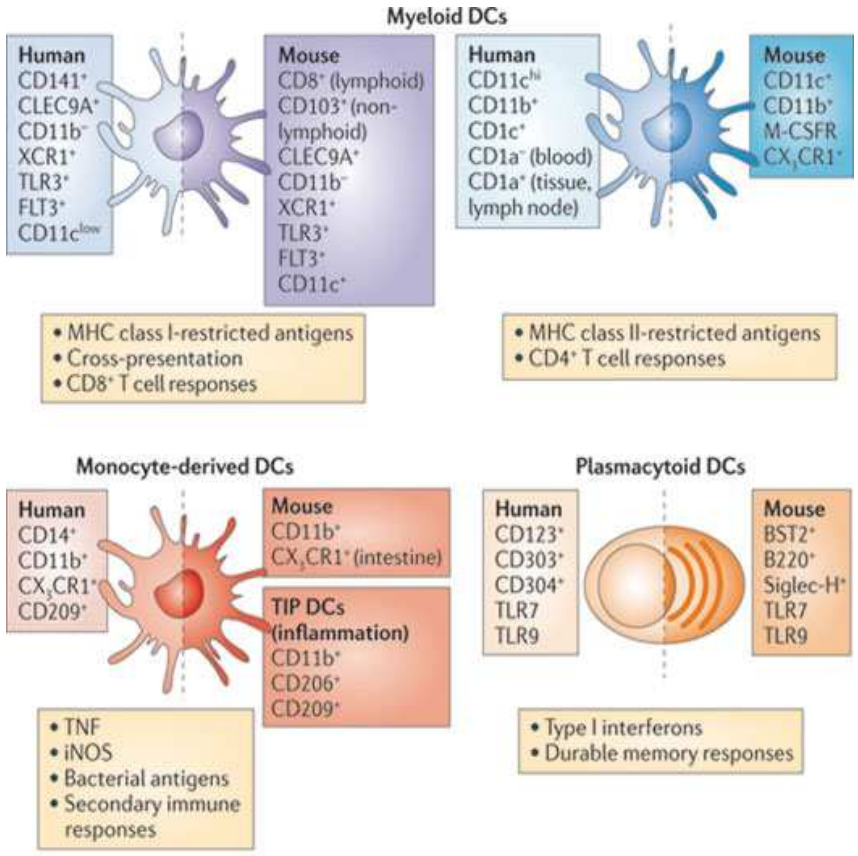
Innate immunity – the Toll-like Receptors



- 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



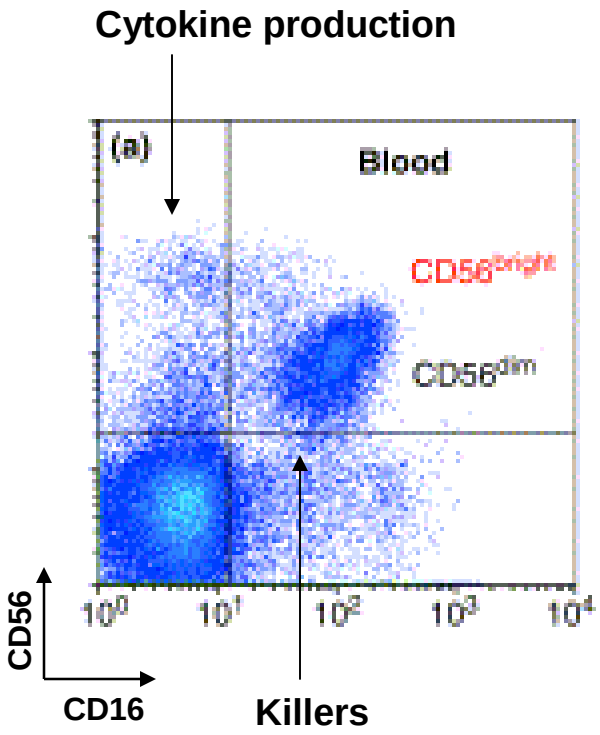
Colin et al. Nat Rev Immunol 2011

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-inlfammatory 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

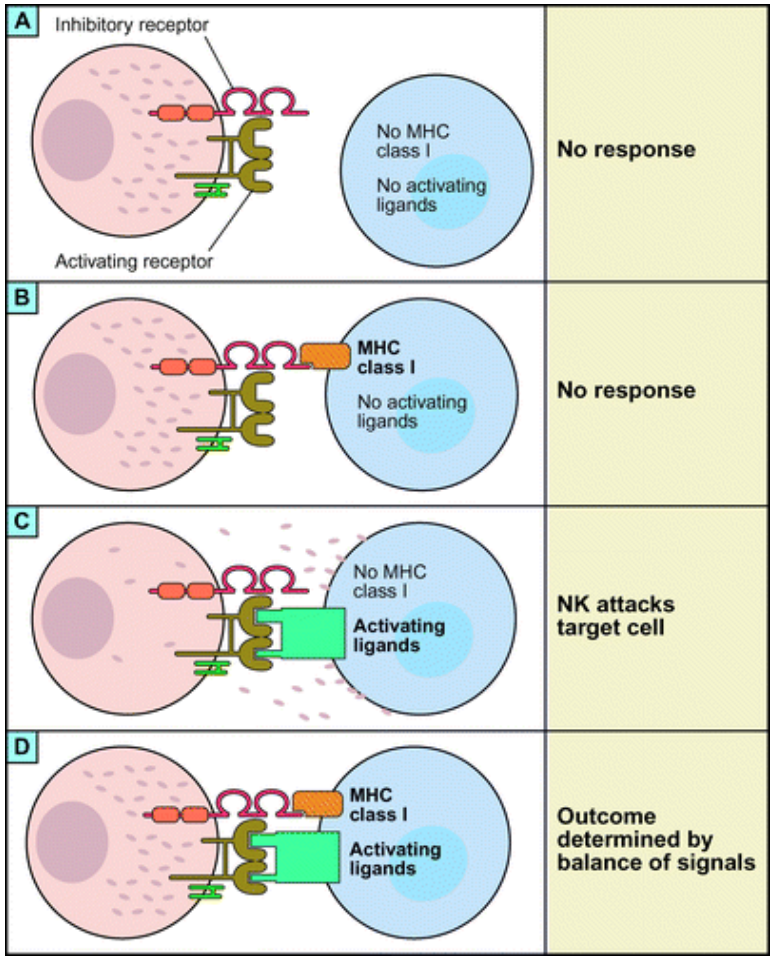
- Natural killer cells
  (NK cells – $CD3^{-}CD56^{+}CD16^{+/-}$ lymphocytes)
  - Develop in bone marrow from CLP
  - Circulate in blood
  - Able to kill lymphoid tumor cell lines in vitro without prior activation
  - Mechanism of killing – secretion of cytotoxic granules containing perforin and granzymes
    - 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
    - Ruggeri et al Science 2002.
  - 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

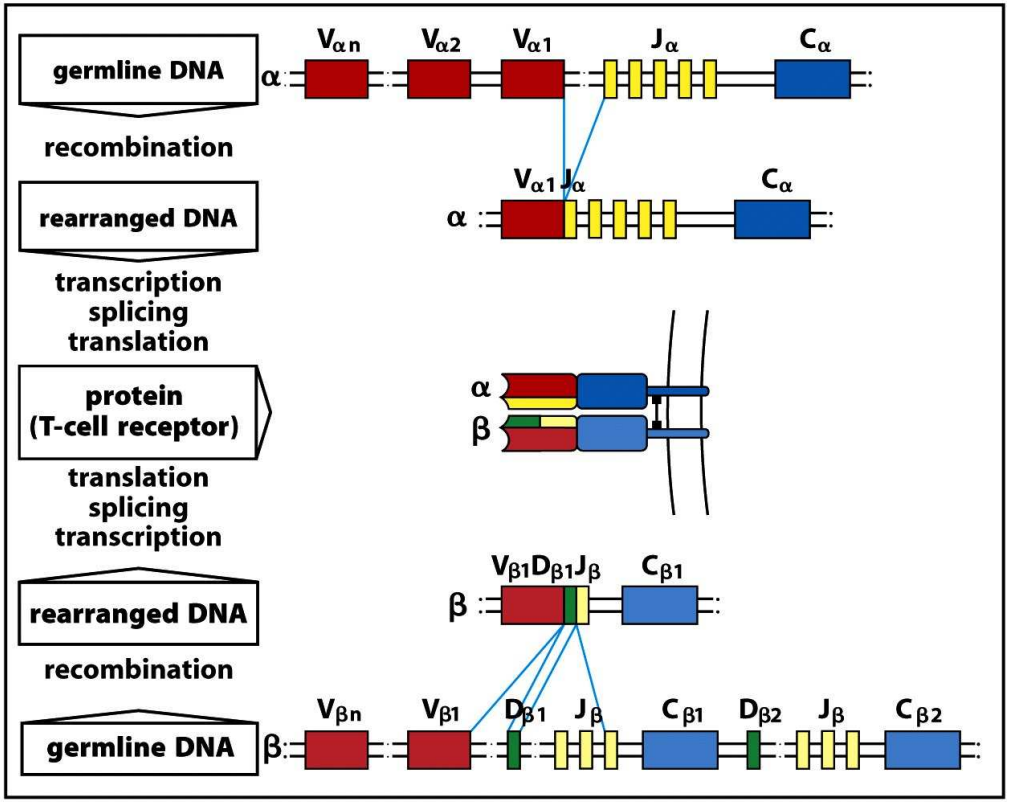


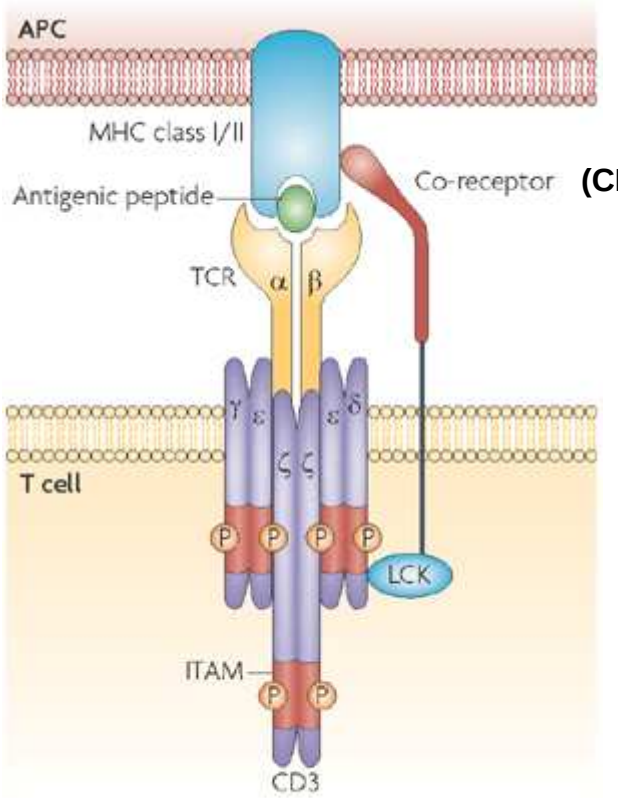
Figure 4-10 Immunobiology, 7ed. (© Garland Science 2008)

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 co-stimulation to act

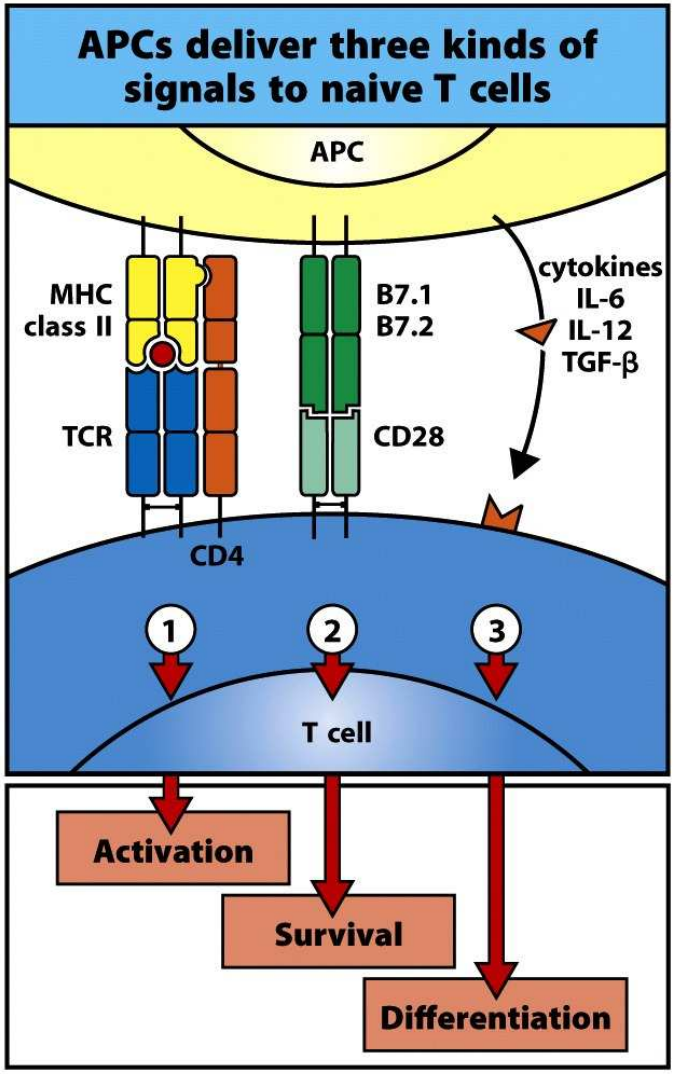


Figure 8-19 Immunobiology, 7ed. (© Garland Science 2008)

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

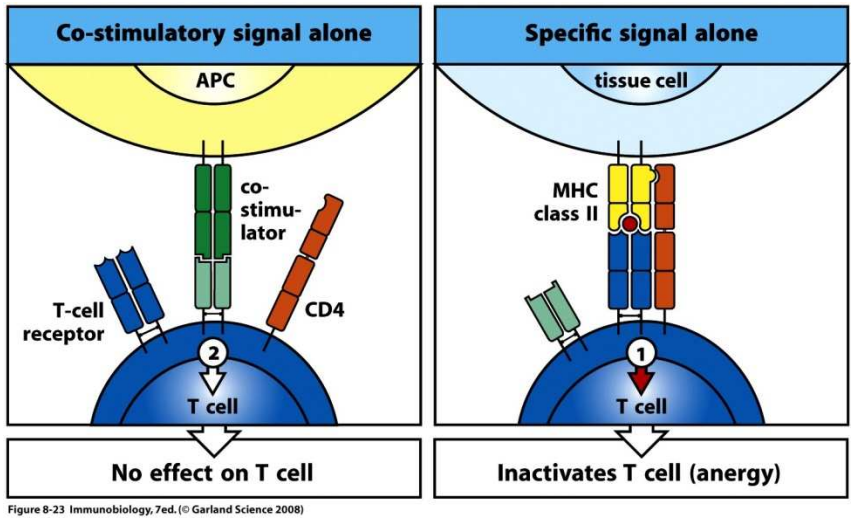


Figure 8-23 Immunobiology, 7ed. (© Garland Science 2008)

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).

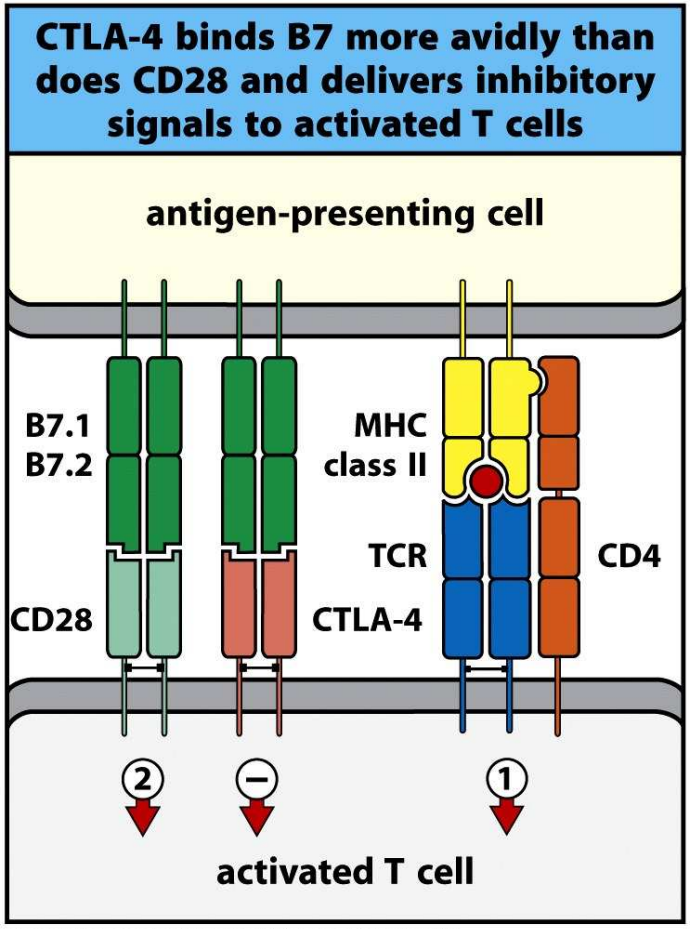


Figure 8-22 Immunobiology, 7ed. (© Garland Science 2008)

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)

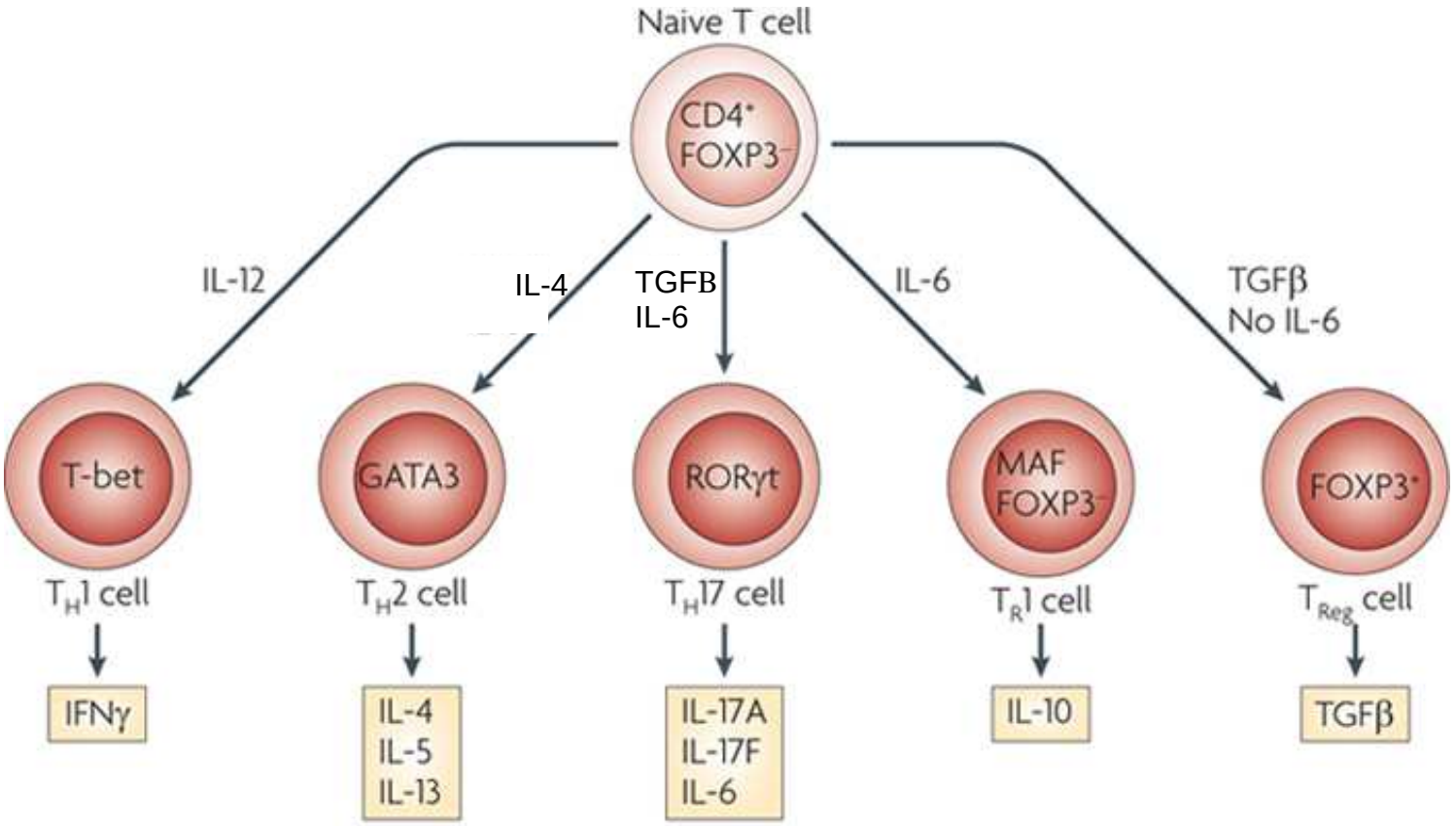
Adaptive immunity – $CD4^+$ T cells are "helpers" in the immune response

- 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-B, 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

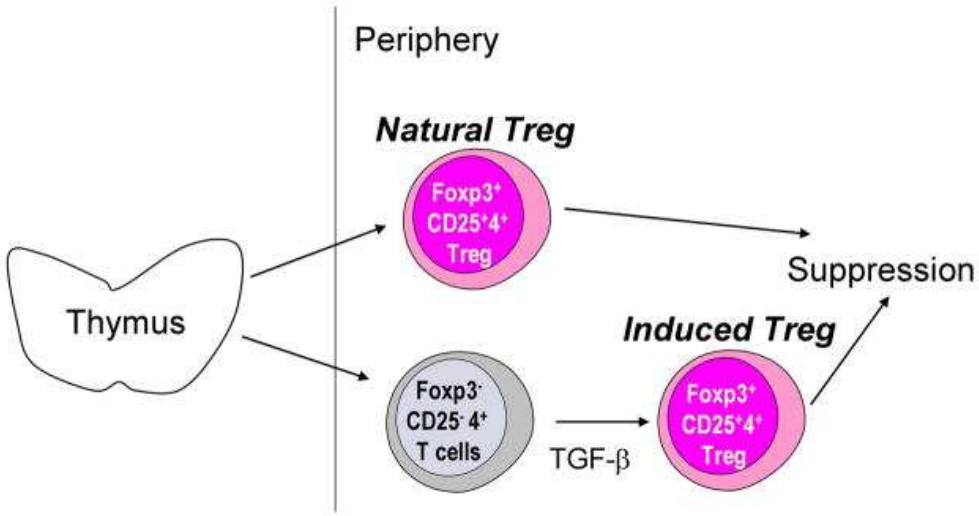
- Subset of $CD4^+$ T cells with suppressive function
  - Definitively described in 1998 by Sakaguchi and colleagues
  - Immunophenotype: $CD4^+CD25^+FoxP3^+$
  - ~ 5-10% of circulating $CD4^+$ T cells
  - Treg development & function controlled by the FoxP3 transcription factor
    - Necessary for Treg 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

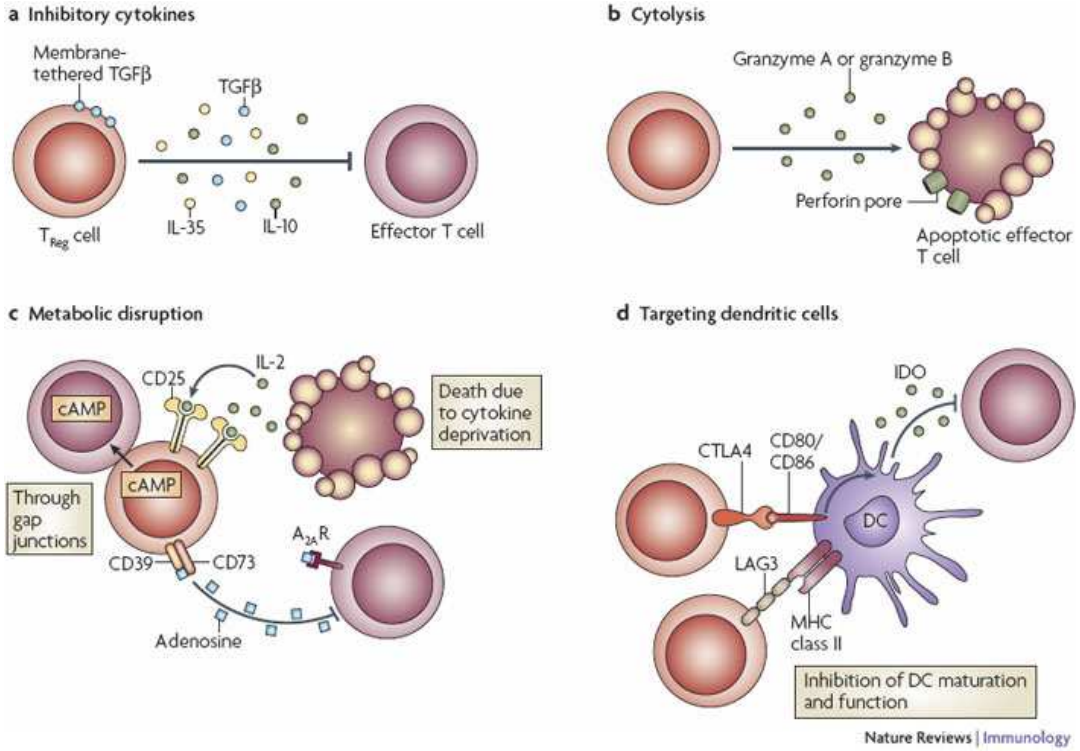
Treg subsets

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



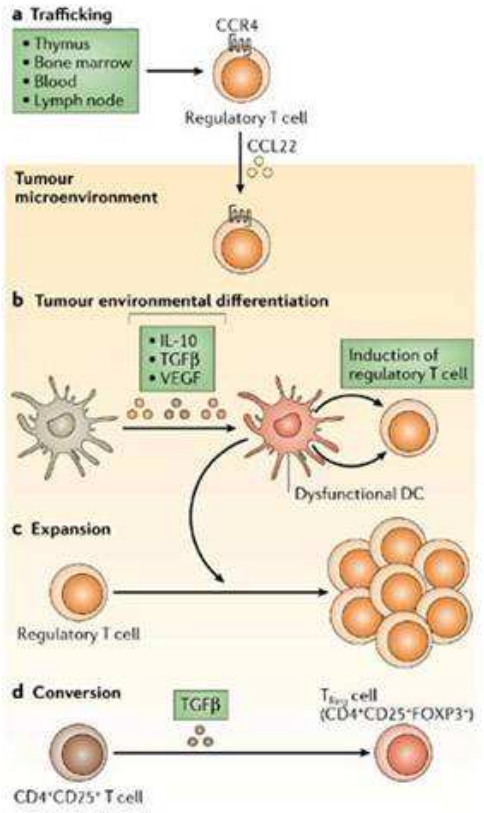
Adaptive immunity – Regulatory T cells – suppressive mechanisms



- 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

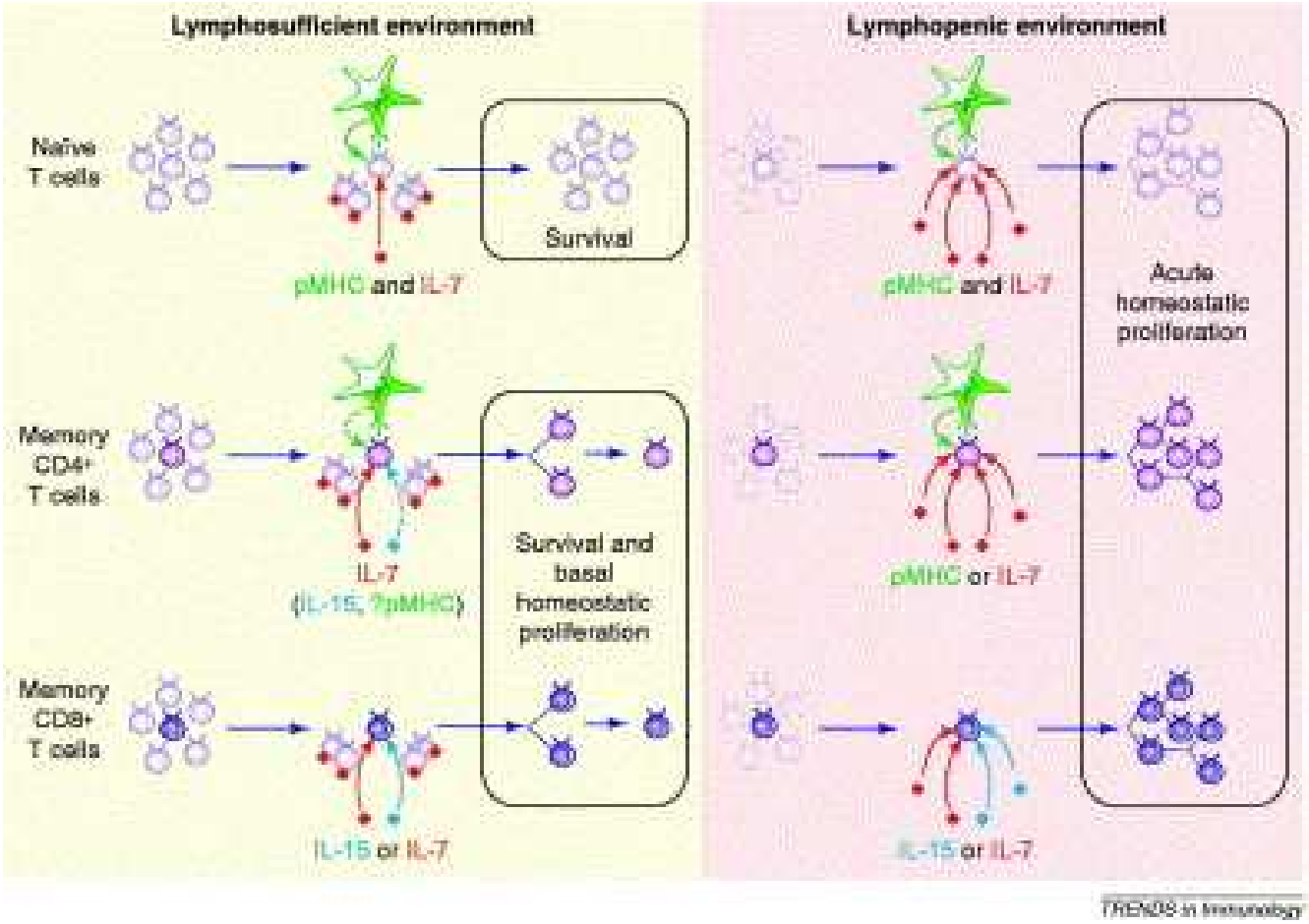


Zou *Nature Reviews Immunology* **6**, 295–307 (April 2006) | doi:10.1038/nri1806

Adaptive immunity – Naïve and memory T cell homeostasis

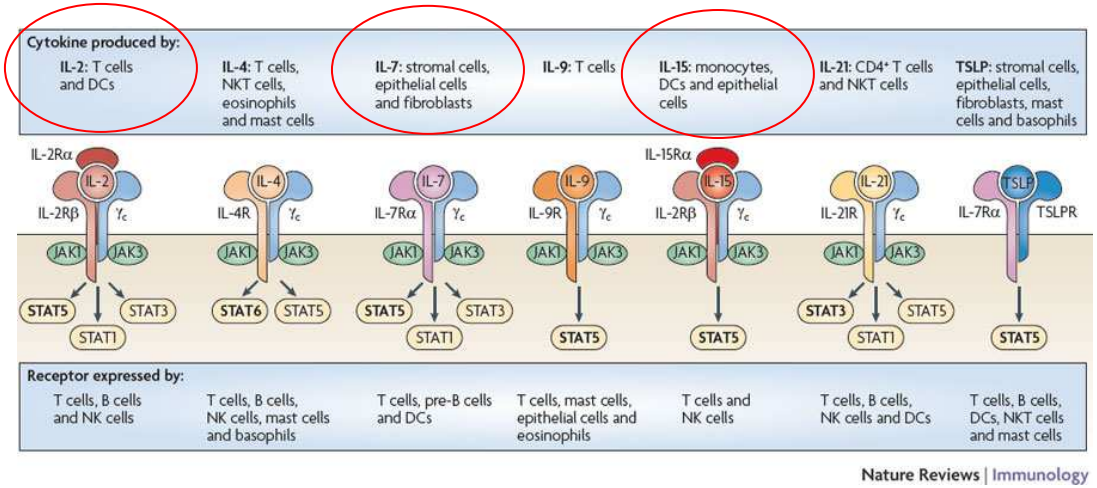
- The size and composition of the peripheral T cell pool is constant
- Naïve and memory T cells survive long-term
  - Proliferation balanced by death
- T cell homeostatis is dependent on:
  - Interaction with self-MHC:peptide
  - Cytokine signals (IL-7, IL-15)
    - Upregulate pro-survival and cell cycle-dependent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

Adaptive immunity – Cytokines required for T cell survival and proliferation



Baccala R et al. Trends Immunol 2006

Adaptive immunity – Receptors for homeostatic cytokines



Nature Reviews | Immunology

- 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

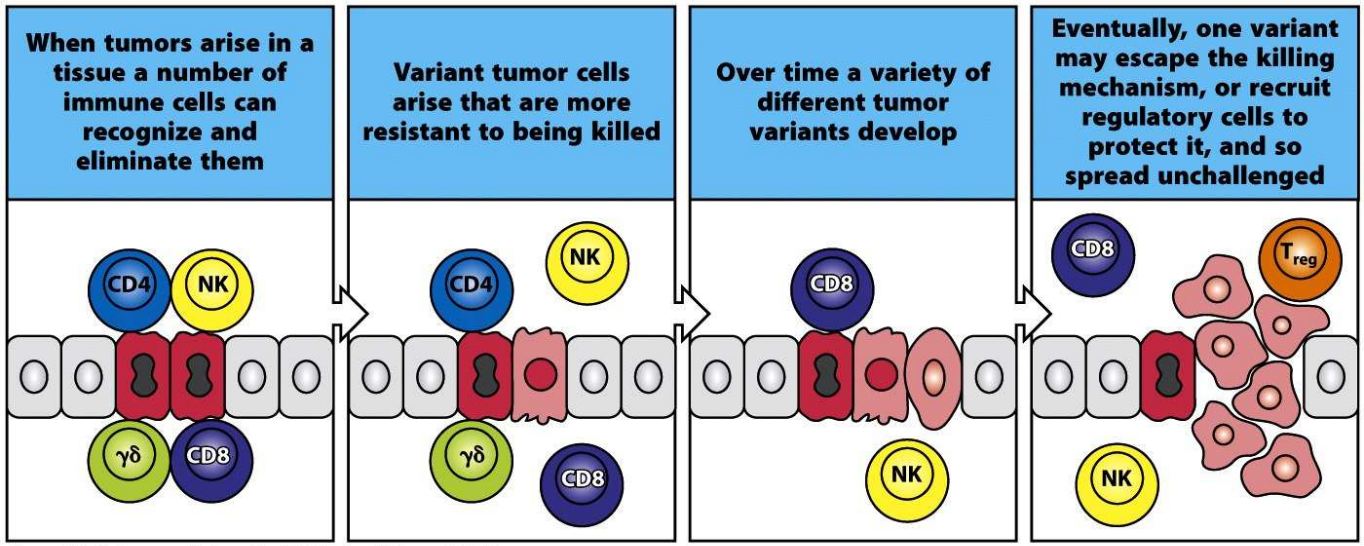
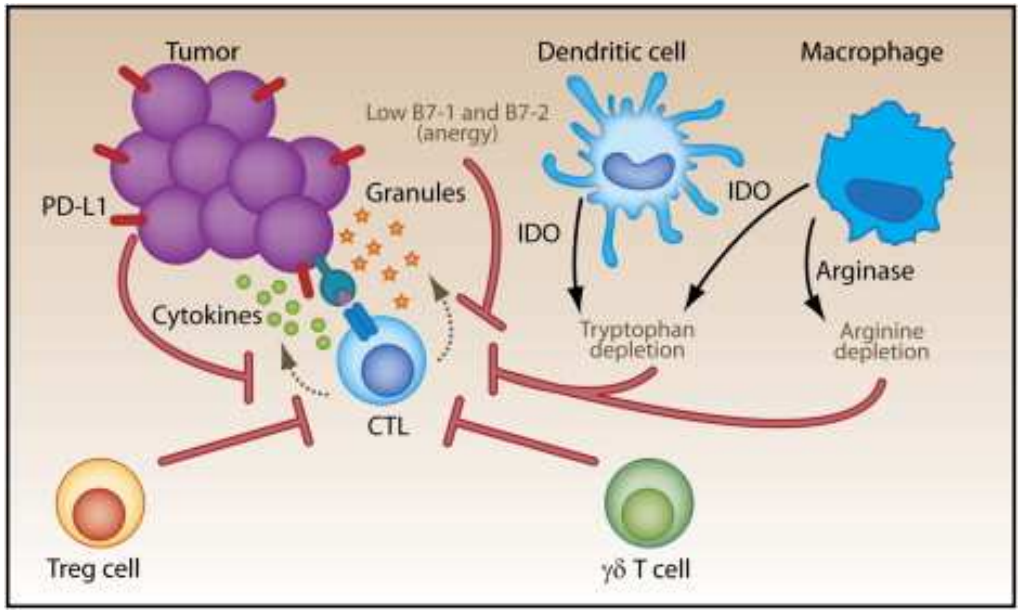


Figure 15-13 Immunobiology, 7ed. (© Garland Science 2008)

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)