Pathophysiology of Aplastic Anemia

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AA is characterized by peripheral blood pancytopenia and a hypocellular bone marrow without dysplasia or fibrosis

Aplastic anemia may arise in the setting of:

- *Inherited/congenital syndromes with a predisposition to bone marrow failure*
- *May develop secondary to toxic stressors in an otherwise normal host,*
- *May develop Without any apparent underlying cause*

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Classification of Aplastic Anemia

*Acquired

- Idiopathic

- Secondary

❖ **Radiation**

❖ **Drugs and chemicals**: chemotherapy; benzene, chloramphenicol; anti inflammatory drugs; antiepileptics.

❖ **Viruses**: EBV, Hepatitis, HIV

❖ **Immune diseases**: Hypoimmunoglobulinemia, SLE (uncommon), Thymoma

❖ **Pregnancy**

❖ **PNH**

❖ **Myelodysplasia**

*Hereditary (IBMFS)

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Acquired AA

Inherited bone marrow failure syndromes (IBMFS)

Hypoplastic myelodysplastic syndrome (MDS)

- IBMFS are more frequent in the pediatric population and comprise roughly 25–30% of cases of bone marrow aplasia in children.

- hypoplastic MDS can be difficult to differentiate from acquired AA (and IBMFS), especially in children.

Pediatr Clin North Am. Author manuscript; available in PMC 2014 December 01.

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Differentiation of Aplastic Anemia (AA) and Refractory Cytopenia of Childhood (RCC)[11]

	AA	RCC
Erythropoiesis		
Bone Marrow Histology:	Decreased Possible single large loci with <10 cells	Decreased Left shifted, mitosis, clustered +/− Dysplastic
Peripheral blood	Decreased reticulocytes	Increased MCV Increased fHb Increased reticulocytes
Granulopoiesis		
Bone Marrow Histology:	Decreased	Decreased Left shifted +/− Dysplastic
Megakaryopoiesis		
Bone Marrow Histology:	Decreased or absent	Decreased Dysplastic Micomegakaryocytes
Dysplastic changes in the bone marrow aspiratte	None	<10% in two cell lineages > 10% in one lineage
Reticulin in the bone marrow biopsy	No increase	No increase
Cellularity the bone marrow biopsy	<25%	Hypocellular
Severity of cytopenia in peripheral blood	Frequently severe or very severe	Frequently severe or moderate
Lymphocytes Bone Marrow Histology:	May be increased focally or dispersed	May be increased focally or dispersed
Blast in the bone marrow aspirate and biopsy	Not increased	<5% (<2% peripheral blood)
Cytogenetics:	Absent, transient	More prevalent than in AA
Numerical or structural chromosomal abnormality		

Retics, reticulocyte count; MCV, Mean Corpuscular Volume; fHb, fetal Hemoglobin

Baumann I, Fuhrer M, Behrendt S, et al. Morphological differentiation of severe aplastic anaemia from hypocellular refractory cytopenia of childhood: reproducibility of histopathological diagnostic criteria. Histopathology. 2012;61:10–17.

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Epidemiology of Aplastic Anemia

- Epidemiologic studies performed in Europe estimate the annual incidence of aplastic anemia is 2/million/year. By comparison, the incidence of acute leukemia is about 50/million/year.

- Aplastic anemia is more common in Asia (4-7/million/year) than in the West. Chloramphenicol, a known cause of aplastic anemia, has been widely used in Asia because of its efficacy and low cost; however, reductions in its use have not been accompanied by reductions in aplastic anemia incidence.

- An increased risk of aplastic anemia was also associated with animal exposures and ingestion of non-distilled water.

- The peak incidence is in adolescence and in young adults as well as in the elderly, with a roughly equal male-to female ratio.

Young N: Acquired aplastic anemia. In NS Y, editor: *Bone marrow failure syndromes*, Philadelphia, 2000, Saunders, pp 1–46.

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Pathophysiology of acquired Aplastic anemia

- Decreased numbers or defective function of the cellular or soluble components required for blood cell production or from exogenous factors that result in damage or elimination of hematopoietic cells.
- In model I, toxins such as irradiation, drugs, or chemicals or direct invasion by viruses might cause hematopoietic stem and/or progenitor cell death.
- In model II, an abnormality of the hematopoietic cells may result in a predisposition to hematopoietic stem cell damage, premature stem cell loss, or insufficient stem cell production.
- In model III, viruses, drugs, toxins, or immune dysregulation may incite a cellular or humoral immunologic reaction against a normal hematopoietic compartment.
- In model IV, abnormalities of the marrow stromal environment may actively inhibit hematopoiesis.

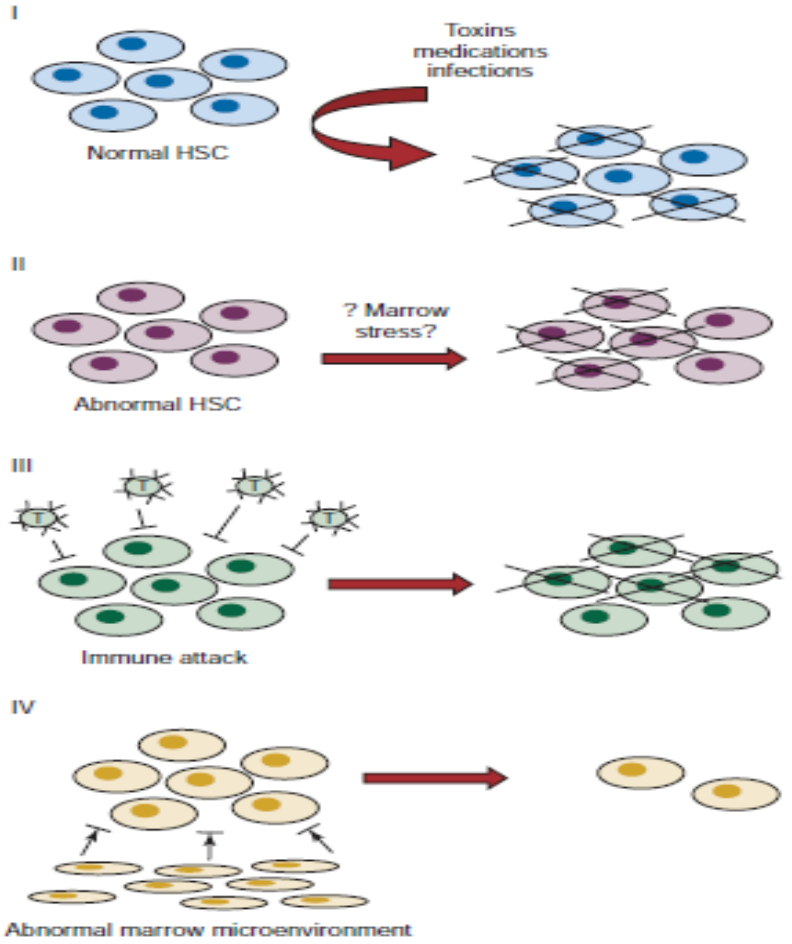

Figure 6-2 Models for the pathogenesis of aplastic anemia. I. Healthy hematopoietic stem cells and progenitors are damaged by exogenous agents such as toxins, medications, or infectious pathogens resulting in marrow aplasia. II. Abnormal marrow cells (e.g., inherited marrow failure syndromes) undergo premature attrition. Marrow aplasia might be exacerbated by external factors. III. Immune-mediated attack (cellular or humeral) eliminates hematopoietic stem cells and progenitors. IV. Abnormal marrow microenvironment impairs hematopoiesis. *HSC*, Hematopoietic stem cell.

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Severity of Aplastic Anemia

- *severe aplastic anemia:* At least two of the following anomalies:

Neutrophil count below 500/µL, platelet count below 20,000/µL, and an absolute reticulocyte count less than or equal to 40,000/µL. In addition the bone marrow biopsy must contain less than 25% of the normal cellularity or less than 30% hematopoietic elements.

- *Very severe aplastic anemia* is defined by a neutrophil count less than 200/µL.

- *Mild* or *moderate aplastic anemia*, sometimes called *hypoplastic anemia*, is distinguished from the severe form by the presence of mild or moderate cytopenias, but still deficient bone marrow cellularity.

Camitta BM, Thomas ED, Nathan DG, et al: Severe aplastic anemia: A prospective study of the effect of early marrow transplantation on acute mortality. *Blood* 48:63–70, 1976.

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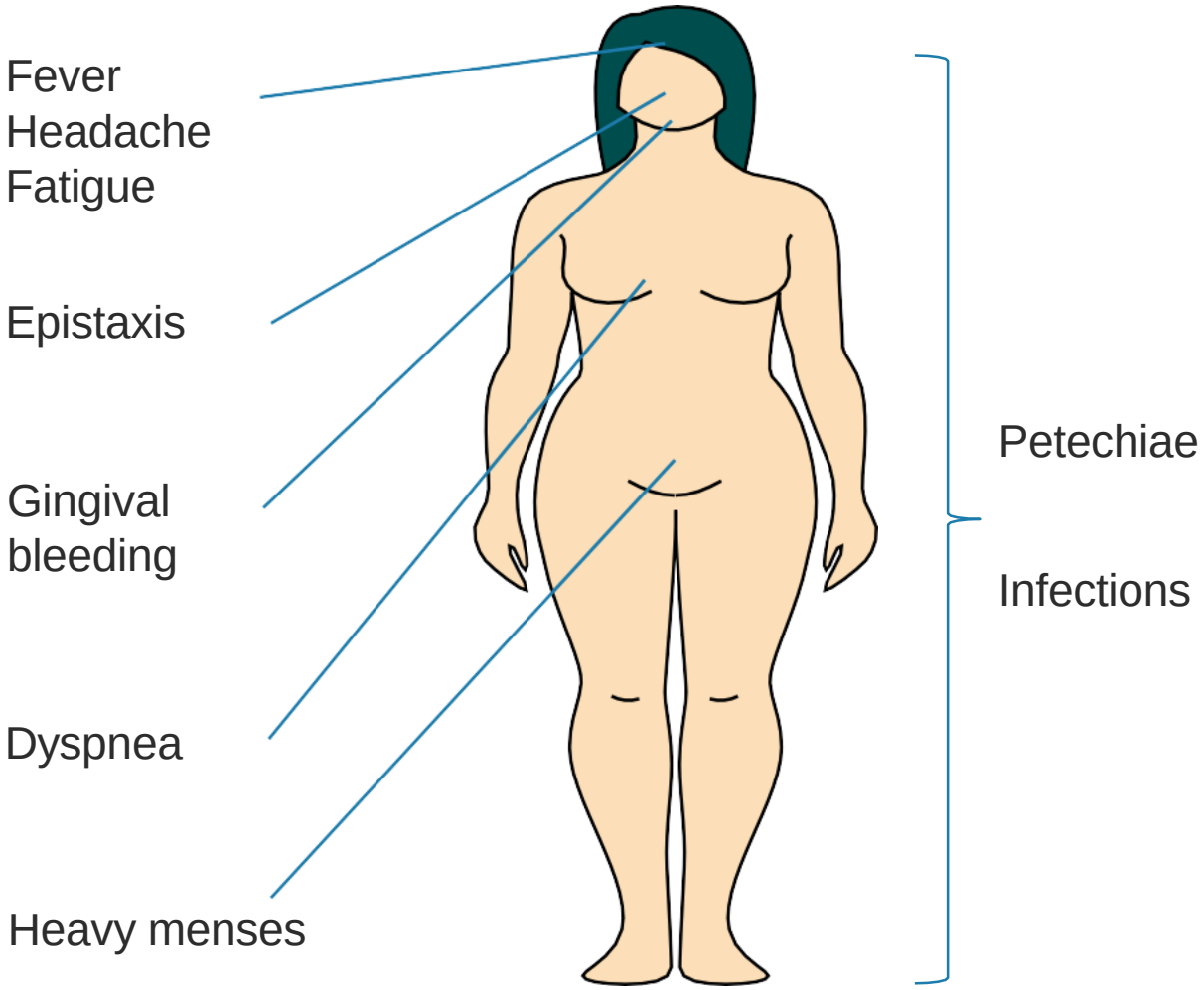
Clinical manifestations of AA



Non-severe AA	Severe AA	Very severe AA
▪ AA not fulfilling criteria for severe or very severe	▪ Marrow cellularity <25%* AND two of the following: ▪ Neutrophils $<0.5 \times 10^9$/L ▪ Platelets $<20 \times 10^9$/L ▪ Reticulocytes $<40 \times 10^9$/L	▪ Same as severe, but neutrophils $<0.2 \times 10^9$/L

*Or <25–50% with <30% residual HSCs

Figure: Brodsky RA & Jones RJ. *Lancet* 2005;365:1647–1656; Young NS & Kaufman DW. *Hematologica* 2008;93:489–492 Table: Killick SB *et al. Br J Haematol* 2016;172:187–207

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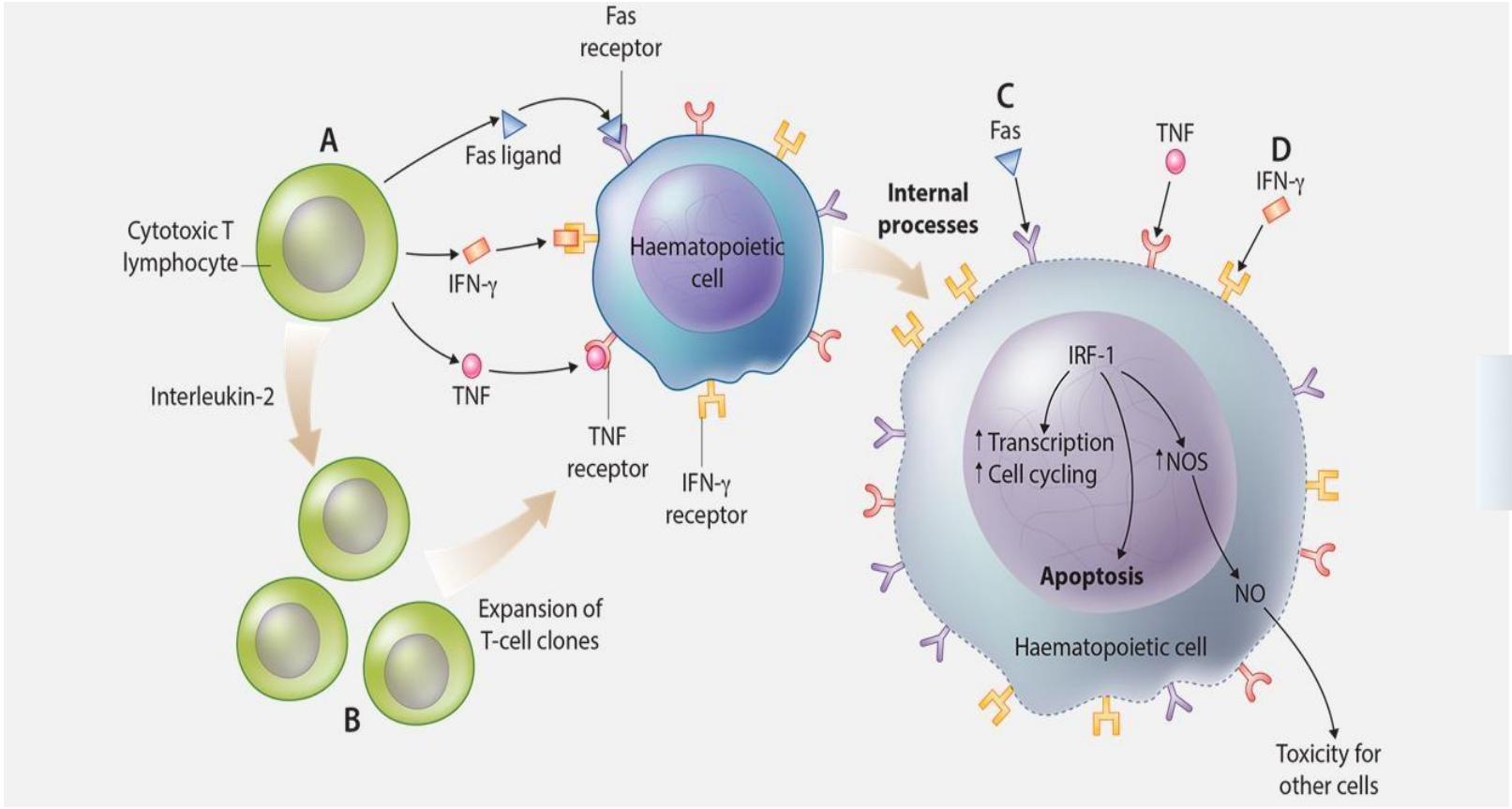
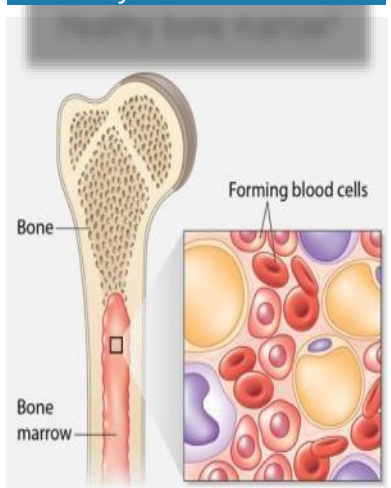
Bone marrow destruction is stimulated by an immune-mediated attack on HSCs

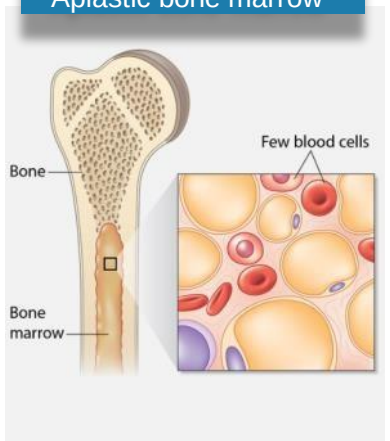
Proposed pathophysiology of AA[1]



Healthy bone marrow[3]



Aplastic bone marrow[3]



An immune response by an expanded cytotoxic T-cell population, targets HSCs and progenitor cells[2]

- **Directed at CD34⁺ cells[2]**
- **T cells induce apoptosis and hematopoietic failure[2]**

1.Young NS & Maciejewski J. The pathophysiology of acquired aplastic anemia. *N Engl J Med* 1997;336:1365–1372. Reprinted with permission from Massachusetts Medical Society ©1997 Massachusetts Medical Society.

2. Young NS *et al. Curr Opin Hematol* 2008;15:162–168; 3. University of Minnesota Medical Center, Fairview, https://www.fairview.org/HealthLibrary/Article/40317

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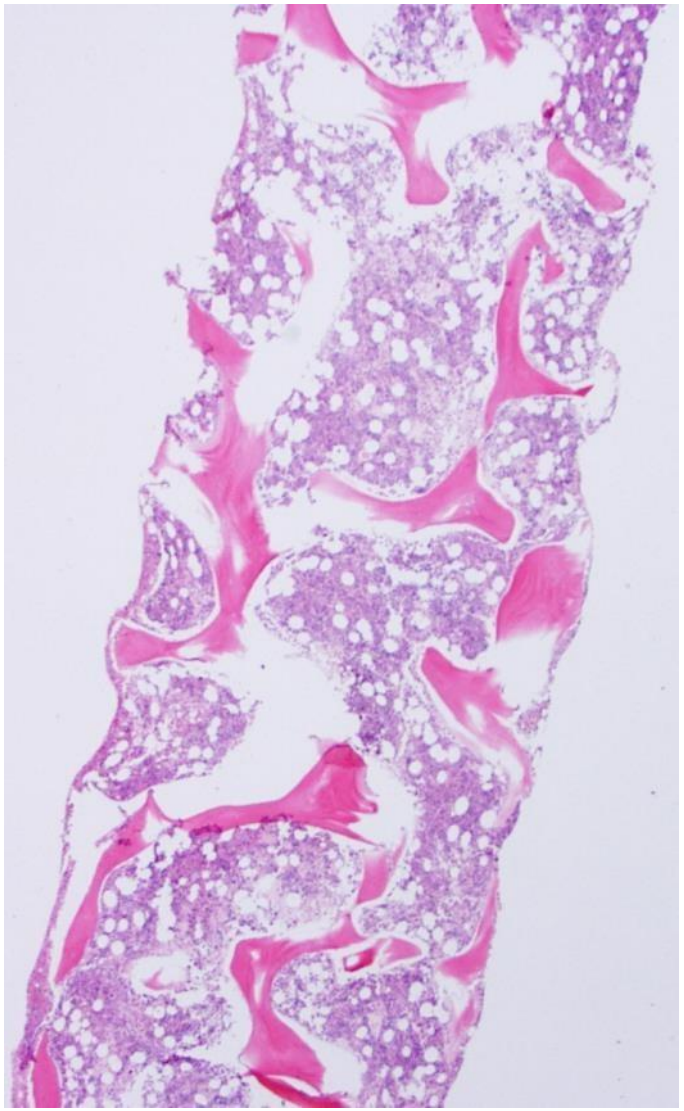
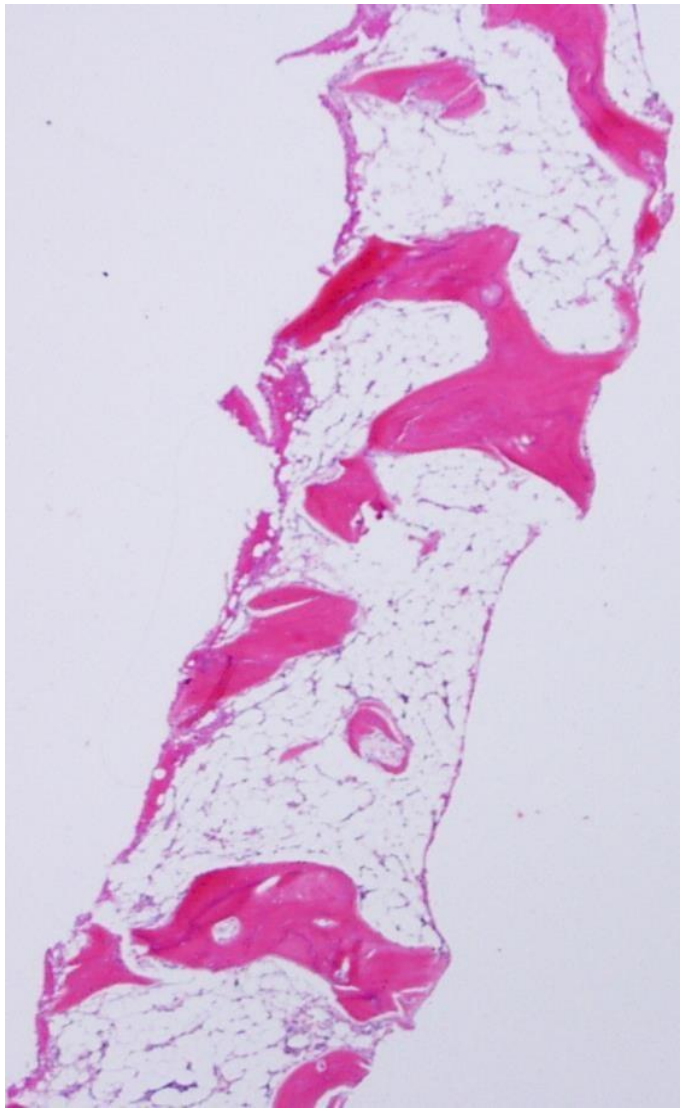
Aplastic anemia is characterized by the destruction of bone marrow





AA, aplastic anemia

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Stem Cell defect theory

- Patients with AA have decreased numbers of marrow progenitor cells, such as myeloid colony-forming cells (CFU-GMs); erythroid colony-forming cells (CFU-Es) and burst-forming units erthyroid [BFU-Es]); megakaryocytic progenitors (CFU-mega); multipotent colony-forming cells (CFU granulocyte, erythrocyte, monocyte, megakaryocyte [CFU-GEMMs]); and *long-term culture-initiating cells* (LTC-ICs).

- Almost all patients show severe reduction in the numbers of CD34+ cells and also poor efficiency for colony formation from purified CD34+ cells. CD34 + cells define a compartment of about 1% of marrow cells (that include progenitors and activated stem cells).

- In line with the theory of "stem cell defect", multiple studies have noted that infusion of stem cells from an identical twin donor was sufficient to treat the aplasia in many cases.

- These results strongly support a **pathogenic stem cell defect** and argue against permanent disorders of the bone marrow environment, including immunologic mechanisms, as the cause of stem cell destruction.
- A primary stem cell defect likely contributes to the observation of clonal hematopoiesis in aplastic anemia as well as late development of other hematologic diseases such as myelodysplasia and acute leukemia.

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Telomere shortening theory

- Telomeres are specialized structures that stabilize the ends of each chromosome to prevent excessive shortening and end-to-end fusions.
- Telomerase is a ribonucleoprotein enzyme that when the levels are diminished or absent, telomeres progressively shorten with each cell division.

- Shortened telomeres have been observed in the leukocytes of aplastic anemia patients.
- Telomere shortening is a common feature associated with bone marrow failure, though telomere lengths are particularly short in patients with dyskeratosis congenita, in comparison to other marrow failure patients.

- Whether telomere shortening in patients with marrow failure represents ***a primary defect or a secondary effect*** of increased cycling of the remaining hematopoietic stem cells remains to be clarified ???
- Shorter telomere lengths have been associated with a higher risk of relapse and clonal progression with decreased survival in patients with AA.

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Pathogenesis of Aplastic Anemia
Immune mediated

- For many years, an immune-mediated pathogenesis has been postulated for AA because Immunosuppressive Therapy (IST) is often successful in the treatment of AA

- Bone marrow lymphocytes from AA patients can suppress normal bone marrow *in vitro*.

- Results from numerous laboratories have demonstrated increased cytokine expression, low CD4 T regulatory cells, oligoclonal CD8 cytotoxic T cells, and to a lesser extent, expansion of specific CD4 cell populations in the bone marrow of AA patients.

- The recent finding of acquired copy number neutral LOH of the short arm of chromosome 6 (6pLOH), represents a likely genetic signature of immune escape

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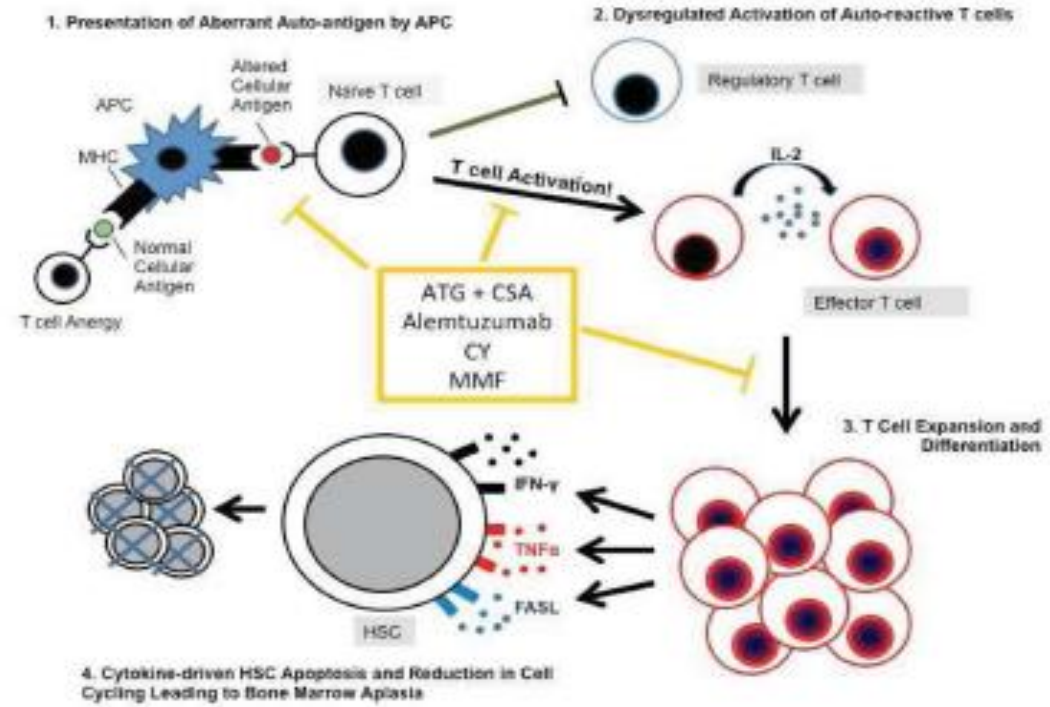

Figure 3.
Current evidence suggests that acquired AA results from the aberrant activation of one or more auto-reactive T cell clones due to alteration of antigens presented by the Major Histocompatibility Complex (MHC) on the surface of Antigen Presenting Cells (APC). This antigen alteration is triggered by viral infection, chemical exposure, or genetic mutation, and leads to the inappropriate activation of antigen-specific effector T cells and decreased activity of regulatory T cells, which normally serve to prevent auto-immunity. T cell activation leads to IL-2-driven expansion and differentiation of T cells into effector and memory T cells. These pro-inflammatory T cells produce a variety of cytokines, including FAS Ligand (FASL), interferon-γ (IFN-γ), and Tumor Necrosis Factor α (TNFα), which 1) induce HSC apoptosis and 2) alter gene regulation and decrease protein synthesis to prevent HSC cell cycling, ultimately leading to bone marrow failure. Immune suppression therapy disrupts T cell-driven HSC destruction by inhibiting T cell responses at several points along this pathway.[85,86]

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Immune dysregulation and lymphocyte alterations/lymphokines

- Several lines of data suggest that immune abnormalities may result in AA.
- It is suggested that abnormal presentation of “self-antigens” leads to CD8 activation against HSC in the bone marrow.
- Multiple reports have demonstrated an association of AA with alterations in lymphocyte numbers or specific immunologic changes.
- Increased TNF and IFN-γ production by peripheral blood mononuclear cells from patients with AA is reported.
- Dominant clones of T-cells/ activated cytotoxic T cells/ reduced (Tregs: CD4+, CD25+, FoxP3+) have reported in AA, which increased after immunosuppression therapy (IST). IST is followed by normalization of lymphocyte subsets with hematologic recovery.

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Immune dysregulation/Lymphokines

- Whether overproduction or dysregulated production of inhibitory cytokines represents a primary etiology *or* a secondary effect of an underlying bone marrow abnormality remains unclear.

- IFN-γ is expressed in the marrow of most patients with aplastic anemia.

- Other inhibitory cytokines, such as *TNF* and *macrophage inflammatory protein-1,* are also overexpressed in aplastic marrow. Both γ-interferon and TNF directly and synergistically inhibit hematopoiesis in vitro.

- Both γ-interferon and TNF increase the potential for programmed cell death within the CD34+ compartment (by increasing Fas antigen expression on target cells= increased apoptosis)

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Microenvironment defect theory

- AA could occur due to a microenvironment that fails to support hematopoiesis: a lesion of “soil” rather than “seed.

- Currently, there is no definitive evidence of a link between defective marrow microenvironment and development of acquired AA.

- More recently interest has focused on defining specific anatomic and/or biochemical spaces (niches) in the bone marrow occupied by hematopoietic stem cells.

- Schofield first termed the phrase ***stem cell niche*** to define a supporting cellular environment in which hematopoietic stem cells reside.

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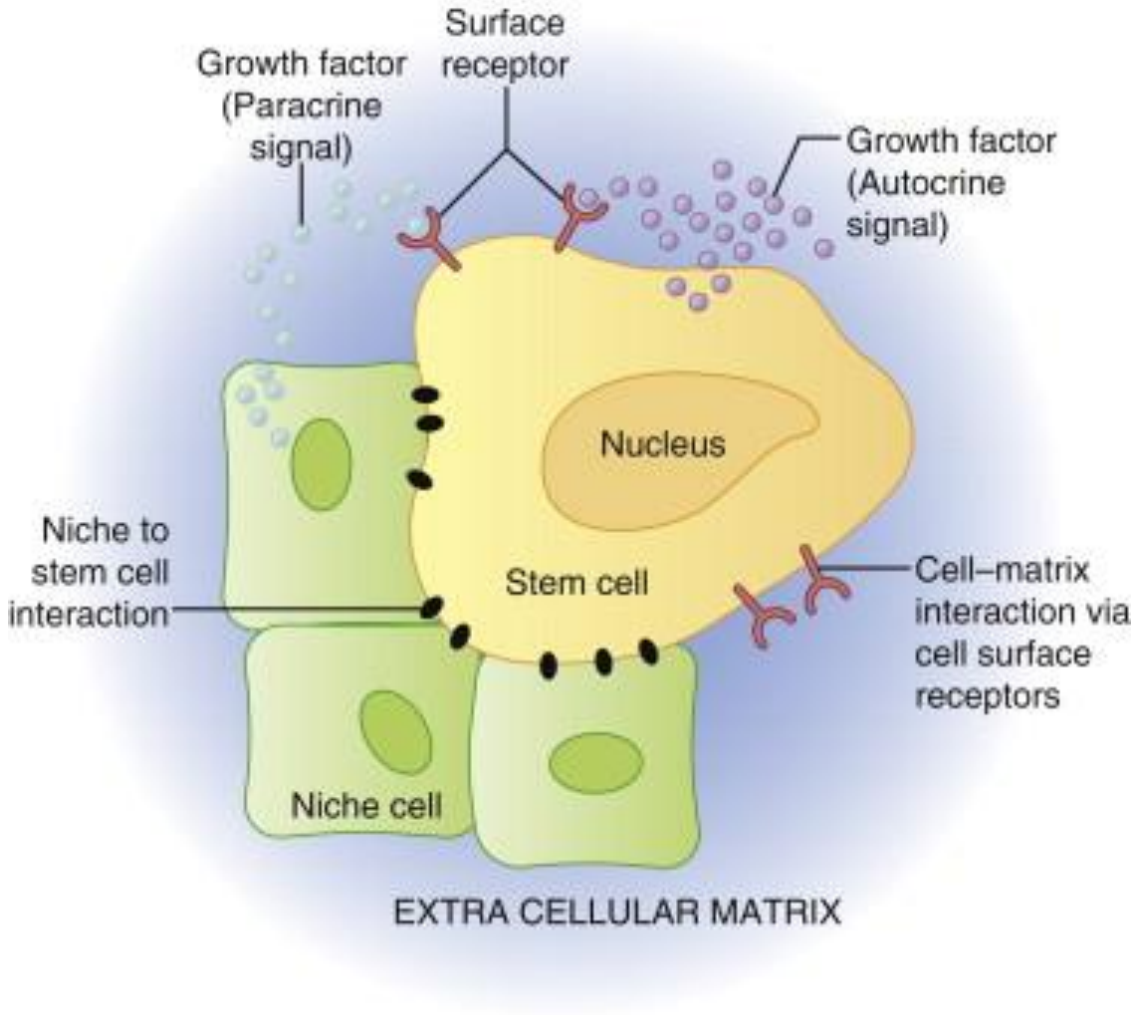
Stem Cell Niche

A stem cell niche can be defined as an environment where supporting cells release factors that promote stem cell maintenance, regulating self renewal and differentiation.



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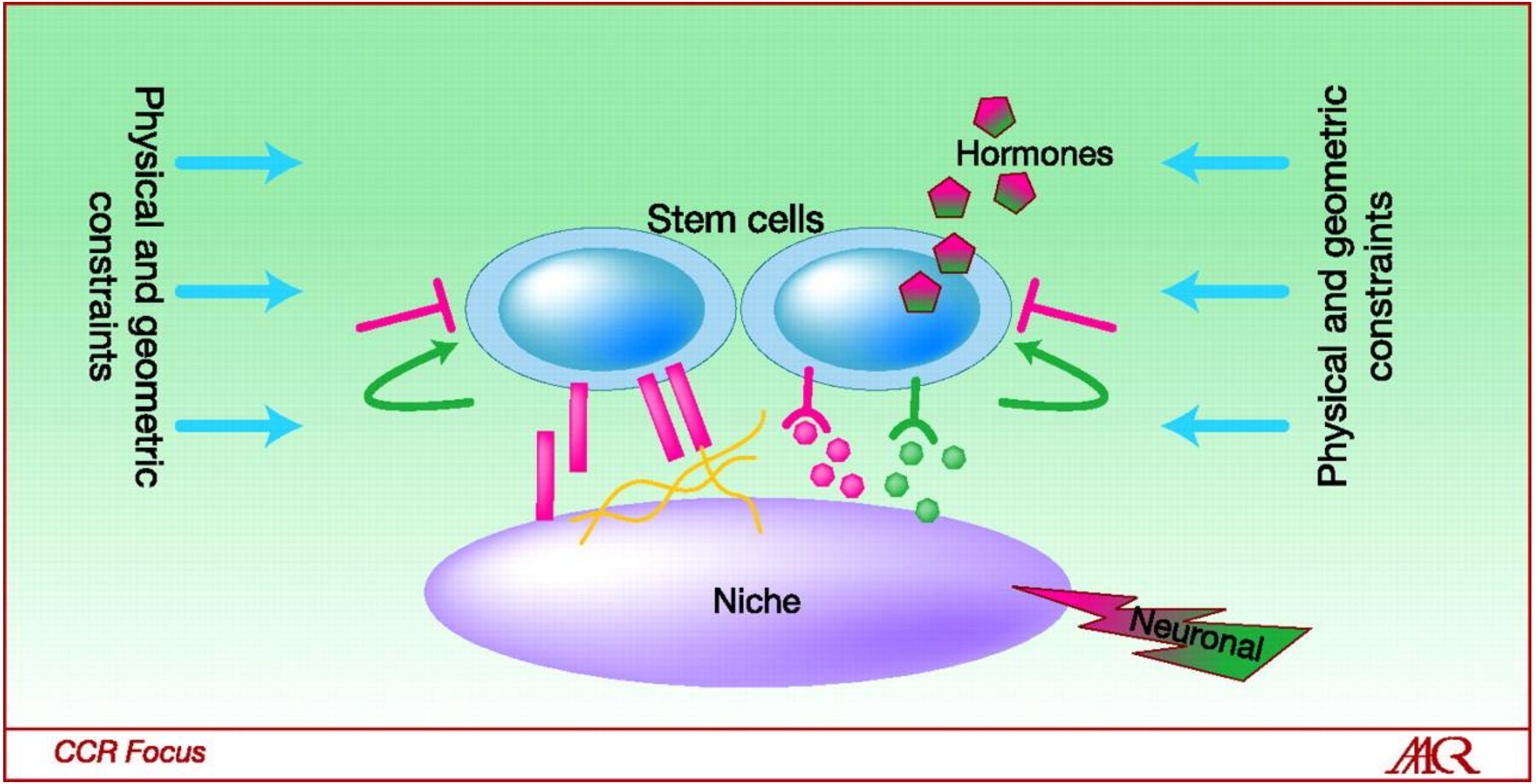
Physical and geometric constraints
Hormones
Stem cells
Physical and geometric constraints
Niche
Neuronal

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osteoblastic niche

- HSCs reside in close proximity to osteoblasts that line the cortical bone of the marrow space

- Osteoblasts may be important in maintaining dormancy of the HSCs. This area of the marrow has been termed "*osteoblastic niche".*

- A subset of osteoblasts; residing in trabecular bone, may be derived from mesenchymal stem cells, and their number correlate with the content of long-term HSCs.

- Ablation of osteoblasts leads to loss of HSCs and failure of hematopoiesis, while manipulations that increase osteoblasts have the opposite effect.

Schofield R: The relationship between the spleen colony-forming cell and the haemopoietic stem cell. *Blood Cells* 4:7–25, 1978.
Yin T, Li L: The stem cell niches in bone. *J Clin Invest* 116:1195–1201, 2006.
Zhang J, Niu C, Ye L, et al: Identification of the haematopoietic stem cell niche and control of the niche size. *Nature* 425:836–841, 2003.

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perivascular niche

- An anatomically distinct HSC niche made up by sinusoidal endothelial cells has been termed *"perivascular niche".*

- This niche may be critical for mobilization and homing of HSCs and/or for progenitor cell proliferation and differentiation.

- Some models suggest that dormant HSCs may migrate from the osteoblastic niche to the perivascular niche via chemokine/growth factor stimulation.

- Overall, whether there are truly differences between these hematopoietic niches remains to be determined, and the role of specific growth factors, adhesion molecules, and chemokines are incompletely understood.

Kiel MJ, Yilmaz OH, Iwashita T, et al: Slam family receptors distinguish hematopoietic stem and progenitor cells and reveal endothelial niches for stem cells. *Cell* 121:1109–1121, 2005.

Hematopoietic growth factors changes in AA

- Hematopoietic growth factor production and plasma levels are usually increased rather than decreased in patients with AA.

- Circulating levels of erythropoietin, G-CSF, GM-CSF, thrombopoietin, and flt-3 ligand are elevated in patients with AA.

- Therapeutic trials with these factors have yielded divergent and incomplete responses casting doubt on the pathophysiology of deficiency of these factors.

- The report of inhibitory effects of marrow adipocytes on hematopoiesis in mouse models is exciting, given the typical fatty replacement observed in AA bone marrows

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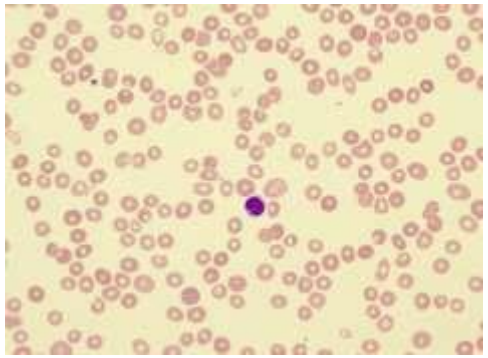
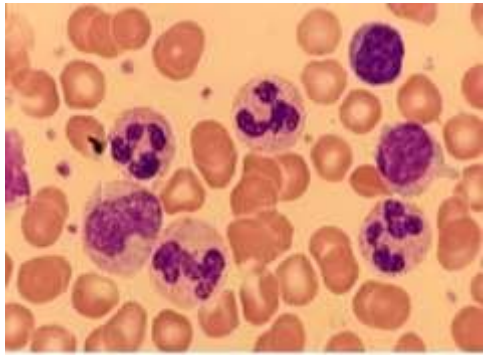
Bone Marrow Failure Syndromes

Idiopathic Aplastic Anemia
Constitutional (Congenital/ Inherited) Aplastic Anemia
Myelodysplastic Syndrome (MDS)
Paroxysmal Nocturnal Hemoglobinuria (PNH)

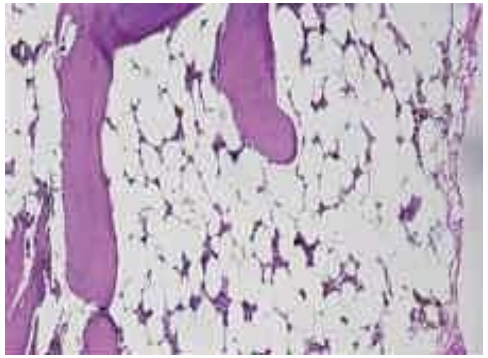
Peripheral blood showing pancytopenia

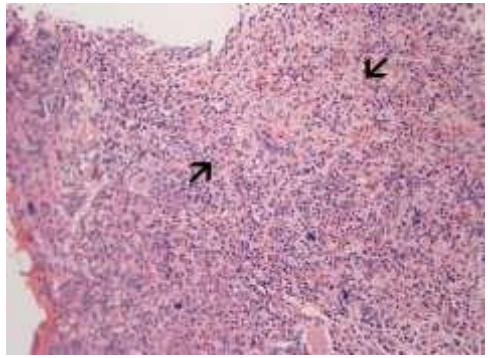




Normal peripheral blood

Fatty bone marrow in aplastic anemia





Normal cellular bone marrow

moon.ouhsc.edu/kfung/JTY1/Com05/Com509-1-Diss.htm
http://www.healthsystem.virginia.edu/internet/hematology/HessImages/Aplastic-Anemia-Pancytopenia-and-macrocytes-40x-website.jpg

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Inherited bone marrow failure syndromes (IBMFSs)

- Bone marrow failure may manifest as an isolated cytopenia (pure red cell aplasia, neutropenia, or thrombocytopenia) or as pancytopenia with the clinical picture of aplastic anemia.

- The affected pathways often involves “housekeeping” functions important for most cell types (DNA repair, telomere maintenance, or ribosome biosynthesis) rather than functions unique to hematopoietic cells.

- Why hematopoiesis is preferentially and sometimes even exclusively affected by a is exciting.

- One common feature of these pathways is activation of p53, suggesting that this molecule plays a central role in the pathogenesis of IBMFS.

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Inherited bone marrow failure syndromes (IBMFSs)

- IBMFS are underdiagnosed, in both pediatric and adults
- The differential diagnosis must be considered when a patient presents with pancytopenia due to apparently acquired aplastic anemia
- we estimate that about 30% of childhood AA is due to “Fanconi Anemia” or other syndromes, the proportion among adults is unknown.
- Appropriate classification of patients is imperative, since it impacts on medical and transplant management, choice of stem cell donors, estimated risks for complications including future neoplasms, and genetic and medical counseling

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Primary stem cell abnormality

- The inherited bone marrow failure syndromes (IBMFS) are often associated with characteristic physical findings; But, some patients may present with isolated aplastic anemia as the sole manifestation of their inherited syndrome.

- In some patients lacking significant physical findings or cytopenia at diagnosis, an elevated MCV may be the only clue to an underlying IBMFS.

- A careful family history for hematologic abnormalities, malignancy predisposition, or other clinical stigmata may be helpful in distinguishing an IBMFS.

Fanconi Anemia (OMIM#227650) - A defect in DNA repair

- This entity was first reported in 1927 by Guido Fanconi, who described 3 siblings with progressive pancytopenia, physical anomalies and predisposition to malignancy (particularly acute myelogenous leukemia).

- The disease is autosomal recessive involving one of the 13 FA genes (FANC-A, B, C, D1, D2, E, F, G, I, J, L, M and N), which regulate DNA repair and cell cycle.

- The disorder is heterogeneous, characterized by hypersensitivity to chromosome-breaking agents (diepoxybutane, DEB).
- Heterozygotes for FA genes (e.g., FANCD1/BRCA2) have an increased risk of breast and other cancers.

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Pathophysiology of Fanconi Anemia

- FA is a multigenic disorder with 13 genes currently identified. With the exception of the X-linked FANCB gene, the remaining 12 FA genes are autosomal recessive.
- The encoded FA proteins function in the repair of DNA crosslinks. Additional functions of the FA proteins are response to stress signaling and apoptosis in response to oxidative damage and inflammatory cytokines.
- FA genes had been previously identified as cancer susceptibility genes involved in DNA repair.
- Nijmegen breakage syndrome, a chromosomal instability syndrome shares many clinical features with Fanconi anemia.
- Bloom's syndrome , a genomic instability syndrome is also characterized by increased sister chromatid exchange.
- Although patients with other genomic instability syndromes, such as ataxia telangiectasia, exhibit genotoxin sensitivity and cancer predisposition, marrow failure is generally not a typical clinical feature.

Blood Rev. 2010 May ; 24(3): 101–122. doi:10.1016/j.blre.2010.03.002.

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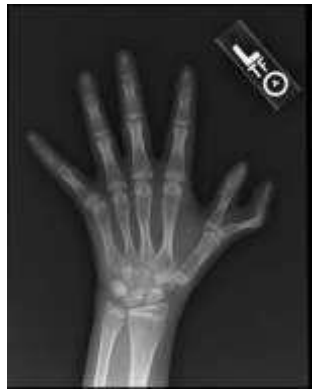
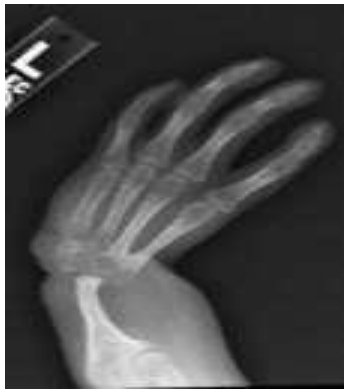
Fanconi Anemia: Birth Defects

- Skeletal anomalies (short stature, abnormal thumbs, abnormal thumbs and radi, microcephaly).
- Altered skin pigmentation (café au lait spots, hyperpigmentation, hypopigmentation).
- Other congenital malformations (abnormal gonads, eye anomalies, renal defects, low birth weight, developmental delay, abnormal ears or hearing).

Café au lait spot



Bifurcated thumb



Absent thumb & radius

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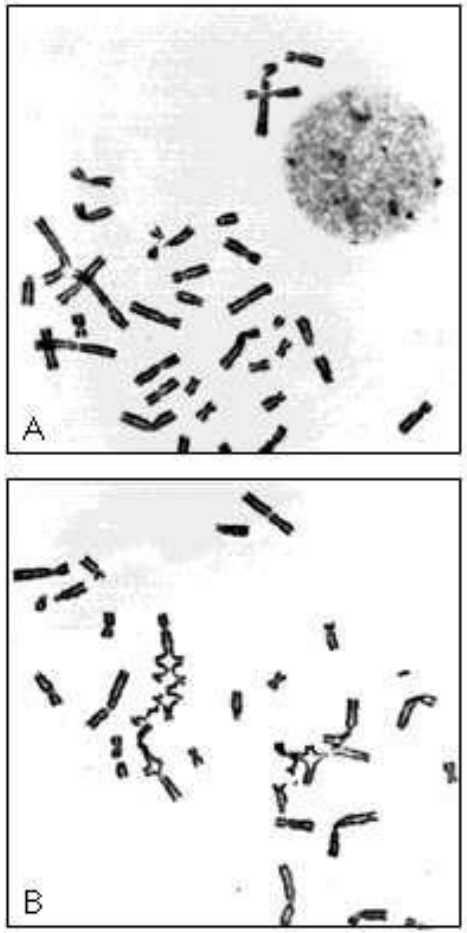
Fanconi Anemia: Chromosome Breaks



Increased chromosome breaks with diepoxybutane (DEB).

Molecular diagnosis has improved the diagnosis of Fanconi anemia.

← treated with 0.1 µg/mL DEB

Arleen D. Auerbach: Diagnosis of Fanconi Anemia by Diepoxybutane Analysis. Rockefeller University, New York. Current Protocols in Human Genetics , UNIT 8.7, 10.1002/0471142905.hg0807s37 , April, 2003.

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Fanconi Anemia

- Diagnosis is usually confirmed by demonstration of chromosomal aberrations in blood lymphocytes cultured with a DNA-crosslinking agent such as DEB or mitomycin C (MMC)

- The next step is determination of the complementation groups . Gene sequencing can then be performed to determine the relevant mutations.

- A significant proportion of patients with FA have hematopoietic somatic mosaicism. For these cases, skin fibroblast cultures are required to demonstrate sensitivity to DNA-damaging agents.

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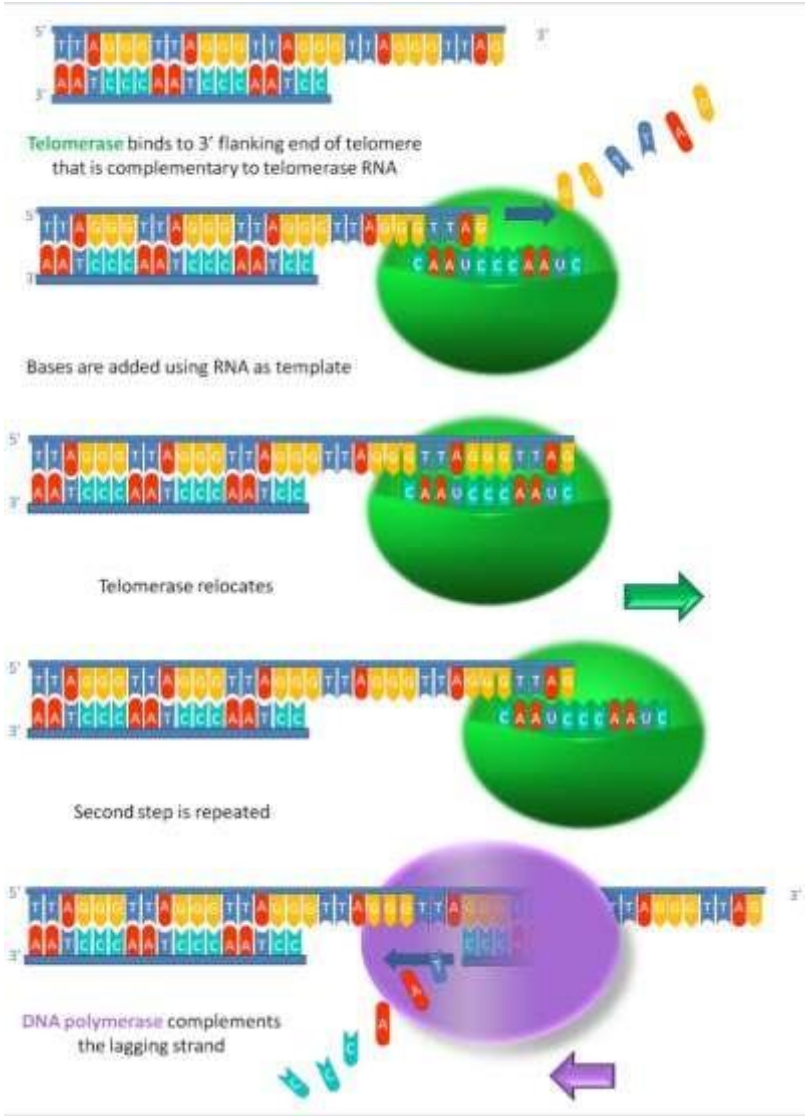
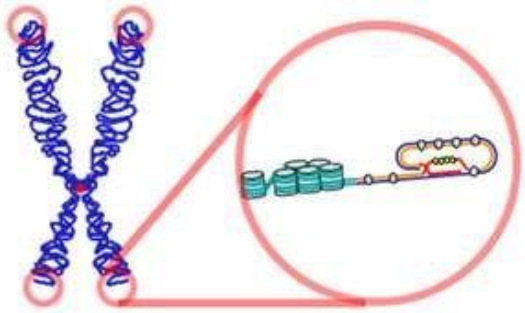
Genetic Susceptibility to Marrow Failure in Dyskeratosis Congenita

- Mutations in telomere repair genes, e.g., *TERT* , the gene for telomerase reverse transcriptase (a ribonucleoprotein polymerase that maintains telomere ends

- Inheritance of such mutations results in short telomeres in the hematopoietic cells, which predispose to apoptosis.



Nail dystrophy in DC



A **telomere** is a region of repetitive nucleotide sequences (TTAGGG) at each end of a chromatid, which protects the end of the chromosome from deterioration.
The telomere is short in dyskeratosis congenita (DC; OMIM#613989).

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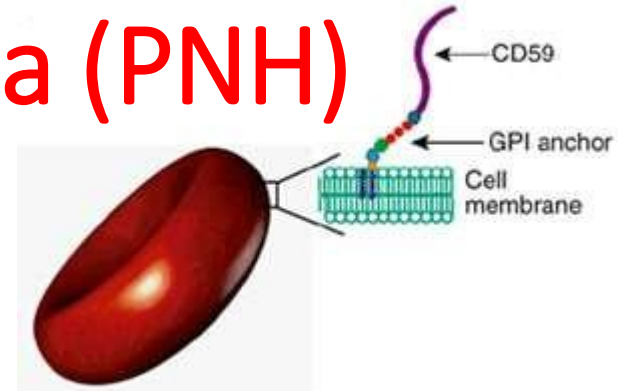
Paroxysmal Nocturnal Hemoglobinuria (PNH)



- PNH is a clonal hematopoietic stem cell disorder caused by somatic de novo (neither transmitted nor parent possessed) mutation in the phosphatidyl inositol glycan class A (PIGA) gene (OMIM#311770) on the X chromosome.
- PIGA protein is required for formation of the phosphatidylinositol (GPI) anchor.
- Its absence results in missing many membrane proteins, including inhibitors of the complement cascade.
- Red cells are especially sensitive to the hemolytic effect of complements.
- The disease usually evolves as an abnormal clone in the milieu of a bone marrow failure syndrome.

J Cell Biochem 1986;30:133-170

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Myelodysplastic Syndrome

- MDS (myelodysplasia) is ineffective hematopoiesis (cellular bone marrow + pancytopenia) resulting from an evolving clone of genetically injured hematopoietic stem cells.
  - Cytogenetic abnormalities exist in most patients; most commonly involving chromosomes 5, 7 and 8.
- Cases could be sporadic (de novo) or result from stem cell injuries, e.g.,
  - Cyclophosphamide (AML-associated with monosomy 5 or 7; (del)5q or (del)7q)
  - Etoposide (AML-associated with rearrangements involving the mixed lineage leukemia, MLL [MIM#602409], gene on chromosome 11q23)
- Patients present with uni-lineage, bi-lineage or tri-lineage (pancytopenia), progressing to acute myelogenous leukemia in many of the cases.

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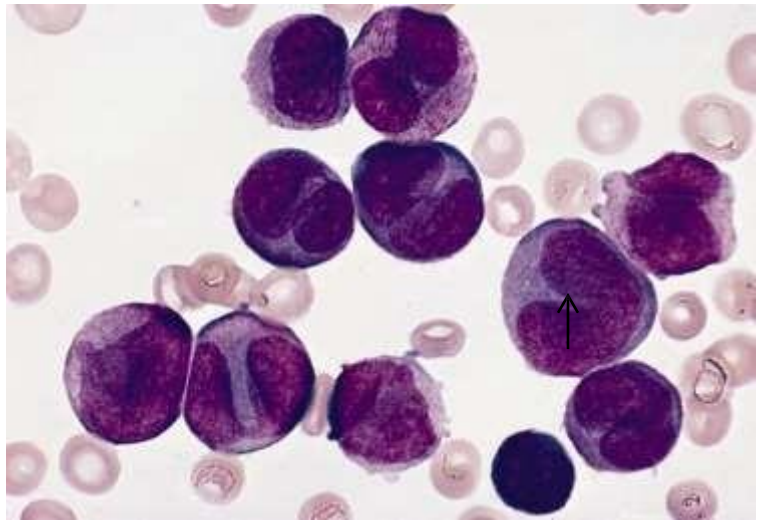
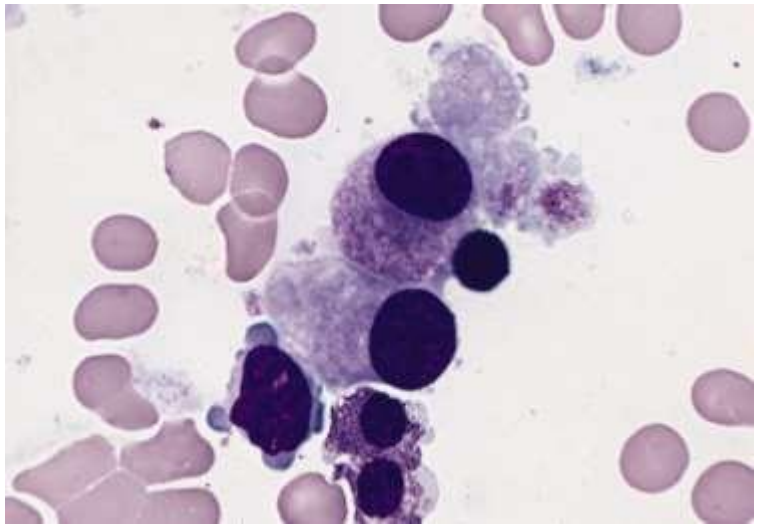
Chromosome 5q Deletion Syndrome (OMIM#153550) [Macrocytic anemia, refractory, due to 5q deletion, somatic]

- It primarily affects older females. It presents with anemia and dysmorphic hematopoiesis (lobulated erythroblast nuclei and hypolobulated micromegakaryocytes). The platelet count is normal or high. The disease is indolent and has low propensity to evolve into AML.
- It requires supportive care (erythropoietin, granulocyte–colony stimulating factor, transfusions and antibiotics).
- Allogeneic stem cell transplantation is curative.



Lobulated erythroblast nuclei
pathologyoutlines.com/images/marrow/048.jpg



Hypolobulated micromegakaryocytes
PEIR Digital Library (Pathology image database

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Diamond-Blackfan Anemia (DBA, OMIM#105650) - Ribosomopathy

- DBS is characterized by isolated anemia (↑MCV, ↑erythrocyte adenosine deaminase [eADA], and ↑hemoglobin F) with severe reticulocytopenia and absence of marrow erythroid precursors. The neutrophil, lymphocyte and platelet counts are normal.
  - It appears in early life and may improve with glucocorticoids.
  - Congenital malformations occur in 50% of the patients (e.g., cleft palate, thumb defect).
- Patients have mutations in RPS19 [**r**ibosomal **p**rotein **S19**; autosomal dominant], RPL11 [ribosomal protein L11], or GATA1; encodes a zinc finger DNA-binding transcription factor that is critical for the development of hematopoiesis).

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