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B cell development and activation

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- Toll-like receptors
  - Recognize bacteria and viruses in innate immunity
  - Bind to pattern molecules not expressed by humans but shared by groups of pathogens
  - Expressed by many cells, including leukocytes
  - Consequences of TLR signaling
    - Production of cytokines and chemokines and subsequent inflammation
    - Production of type I interferons
    - Killing or inhibition of viruses and intracellular bacteria
    - Up-regulation of co-stimulatory molecules that help activate T and B lymphocytes
  - NOD receptors
    - Expressed in cytoplasm --> viral defense
    - Contain nucleotide-binding olgiomerization domain
- Cells associated w/ innate immunity
  - Macrophages and neutrophils
  - "B1" B cells - make most of the antibodies in serum; bind to common epitopes
  - Dendritic cells
  - γ-δ T cells
  - NK T cells
    - Lymphocytes slightly larger and more granular than T and B lymphocytes
    - Kill cells that do not express MHC class I molecules
    - Some have Fc receptor to bind antibody and kill cell (antibody-dependent cell-mediated cytotoxicity)
- B Cell Differentiation
  - Occurs in bone marrow from pluripotent stem cells
  - Stem Cell has nothing on it
  - Pro B cell gains CD19, CD20, other CD antigens
    - Cluster of differentiation
      - Found on groups of cells at same stage of differentiation
      - CD45 on all cells, but can be as specific as CD8+ T cells (killer T)
      - Can be used to define state of development of a cell
      - Detected by monoclonal antibodies
    - Flow Cytometry (FACS) - expression of a cell surface molecule
      - Binds an antibody tagged w/ fluorescent molecule to a cell surface molecule
      - Analyze amount of antibody bound
      - Laser beam aimed at stream of fluid w/ three detectors
        - Forward scatter - how big
        - Side scatter - granulation
        - Emission spectrum - amount of antibody on cell
      - Each cell is represented as one dot
      - CD19 vs. IgM graphs on pg80
    - Early pro-B cell undergoes D-J rearrangement on H chain
    - Late pro-B cell undergoes V-DJ rearrangement on H chain
  - Pre B cell has μ chain in cytoplasm
    - Pre B cell receptor
    - Positive signaling w/ μ heavy chain only
    - H chain is VDJ rearranged, begin light chain gene management
    - Small pre B cell undergoes V-J rearrangement of L chain
  - Immature B cell has IgM on surface

        - VJ rearranged on L chains, VDJ rearranged on H chain
        - Expression of IgM receptors
        - Bruton's agammaglobulinemia
            - x-linked disease
            - Failure to produce antibodies
            - Repeated infections
            - Almost no production of B cells in bone marrow
                - Mutation in Bruton's tyr kinase activated upon engagement of the pre B cell receptor
                - Signal from μ on surface of pre B cells doesn't reach nucleus - positive signaling fails
    - Mature B cell has IgD and IgM on cell surface
        - B cell development depends on adherent cell part of bone marrow and cytokines (IL-7) --> B cell development requires a signal
        - Final product is naïve
        - Antigen independent B cell differentiation in bone marrow results in 10 mm diff clones of B cells each w/ different epitope (receptor repetoire)
        - Each antibody is made by one clone of B cells hence immunoglobulins are clonally distributed
    - Immature B cells w/ an Ig that binds to a self antigen sends negative signal
        - Results in deletion of immature B cells in bone marrow
        - Clonal deletion - part of self-tolerance for B cells
    - Allelic exlcusion
        - In a b cell clone only one of two antibody loci is expressed
        - A B cell could express two heavy chains and four light chains (κ and α), it only expresses one of each
    - Where does the B cell diversity come from?
        - Constant region - one gene; if there were two genes, single amino acid changes would occur and there would soon be two isotypes
        - Variable region - many genes for the three kinds of variable regions
        - Vκ encodes aa 1-95
            - 1500 kb of DNA; 79 genes
            - Half are pseudogenes
            - V genes are 5 to 20 kb apart
            - Most 3' Vκ is 23 kb 5' of Cκ
        - Jκ encodes aa 96-107
            - 5 regions
            - Lies 1.2 kb 5' of Cκ
        - Cκ encodes aa 108-214
        - Recombination Signal Sequences
            - Each V is followed by: V codon 95CACAGTG--spacer--GGTTTTTGT
            - Each J preceeded by: ACAAAAACC--spacer-CACTGTG-J codon 96
            - True for variable regions and J segments associated w/ heavy chain, κ and λ light chain genes
            - V(D)J recombination mediated by lymphoid recombination activating genes: RAG1/2
            - Mutation in genes lead to severe combined immunodeficiency (no B or T cells) or Omenn's syndrome (milder)