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Immunology Lecture 6 Regulation of B Cell Development and the Humoral Response I

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### B-cell lymphocyte development

- § humoral arm of the immune response
- § make soluble antibodies
- § takes place continually throughout the life time of vertebrates
- § development in bone marrow
- § circulate until they recognize an antigen epitope (then proliferate and make antibodies)
- § die after 2-3 weeks if no stimulation from antigen

Exquisitely regulated to limit time and amount of antigen response. The end result is a specific antibody to a particular pathogen. A Three Bears scenario@.

### Three unique aspects of immune response

1. specificity (huge diversity of recognition possibilities)
2. tolerance (distinguish self from non-self)
3. memory (basis for immunization allows for more vigorous and more rapid response to antigen)

### Clonal selection (B-cell development)

1. Single progenitor cell gives rise to large number of lymphocytes
  - each lymphocyte expresses single specific antibody (diversity)
  - random selection of different VDJ segments
  - huge number of different clones (greater than  $10^8$ )
  - diversity established independent of any antigen
2. Deletion of clones that recognize self antigens (tolerance)
3. Pool of naive lymphocytes (primary repertoire)
4. Upon contact with recognizable epitope, proliferate and differentiate and secrete antibodies

Diversity is established independent of any antigen

Diversity exists all the time and antigens then select from clonal population

Diversity is fostered through DNA rearrangement

### Antigen-Independent B-Cell Development (bone marrow)

1. DNA rearrangement (VDJ) establishes the primary repertoire (diversity)
2. Allelic exclusion ensures that each clone expresses a single antibody on the surface (specificity)
  - there are originally two copies of each heavy and light cell gene (one on each allele)

- only one allele of each heavy chain and each light chain will be expressed
  - the other alleles will be unexpressed
3. Deletion of self-reactive clones establishes tolerance

Bone marrow stromal cells support early B lymphopoiesis

1. early B cells nestle down with stromal cells
2. critical signals
  - (KIT (early B-cell) / stem cell factor (stromal cell))
  - IL-7 (released from stromal cell)
3. B cell leaves stromal cells when Ig receptor is expressed

Critical DNA rearrangements are driven by B-cell developmental signals.

1. Highly regulated order where heavy chains rearrange first
2. Begins as stem cell in bone marrow
3. Early pro-b cell rearrange heavy chain genes (multiple v, 20 or so d, 4 j, and the constant regions)
  - first A<sub>d</sub> to j<sub>h</sub> on both alleles
  - one allele rearrange A<sub>v</sub> to d<sub>j</sub> (cell commits to b cell lineage)
  - mu heavy chain associates with two surrogate light chains

Heavy chain rearrangement in context of allelic exclusion:

- § DJ rearrangement on both alleles
- § V-DJ on one allele (the two alleles for the heavy chain are in different places in the nucleus and one will always undergo A<sub>v</sub>-d<sub>j</sub> arrangements before the other)
- § After adding and subtracting bases in a random way, there are now two possibilities:
  1. 1/9 chance it will be in the correct reading frame (A<sub>d</sub>j<sub>h</sub> 1/3 x A<sub>v</sub> to d<sub>j</sub> 1/3 = 1/9) productive rearrangement
  2. 8/9 out of frame non productive rearrangement

If productive rearrangement:

- § Mu and preBCR (surrogate light chain) expressed on cell surface
- § Three signals
  1. STOP heavy chain rearrangement
  2. Proliferate
  3. Begin light chain rearrangement
- § If productive on one allele, the other heavy chain is never rearranged

If non-productive rearrangement

- § A<sub>v</sub>-D<sub>J</sub> rearrangement on 2<sup>nd</sup> allele (1/9 correct reading frame)
- § If productive rearrangement:
- § Mu and pre BCR (surrogate light chain) produced
- § only two signals:
  - a. Proliferate
  - b. Begin light chain rearrangement

If not productive with V-DJ rearrangement on 2<sup>nd</sup> allele and both alleles are out of frame, which is the most likely scenario, no mu will be produced and the cell dies. This is a very wasteful process, but ensures allelic exclusion.

Light chain rearrangement (lambda and kappa)

1. 4 possible alleles (more likely to succeed than heavy chain rearrangement) and only need one successful “v to j” rearrangement

kappa	PR, GL	NPR, PR	NPR, NPR	NPR, NPR
lambda	GL, GL	GL, GL	PR, GL	NPR, NPR
		Continue Development		Death

2. each allele has a 1/3 chance of a productive rearrangement
3. kappa usually proceeds lambda
4. productive rearrangement produces a light chain which then associates with a heavy chain to express IGM
5. successful rearrangement then produces signals to stop further light chain rearrangement and further B cell development

There are two checkpoints that confer allelic exclusion and depend on successful heavy and light chain rearrangement

- 1 Making of Pre B cell receptor (mu chain consists of a heavy chain with an associated invariant light chain expressed on surface) Also, two signaling proteins Ig alpha and Ig Beta are expressed on cell surface (Ig alpha and Ig Beta associate with fc portion of mu chain). Signal B cell to stop heavy chain gene rearrangement and move on to light chain rearrangement
2. IgM on surface expressed with lambda or kappa light chain (stop other light chain rearrangements) Critical step for further B cell development.

**A gamma globulin anemia** the inability to develop B cells. missing kinase tyrosine signals (Ig alpha and Ig Beta associate with tyrosine kinases when cross linked during normal binding with antigen. Activation of tyrosine kinases responsible for intracellular signal cascades) Missing or nonfunctional molecules in tyrosine kinase cascade leads to non development in pre B cell receptor cells because signals stop. Don=t get pre b cell receptor so can=t go on and rearrange their light chains. No B cells.

Tolerance is established in the bone marrow

- § Random arrangements of receptors means we could have some receptors that recognize self
- § If Ig M receptor on B cell does not recognize self antigens, then the B cell is allowed to continue proliferate, to continue to develop and to leave bone marrow to the periphery
- § If B cell recognizes self antigen present in bone marrow it has the option of:
  - § light editing (which occurs in over 50% of the cases)

- § there is a possibility of rearranging an up stream v or a down stream j from the initial v and j selected. If this edited vj rearrangement changes the specificity of the B cell receptor so that it does not recognize self the cell can live.

#### Light Chain Receptor Editing to Change the Specificity of Self reactive Clones

1. strong ligation of IgM by self antigen
2. arrest of B-cell development and continued light chain rearrangement: low cell-surface IgM
3. a new cell receptor specificity is expressed
4. if the new receptor is still self-reactive the B cell undergoes apoptosis
5. if the new receptor is no longer self-reactive, the immature B cell migrates to the periphery and matures

Light chain editing possible numerous (recombination sequences)

Heavy chain editing harder because the D=s have recombination sequences on both sides. The D that gets recombined uses both of the recombination sequences surrounding it and they are no longer available to recombine.

#### Ig gene status at different stages of development

<u>B Cell</u>	<u>Heavy Chain genes</u>	<u>Light-chain</u>	<u>Intracellular proteins</u>
early pro-B cell	DJ	germline (LC)	RAG-1, RAG-2, TdT, lambda 5 and V pre B (surrogate light chains)
late pro B cell	V-DJ rearrangement	germline	TdT, lambda 5 and V pre B
large pre B Cell	VDJ	germline	lambda 5 and V pre B
small pre B cell	VDJ rearrangement	V-J	mu, RAG-1, RAG-2

#### Antigen Independent B-Cell Development in Bone Marrow

1. DNA rearrangements establish the primary repertoire, creating *diversity*
2. Allelic exclusion ensures that each clone expresses a single antibody on the surface, establishing *specificity*
3. Deletion of self-reactive clones establishes *tolerance*

#### Antigen-Dependent B Cell Development in Periphery (spleen and Lymph Node)

1. Antigen and T Helper cells give B cells two signals
  - a. proliferate
  - b. differentiate
2. T cell dependent responses are refined two ways
  - higher affinity antibodies
  - IgG/A/E (switched) isotypes (All early B cells express Ig M)
3. Two products of B cell development (effector cells)
  - plasma cells secrete Ig (final effector)
  - memory cells respond to antigen for the second time

Peripheral development occurs in the spleen or in the lymph nodes (anatomical structures similar

in spleen and lymph nodes).

Mature B cells travel to the lymph node/spleen via the bloodstream. Exit arterial into T area of spleen/lymph node and encounter T cells. B cells that encounter cognate antigen and antigen specific T helper cell form primary foci from which proliferating cells migrate to the primary follicle, forming a secondary follicle with a germinal center. B cells that do not encounter cognate antigen continue to circulate through spleen and various lymph nodes for 2 to 3 weeks and then die.

If they do encounter cognate antigen:

- 5% go to marginal zone in spleen to encounter antigens that filter through the blood
  - T independent antigens such as bacterial poly saccharides encountered in these marginal zones and develop plasma cells that secrete low affinity IgM (first response)
  
- most B cells go the germinal center to develop
  - need T cells help
  - affinity maturation
  - Isotype switching
  - 10-12 days
  - memory cells and plasma cell
  - high affinity switched isotype

B cell can be activated by T cell dependent antigens and T cell independent antigens (usually two signals [antigen and T helper cell] are required to activate B cell to develop into plasma cell

1. T cell dependent
  - majority of cases require T helper cells to activate receptor response
2. T cell independent
  - some polysaccharides don't need 2<sup>nd</sup> signal
  - repeating epitopes trigger other receptors on the B cell to give second signal

The B cell receptor

1. Binds antigen, internalizes antigen, breaks antigen into small polypeptides and presents one or more polypeptides in the context of MHC II (T cell can only antigen presented on MHC)
2. Bound antigen gives signals to the B cell to proliferate and differentiate

T cell and B cell communication

(B cells signal T cells by presenting antigen with MHC II)

T cells provide 2 kinds of help to B cells:

1. Cell-cell signals from CD40L (on the T cell) /CD40 (B cell) and other surface molecules
2. secreted cytokines

T cell: B cells synapse

1. Helper T cell adheres to the B cell and begins to synthesize IL-4 and CD 40 ligand

2. The helper T cell reorients its cytoskeleton and secretory apparatus towards the B cell
3. IL-4 is released into and is confined to the space between the B cell and the T helper cell

#### Germinal Center

1. Affinity maturation
  - somatic hypermutation
  - selection for high affinity clones
2. isotype switch recombination
3. peripheral tolerance
4. final maturation to memory or plasma cell