LECTURE 38
HEMOPOIESIS, part 2

Chapt. 22, p. 1289-1296
Figs. 22-34 to –39; Tables 22-1 & 22-2.

HEMOPOIESIS: generation of blood cell (bc) types

- **hematopoietic stem cell (hsc):**
  - small subset of bone marrow (bm) cells
  - **Fig. 22-34:** X-ray-irradiation kills hsc's; lethal w/in a few days. Transfusion of bm cells from healthy, immunol. compatible donor can replenish all bc types in X-irradiated mouse & rescue from lethality
  - expt'l basis for bm transpl'ts in treatm't of human **leukemia**
  - Isolation/identif'n of hsc from bm: use **FACS** (fluorescence-activ'd cell sorter) to sort bm cell types based on diff't surface proteins, transf. fract'ns back into irradiated mouse to see which rescue
  - **multipotent progenitor** of blood cell lineages; fates of progeny cells can be traced experimentally
    - **retroviral DNA** or other **genetic marker** inserted in chromosome; marks all progeny cells

FIG. 22-35: Commitment is a stepwise process

- **Multiple cell division steps** generate progeny cells with progressively restricted fates
- **First step is commitment of hsc daughter cell to:**
  - common **lymphoid** lineage progenitor fate or
  - common **myeloid** lineage progenitor fate
- Further division steps: yield **"committed progenitor cells"** that produce just one cell type (T cells, B cells, neutrophils, etc)
- Steps marked by **changes in gene regulatory proteins** req for prod'n of subsets of bc types; complicated combinatorial regul'n involving GATA family TFs
  - Switch on genes for chosen diff'n pathway
  - Switch off genes for other diff'n pathways
  - Example: *Pax5 -/-* mice can't generate mature B lymphocytes; *Pax5 -/-* B-progenitors can be induced to produce non-B bc types; Pax5 req to activate B lymphocyte specific genes & also to repress genes driving other fate options (textbook doesn't say so, but new data suggests Pax TFs is regulated by Notch signaling)
- **Commitment:**
  - **Committed progenitor cells** go thru **multiple rounds of rapid division** (like epider. transit amplif cells) before generating differ'd cell types:
  - **amplifies** nr. of mature bc's attained from 1 hsc cell division
  - allows infreq't stem cell division (common to most tissues); reduces times stem cell replicates its DNA/potentially incurs replic'n errors

Fig. 22-36: Stem cells require contact signals from stromal cells

- **hsc's survive in culture** only if appropriate **signals** supplied
- long term mainten, reqs **cell:cell contact w/ appropr. supporting cells**
  - hemopoeisis goes on for months/yrs in vitro if hsc's cultured on **bm stromal cell layer**
  - suggests continual renewal of hsc's req's direct contact w/ str.cells

- **stem cell niche:** stromal cell:hsc contact keeps hsc in its multipotent, uncommitted state (similar to epidermal basal cell req for contact w/ basal lamina)
  - **Notch/Delta** signaling: hsc's & progenitors express Notch1; sromal cells express ligands
  - **Kit/SCF** signaling (espec. for rbc dev): hsc's & progenitors express Kit (an RTK) stromal cells express SCF (ligand for Kit); mutations in either cause **anemia** (few rbc) & defective melanocyte & germ cell production
  - In both cases, ligands are **membrane bound**

Factors that regulate hemopoiesis can be analyzed in culture

- **Table 22-2: Colony Stimulating Factors**
  - Identified/purified using **colony forming assay:**
    - hemat. progenitor cells **dispersed in semisolid matrix**; single cells survive, mitose to form clones ONLY if approp. soluble factors provided (clone size & cell type generated is progenitor-specific)
  - are **glycoprotein signaling proteins**: some soluble in blood/hormone-like, others in bm (membrane bound F's also included).
  - if approp. CSF's not present, commit'd progenit. cells die by apopt.
  - Recombinant CSF forms widely used to treat human patients/stimul. hemopoiesis/boost resistance to infection

Best known hormone-like CSF: erythropoietin (Epo), for erythropoiesis

- produced by **kidney; inducible by hypoxia (low O2) or loss of rbc's;** more Epo secreted into bloodstream to increase rbc production
- **Epo** required by **late** erythr. progenitors (colony forming cells (**CFC's**); each generates 80 rbcs) but NOT by **early** progenitors **(BFC's)** (latter need IL-3; each generates 5000 rbc's if IL-3 provided & Epo provided later)

Fig. 22-37: red blood cell (rbc) development

- Erythroblast (developing rbc) extrudes nucleus to become **immature erythrocyte (aka, reticulocyte)** which leaves bm, enters blood stream
- **Reticulocyte** loses mitochondria, ribosomes, & ER w/in 1-2 days, becomes **mature erythrocyte**
- **Erythocyte clones** dev in bm on **surface of macrophage** which phagocytoses the discarded nuclei

Neutrophils & Macrophages: generated from granulocyte/macrophage **(GM) progenitor cell** which requires mult. CSF's, some in combination, produced by various cell types (concs. of these CSF's increase rapidly in blood in response to bact. infect'n).

- **Fig. 22-38: Sharing of subunits by CSF receptors.**
  - Human IL-3 receptors & GM-CSF receptors have diff't α subunits & common β subunit. Binding of ligand to low affinity receptor (α subunit) triggers assembly of high affinity heterodimeric receptor.
- **Fig. 22-39:** Parameters regulating production of specific bc types via CSF's