B CELL DEVELOPMENT, continued

Ig and the stages of B cell development

\* Survival of B cells depends on **productive** gene rearrangement.

\* The chances of any given gene rearrangement being successful is one in three (due to there being three reading frames in a gene, only one of which encodes functional protein, and junctional diversity creates imprecise joining)

\* B cells that produce **unproductive rearrangements** at both H chain loci, or at all four L chain loci, are programmed to die by apoptosis

Figure 6.6

**Early pro-B cell**

- D and J rearrangement of the heavy chain starts on both chromosomes.

**Late pro-B cell**

- V-DJ rearrangement of the heavy chain on one chromosome.

- If that fails (unproductive, no protein produced), V-DJ rearrangement on the other chromosome. Heavy chain cannot try another VH because RSS is lost from DJ segment

- Probability of B cell survival during the pro-B cell stage

\* About half of the B cells are eliminated at the pro-B cell stage due to non-productive rearrangements.

**Large pre-B cell**

- If the VDJH recombination is successful in producing a functional heavy chain protein, the B cell expresses the pre-B cell receptor on the cell surface.

- pre-BCR: the  heavy chain is expressed on the cell surface with the **surrogate light chain**,which is composed of two proteins, **5** and **VpreB**. Also, **Ig**and **Ig** (analogous functions to CD3 and zeta chains associated with TCR) are in the complex. (Fig. 6.7)

- Pre-BCR signals to the B cell that a complete heavy chain has been formed and that no further rearrangement must occur for heavy chain🡪 **allelic exclusion**.

* **Btk** (Bruton's tyrosine kinase) is involved in this signaling since Btk-deficient individuals (disease: X-linked agammaglobulinemia; **XLA**) have a block in the pre-B cell stage and therefore few mature B cells.

- Expression of the pre-B receptor is followed by several rounds of proliferation, which results in ~100-fold expansion of B cells. Each of these will then rearrange its light chain - One type of heavy chain can therefore be joined with potentially 100 different light chains in 100 different B cells.

- Pre-B receptor expression is transient. In small pre-B cells the µ chain and Ig/Ig are retained in the ER, awaiting a functional light chain

**Small pre-B cell**

- Once proliferation is stopped, light chain rearrangement occurs in the small pre-B cell stage

- As with the heavy chain V-DJ rearrangement, light chain V-J rearrangement occurs one chromosome at a time (back to Fig. 6.10). Usually  first.

- However, if one V-JL rearrangement is non-productive, another V-J rearrangement on the same chromosome can happen (Fig. 6.9) using different V and J. Successive V-JL rearrangement attempts can happen on one chromosome before going on to the next chromosome. Together with using either  or , this allows a good portion (~85%) of the pre-B cells to accomplish a successful light chain gene rearrangement.

- Therefore once a B cell reaches the pre-B cell stage, it has a good chance of developing into an immature B cell.  and  isotypes have no functional differences but increase efficiency of B cell dev.

**Immature B cell**

- Immature B cells express IgM on the cell surface.

- Only a small portion will survive to become functioning, mature B cells.

- B cells start undergoing selection at this stage.

- **Allelic exclusion**: if the first allele is successfully rearranged, the other allele with not rearrange (1 B cell, 1 Ab specificity) (Fig. 6.8)

- mediated in part by shutting off expression of RAG-1 and RAG-2 (Fig. 6.12). They are expressed during the pro-B cell stage (necessary for the heavy chain rearrangement), inactive in the large pre-B cell stage when cells are proliferating, and then again re-synthesized for the light chain rearrangement (small pre-B).

Other regulated genes in B cell development

* Ig and Ig: They are expressed from the pro-B cell stage through the lifetime of a B cell.
* 5/VpreB expressed in pro-B cells and large pre-B, then turned off in small pre-B

Review: two **checkpoints** in B cell development ensure that B cells survive only if functional heavy and light chains produced (Fig. 6.11)

Summary slide: Fig. 6.16

* **B1 B cells** (Fig. 6.15)
* produced by a different developmental pathway
* arise early in embryonic dev, so called B1 to distinguish from later “classical” B cells called B2
* distinguished by expression of the surface molecule CD5 and by anatomical location (mostly in body cavities rather than blood and lymph). For example, peritoneal and pleural cavities have lots of B1 cells.

Also have restricted junctional diversity and secrete mainly IgM

B Cell Selection

**self antigens** = normal components of the cells and tissues that bind to surface Ig on B cells

often categorized as **monovalent** vs. **multivalent**

* examples: a protein in plasma such as albumin is a monovalent self Ag
* IL-7 secreted by bone marrow stromal cells is a monovalent self Ag
* a cell surface adhesion molecule on bone marrow stromal cells is a multivalent self Ag because a given stromal cell expresses many of these in the membrane and are not freely diffusing

Fate of immature B cells reactive with self Ag: (Figs. 6.17-6.19)

1) **Clonal deletion**: multivalent self Ag can crosslink (cluster) surface IgM and cause apoptosis.

2) **Receptor editing**: multivalent self Ag can crosslink (cluster) surface IgM on immature B cells reactive to multivalent surface self-antigen can further rearrange their Ab L chain genes to escape selection. RAG gene expression is induced again (Fig. 6.18) Not always functional rearrangement or new L chain might not overcome self reaction. Receptor editing is most common outcome, but if it fails then clonal deletion occurs.

3) Soluble self Ag bind IgM and cause B cell **anergy**. (Fig. 6.19)

- Characteristics of anergic B cells:

A) They retain their IgM in the cytoplasm and do not transport the protein to the surface.

B) IgD is still expressed but does not signal B cell activation when bound to Ag.

C) Generally die rapidly in peripheral lymphoid tissue—see below.

Note that the difference between surface IgM crosslinking and monovalent interaction produces a different signal to the cell to cause death vs. anergy.

4) No self-reaction: cells go on to become mature B cells (Fig. 6.17)

5) **Immunological ignorance**: some self-reactive immature B cells have very weak affinity to self Ag, or high affinity for a self Ag that is not accessible during the B cell “education” process, and these cells go on to mature. They will be held in check by the lack of T cell help, or continued inaccessibility to self Ag. Balance between elimination of ALL self-reactive cells and ability to have enough Ag specificity repertoire so that the body can fight off infection. They may be seeds of autoimmune cells later. Example: anti-DNA antibodies cause tissue damage in lupus.

**Mature B cells**: B cells that have undergone selection now express IgM and IgD on the surface by alternative splicing. They are also called **naive** B cells until they encounter antigens.

Recirculation

blood, secondary lymphoid tissues, lymph (Fig. 6.21, right panel)

within lymphoid tissues, B cells and T cells both exit the blood via the high endothelial venule (**HEV**) to enter the T cell area. T cells stay but B cells then migrate to the **follicles**. If no antigen encountered, B cells exit via the medulla and efferent lymphatics (Fig. 6.20).

Summary (Fig. 6.25)