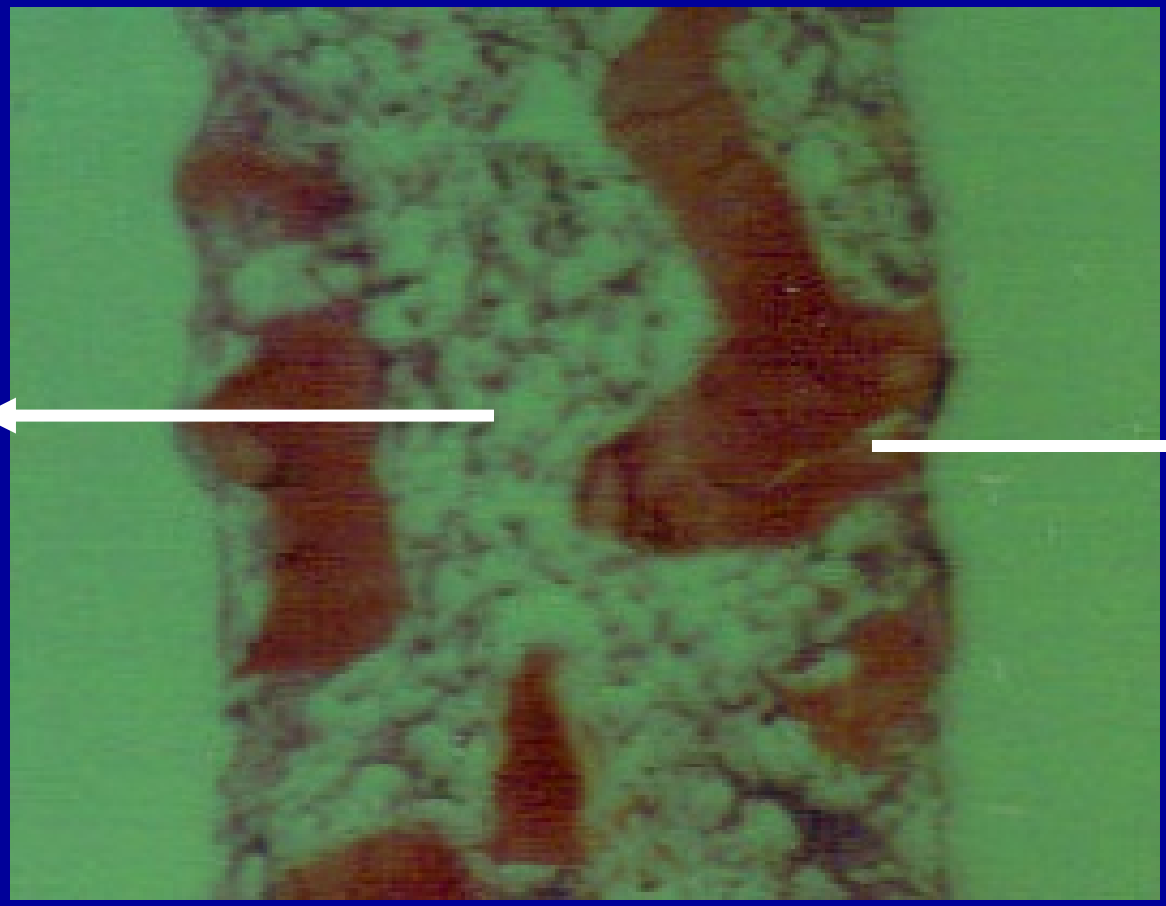
APLASTIC ANEMIA,
one of the states of Bone Marrow failure

Marion S. Sternbach, MD, FRCP(C), FACP

Aplastic Anemia - Definition

- Aplastic anemia was described by Ehrlich in 1888 in a pregnant woman.
- The term is a misnomer, since all three hematopoietic cell lines disappear gradually from the bone marrow without replacement by other cell lines.
- This defines it and differentiates it from hematopoietic replacement by : fibrosis, tumors, leukemias, granulomas, fungi, etc.

Aplastic Anemia - pathology



Aplastic Anemia (AA) – Classification and Etiology

- A. Acquired.
- B. Congenital
- Incidence : Rare hematological disease in Caucasians 3-5 / Million population/ year.
- In Orientals up to 10 - 15 / Million population / year.

Aplastic Anemia (AA) – classification and etiology continued

- 1. Cytotoxic drugs, organic solvents (benzene), fumes, (lindane, glue vapors), radiation.
- 2. Idiosyncratic drug reactions:
- Chloramphenicol,
- Gold,
- Phenylbutazone, Indomethacine,
- Sulfa,
- Anti-epileptic drugs,
- Arsenicals.

Aplastic Anemia (AA): classification and etiology continued (3)

- 3. Viral Infections:
- Parvovirus B 19 – pure red cell aplasia,
- Hepatitis: Non-A, non-B, non-C
- HIV,
- EBV
- 4. Immune disorders:
- Eosinophilic fasciitis,
- SLE,
- GVH

Aplastic Anemia (AA): classification and etiology, continued (4)

- **5. Miscellaneous:**
- **Paroxysmal Nocturnal Hemoglobinuria (PNH) , survival of a more adaptive stem cell population.**
- **Thymoma and Thymic Carcinoma – mostly pure Red Cell aplasia.'**
- **Pregnancy, most likely immune.**

Congenital Aplastic Anemias

- 1. Fanconi Anemia: > 1850 in literature,
- physical findings and chromosome breakages.
- 2. Dyskeratosis Congenita: 425 in lit.
- Physical findings; telomere shortening.
- 3. Diamond-Blackfan anemia 825 in lit.
- Pure red cell aplasia, phys. Findings.
- Adenosine deaminase deficiency.

Congenital Aplastic Anemia continued (2)

- 4. Schwachman-Diamond syndrome:
- 500 in lit., phys. Findings, malabs., pancreatic insufficiency. Neutropenia.
- 5. Severe Congenital Neutropenia: 374 in lit. ; Bone marrow – promyelocyte arrest.
- 6. Amegakaryocytic Thrombocytopenia
- 100 in lit. No phys. Findings.; absent or abnormal megakaryocytes in BM.

Congenital Aplastic anemias continued (3)

- 7. Thrombocytopenia, absent radii syndrome: 280 in lit. ; phys. findings – mostly bony. Absent megakaryocytes in bone marrow.
- All congenital AA-s have the predisposition to evolve into MDS or leukemia.
- Fanconi's pancytopenia has a special susceptibility for solid tumors.

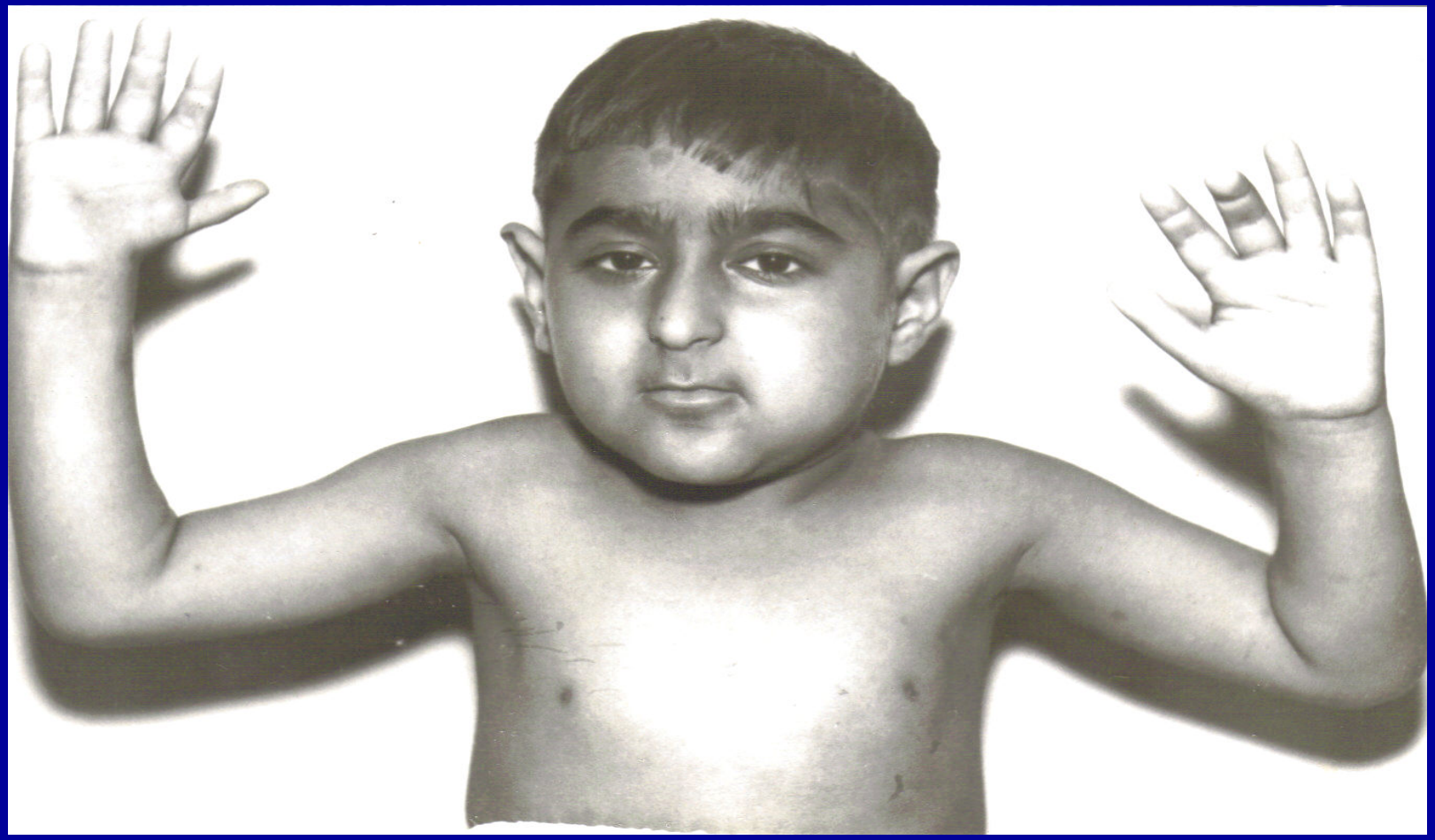
Aplastic Anemia : Pathogenesis of Idiopathic AA

- 1. Stem Cell anomalies:
- A. Quantitative,
- B. Qualitative.
- 2. Stroma support anomalies,
- 3. Cytokine anomalies: e.g. Interferon Gamma, TNF Alpha, IL -6, IL – 2, lead to polyclonal T- cell expansions,
- 4. Cytotoxic – NK lymphocytes

Aplastic Anemia : Patient presentations.

- 1. Fanconi's Pancytopenia:
- During my Pediatric rotating internship in 1963, I encountered a Moroccan family where the propositus, at 8 years was brought in with: severe anemia : Hb.-60 g/l with macrocytosis, leuco-neutropenia and thrombocytopenia, covered by purpura and ecchymoses. He also had mild renal impairment, which improved with good hydration.

Aplastic Anemia – Patient presentations (2)



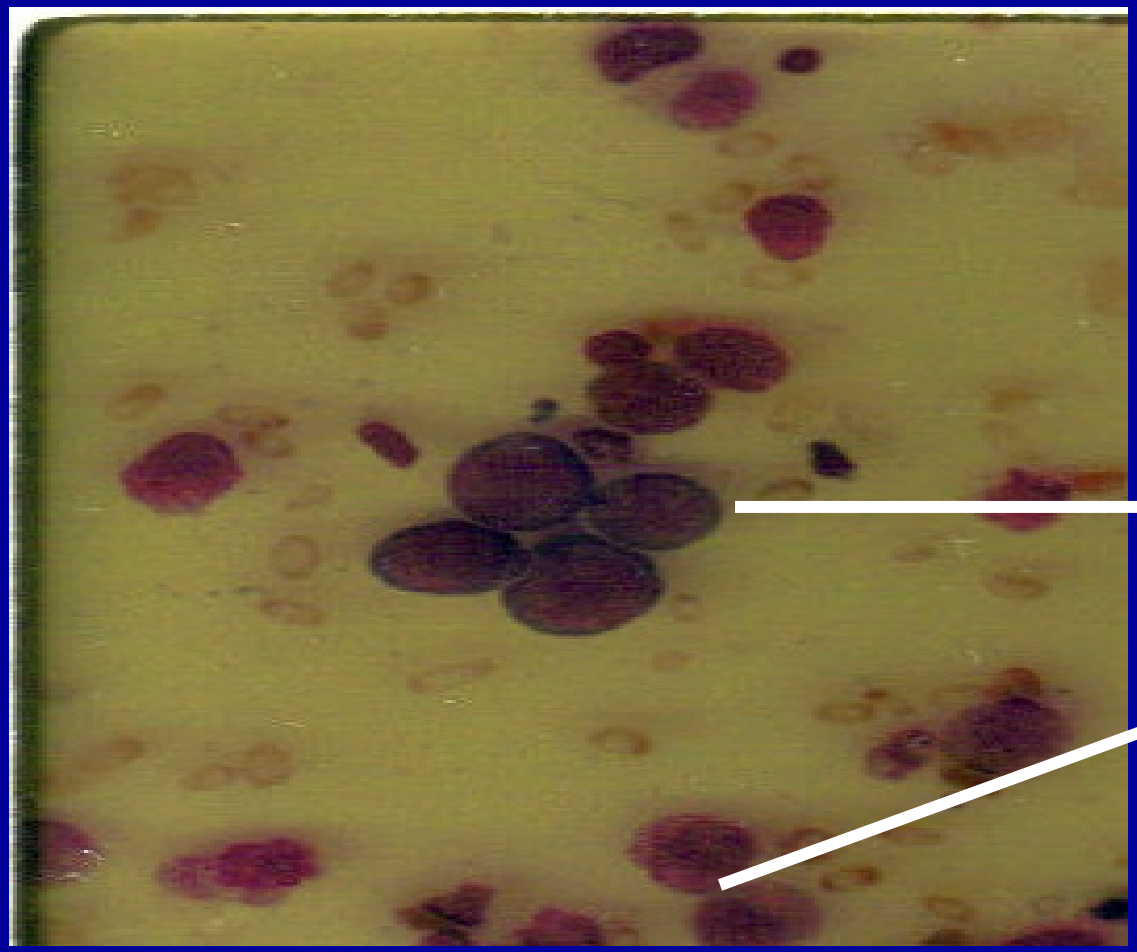
Aplastic Anemias : Patient presentations (3)

He also had “ Café au lait “ spots on his trunk and chest,

High arched palate, micro-cephaly, short stature, coxa vara and on further investigations “ horse shoe “ kidneys.

His bone marrow aspirate was megaloblastic, not empty at that stage.

Aplastic Anemia : Fanconi bone marrow

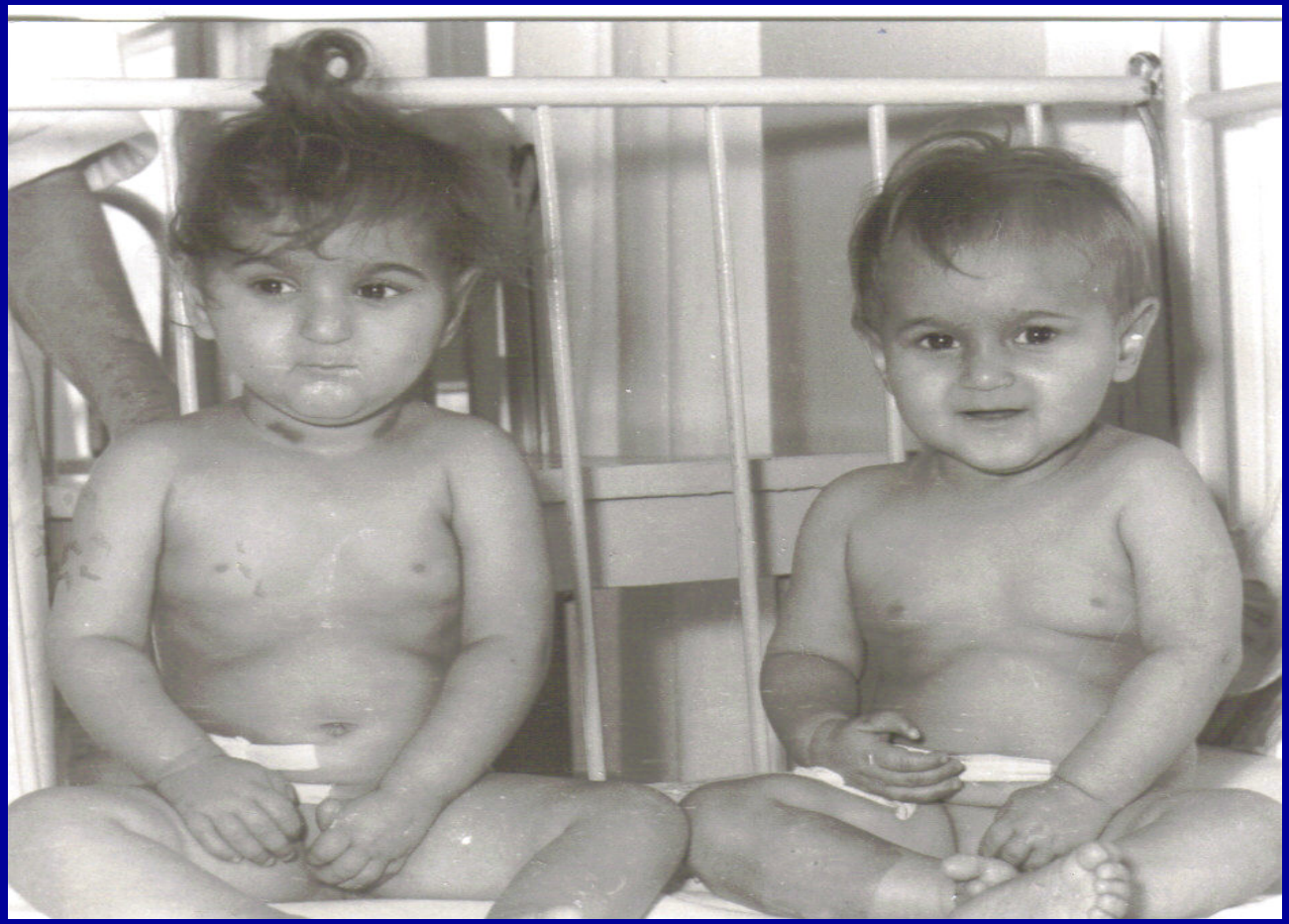


Aplastic Anemia : Patient presentations (4)

- Ely was found to have also an elevated Hb. F and family history revealed that there were a couple of twins, who looked very much like their brother and bruised easily.
- At age 18 months they were also pancytopenic, had dysmorphic features and were bruising a lot.

Their labs and bony anomalies were similar.

Aplastic Anemias: Patient presentations : The W. twins (5)



Aplastic anemia: patient presentations: W.twins' hands.

- Deformed and extra thumbs.

Aplastic anemia: Patient presentations – Fanconi's Pancytopenia (cont.)

- These twins had also renal anomalies.
- A first female cousin, 5 year old at a time had the same dysmorphic features and pancytopenia, as well as skeletal and renal anomalies.
- In addition all of them had elevated Hb. F, hypoplastic, megaloblastic bone marrow and developed in addition FMF with renal amyloidosis, documented by renal biopsies.

Aplastic anemias: Fanconi's pancytopenia in 4 members of a family.

- **Therapy:**
- Consisted of Oxymetholone,
- Good i.v. and oral hydration for the kidneys and FMF
- Transfusions, when Hb. Dropped to below 70 g/l
- Antibiotics i.v.or p.o depending on infections.
- They all eventually died from renal amyloid with renal failure.

Aplastic anemia: patient presentation – Acquired AA 1

- **TV – 10 year old Caucasian boy, previously healthy, fit, hockey player.**

Admitted to hospital for fatigue, listlessness, headaches, anorexia, bruising.

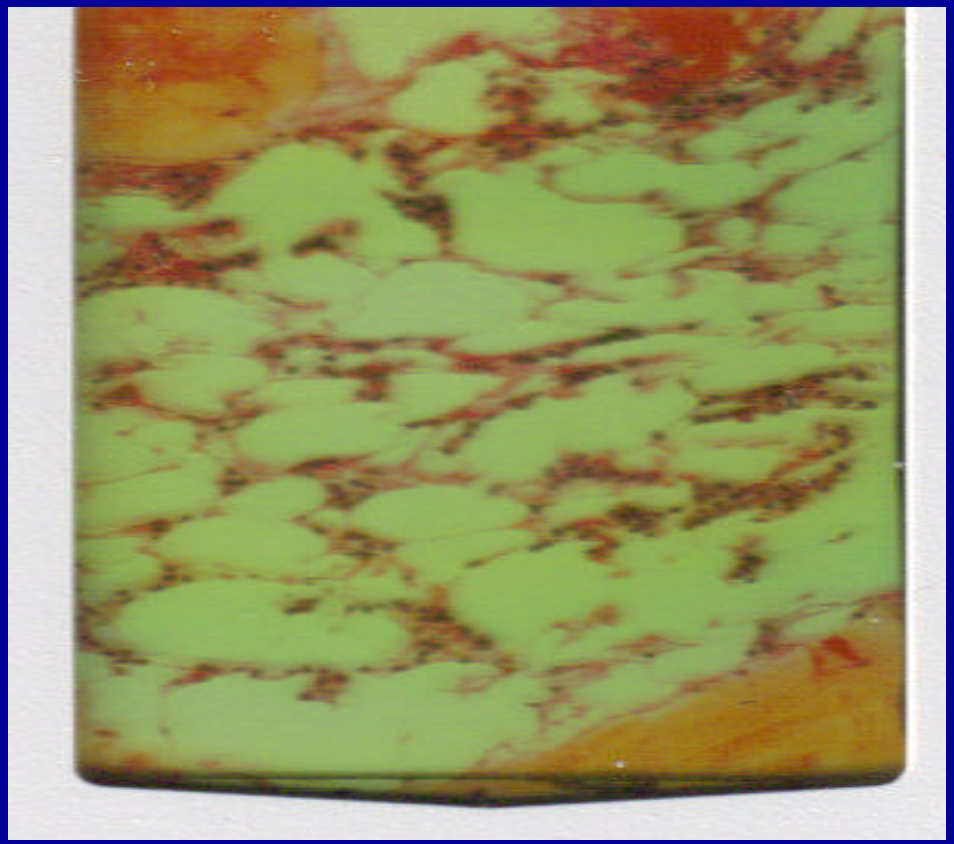
Exam: Pallor, purpura, ecchymoses, some shoddy cervical lymph nodes. No hep-spl. Megaly.

Hb. 72 g/l, Leuc.-1.8, Plat.-15, Retics – 20.

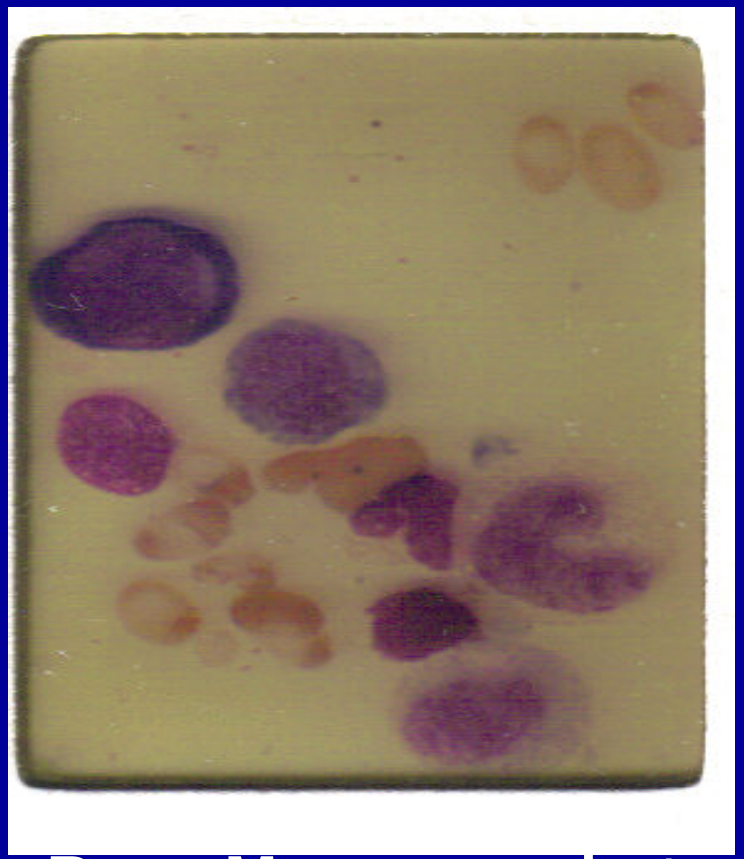
Aplastic anemia: acquired AA – patient 1.

- **Past History: Was term baby, had all immunizations, normal mile stones, excellent student, athlete.**
- **Healthy 12 year old brother and healthy, unrelated parents.**
- **Helped his father for over 3 weeks in their house renovations, was exposed to organic solvents and paints.**

Aplastic Anemia: Acquired AA
Patient 1. Bone marrow Biopsy



Bone Marrow biopsy



Bone Marrow aspirate

Aplastic Anemia: Acquired AA Patient 1.

- Therapy with steroids was initiated.
- Within approx. six weeks, macrocytic counts started recovering.
- PNH developed with real nocturnal brown urine.
- Ham test was positive.
- Patient followed for two years.
- Complete recovery.

Aplastic anemia: Acquired
Patient 2.

- FS, 7 year old grade 2 student, previously healthy, complained of fatigue, headaches, bruising.
- Phys. Exam. – Neg., exc. For purpura, petechiae, ecchymoses on legs.
- Severe Pancytopenia,
- Empty bone marrow.
- Steroids initiated. No response.

Aplastic anemia: Acquired, Patient 2.

- Regular follow up with bone marrow aspirations and biopsy every 3-4 months. Marrow empty for over 1 year.
- Developed within 18 months ALL with incomplete response to conventional Vincristine, Prednisone, Daunorubicin, L-Asparaginase, CNS – Methotrexate prophylaxis.
- Deceased within two years.

Aplastic Anemia: Acquired AA Patient 3.

- 16 year old student, known at MCH for previous “ Fe. Deficiency” anemia at age 12 years. Treated with Fe++
- Now very severe pancytopenia: Hb – 68 g/l, Leucocytes – 1.0 with PMN-< 500,
- Platelets 10, Retics – 0
- Exam- Pallor, bleeding, systolic ejection murmur at all cardiac foci, no nodes and no hep-spl. Megaly. B.M empty.

Aplastic anemia: Acquired AA Patient 3.

- The year is 1978, no previous BMT done yet in Canada.
- We decide to try it in Montreal, since patient has 5 sibs.
- HLA identical, MLC compatible brother served in NATO forces in Lahr, Germany, brought home.
- Meanwhile Hb. Dropped to 32 g/l with cardiac failure and early papilledema in fundi.
- No transfusion administered.

Aplastic anemia: Acquired AA, patient 3. - BMT

- BMT expertise available at MGH (Dr.Rybka worked in Seattle)
- Patient received conditioning with Cyclophosphamide 200 mg/kg X 4 days., followed by BMT
- Excellent, successful engraftment with minimal GVHD, treated with MTX.
- Two years later joined armed forces, four years later, fathered first baby.

Aplastic Anemia: Acquired AA post-Hepatitis B, patient 4.

- 14 year old student developed after episode of Hepatitis severe, fast progressing pancytopenia.
- Exam, apart from minimal liver enlargement and recovering LFT, unremarkable.
- Bone marrow – almost empty.
- Sister HLA identical and MLC compatible, seen and examined at Mac.

Aplastic anemia: Acquired post-Hepatitis B. Patient 4.

- Meanwhile patient administered small dose of steroids.
- 8 weeks later, one week before planned BMT, suddenly:
- Reticulocytosis, Hb. Up to 85 g/l, Leucocytes 3.4 with PMN > 500, Platelets – 78,000, Retics – 48,000
- Ham test strongly positive.
- Patient followed now for 10 years with low normal counts. PNH intermittently positive.

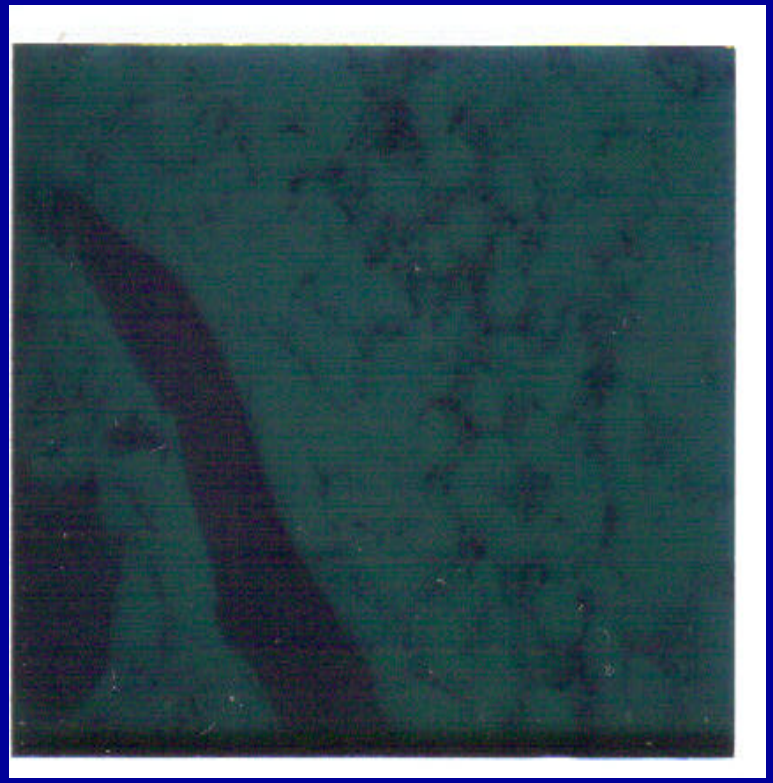
Aplastic Anemia: Acquired AA patient 5

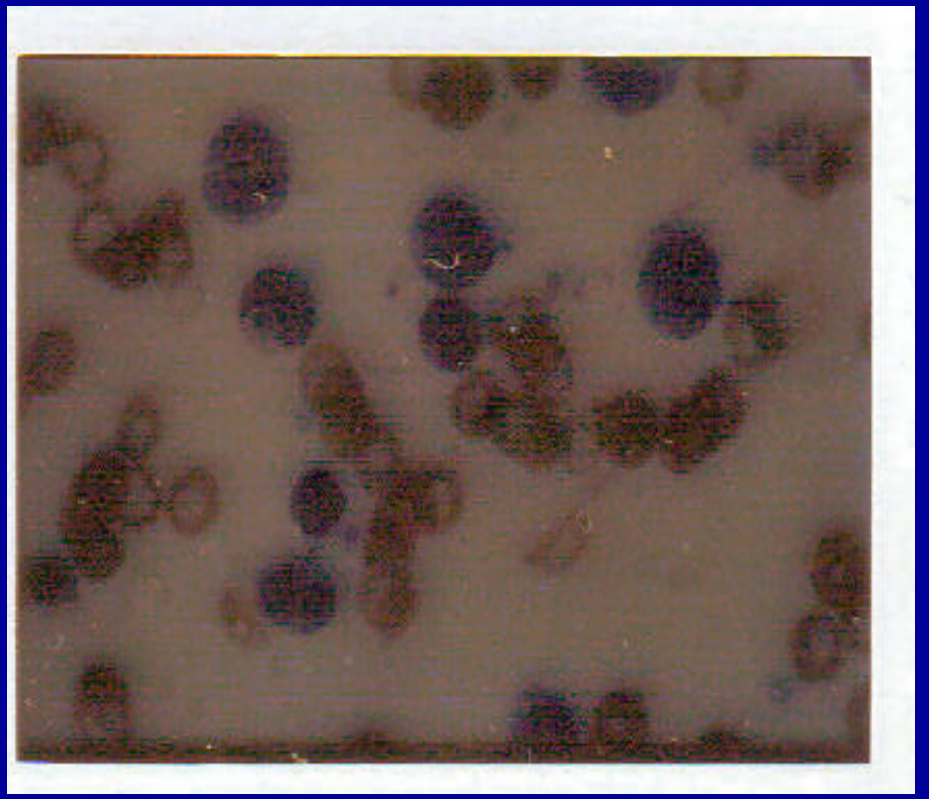
- 48 year old man, president of steel company, treated in Montreal for 4 years for Wegener's granulomatosis.
- Developed gradually ESRF, treated here with hemodialysis.
- Gradually developing Pancytopenia,
- Cyclophosphamide D/C-d, Prednisone continued.

Aplastic Anemia: Acquired AA, patient 5.

- Followed by me for several months.
- Bone marrow becoming more and more empty with dyserythropoiesis and MDS in the few progenitors left.
- Referred by me to 3 BMT centers. Nobody wanted to transplant him because of renal failure and MDS ?
- Patient developed Acute Myelo-Monocytic leukemia with ocular infiltration and did not respond to chemotherapy.

Aplastic anemia: Patient 5 evolving into MDS and AMML

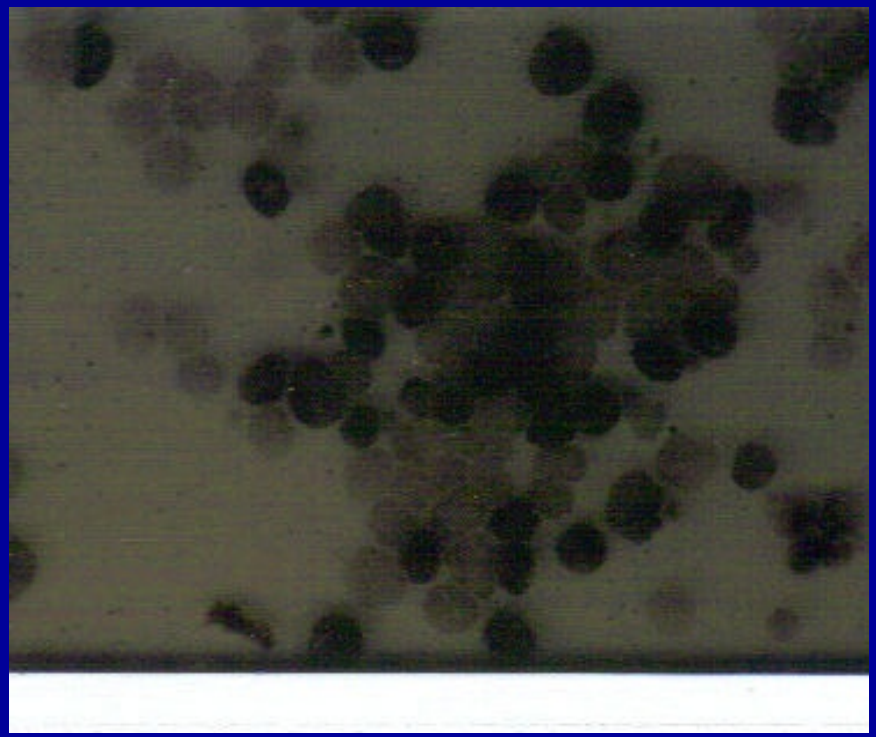


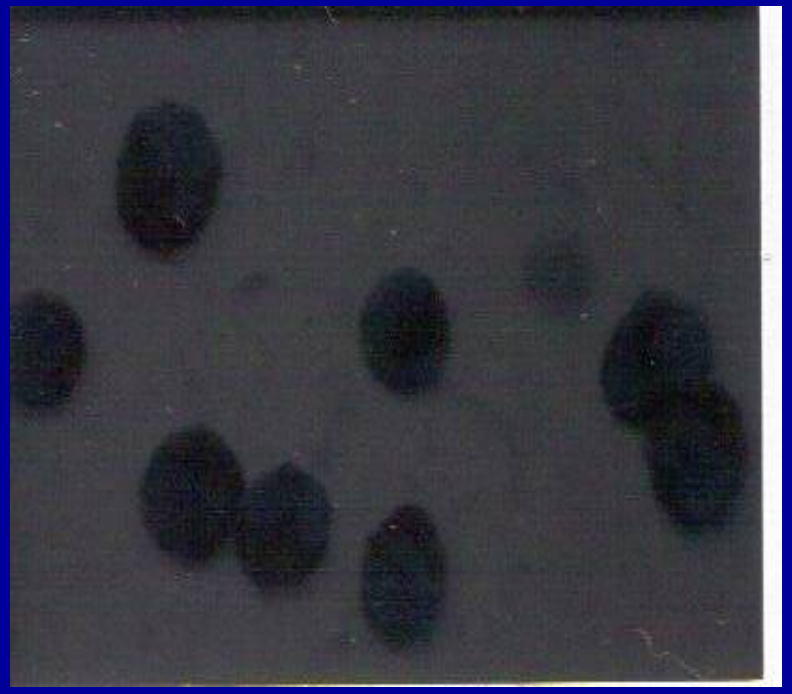


Marrow aplasia, two years later AMML

Aplastic anemia – MDS – AMML, patient 5.

- **Alpha Naphtol Acetate Esterase**





Chloro Acetate esterase

Aplastic Anemia: Pathophysiology

- 1. Relationship of medical drug use to AA is due to deletions in the drug-metabolizing glutathione-S –transferase genes : GSTM1, GSTT1, that permit accumulation of toxic drug intermediates.
- 2. Benzene, pesticides are well documented.
- 3. In Asia: unbottled water, ducks, geese, animal fertilizer and some pesticides – are suggestive of an infectious etiology.

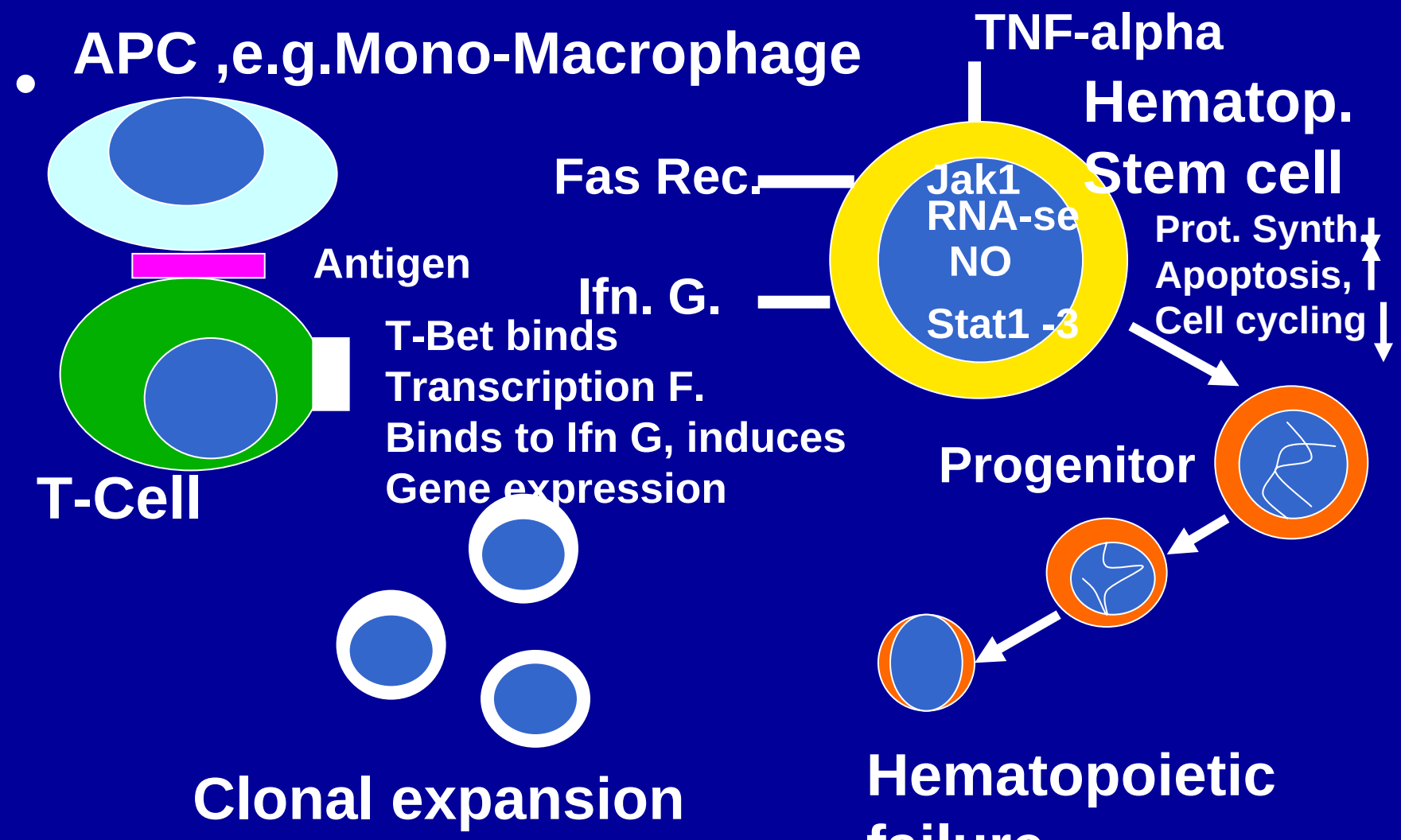
Aplastic anemia: Pathophysiology.

- 3. Auto-immunity – Autoantigens:
- AA pts' sera tested ag. A peptide library by genes in fetal liver or leucemic cell lines:
- Kinectin – bound to antibodies of about 40 % of AA pts.
- Diazepam binding related protein-1= essential enzyme for oxidation of unsaturated fatty acids
- Kinectin reacting cytotoxic T-cells generated in vitro, inhibit hematopoietic colony formation.
- But in vivo these cytotoxic T-cells have not been found in AA pts.

Aplastic anemia: Pathophysiology.

- Most cases of “ Idiopathic “ AA are immune mediated diseases.
- Cellular and molecular pathways have been mapped to:
- A. Effector T-lymphocytes,
- B. Target Hematopoietic stem cells and progenitors
- C. Environmental precipitants,
- D. Genetic risk factors and
- E. Individual characteristics of immune response.

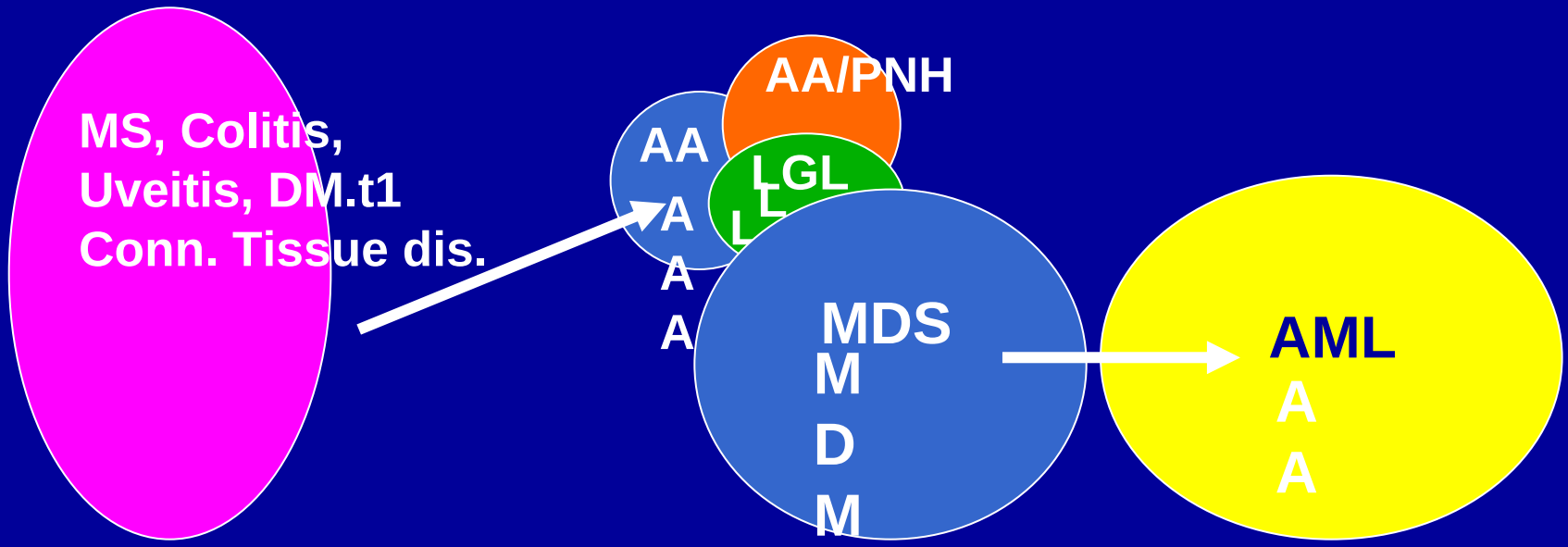
Aplastic anemia: Immune destruction of hematopoiesis.



Aplastic anemia: Immune destruction of hematopoiesis

- Evidence for this:
- 1. Recovery from AA after BMT graft rejection, due to immune suppression of conditioning regimen.
- 2. T-cells from AA pts. Inhibit CFU in normal bone marrows.
- 3. Currently good clinical response to immune suppression with ATG and CyA.
- 4. B.M. recovery of AA in pregnancy after delivery, 20% relapse risk in future pregnancy, occas. fatal.

Clinical and pathophysiol. Relationships betw.B.M failure and autoimmune diseases.



Aplastic Anemia: Clonal evolution

- 1. An abnormal expansion of suppressor T- cells may cause HSC depletion and clonal anomalies.
- 2. HSC defects may be associated with abnormalities of the microenvironment.
- 3. CD4+CD25+FOXP3+ regulatory T cells are deficient in some of AA pts.

Aplastic anemia: Clonal evolution

- Glycosyl-Phosphatidil-Inositol (GPI) anchor is an important surface glycolipid, normally positive in normal hematopoietic cells.
- Rare GPI- cells exist even in normal individuals and are predominant in PNH. (CD55 and CD59), derived from a somatic mutation in an X-linked gene called PIG-A.
- CD 52 is a GPI linked protein, the target of Alemtuzumab (Campath).

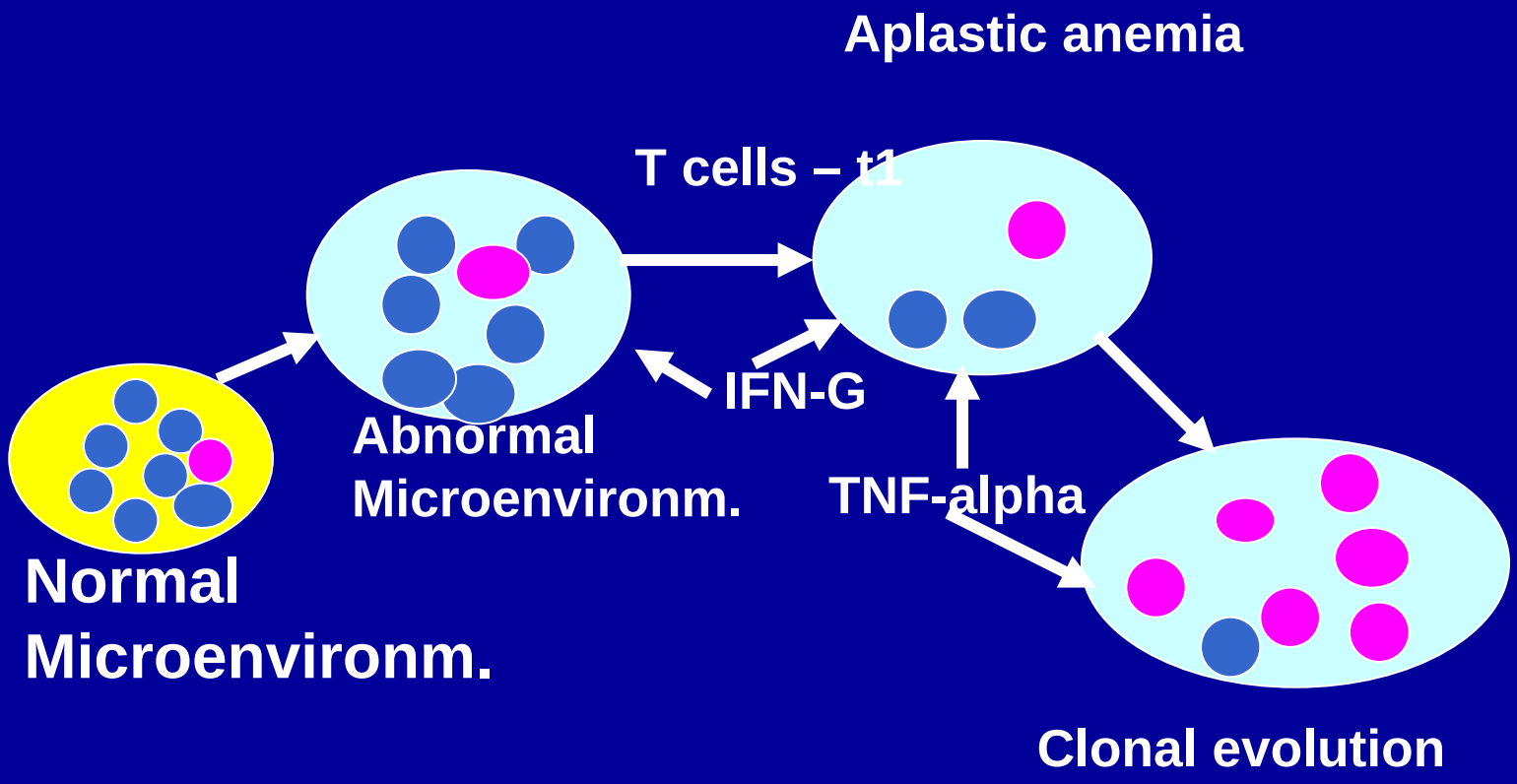
Aplastic anemia: Clonal evolution

- Following treatment with Campath, CD52 – T-cells are identified, which are also GPI- neg.
- It appears that GPI-neg. stem cells are spared from the autoimmune attack, suggesting that the “Autoantigen” may be GPI – linked.
- This may explain the PNH recovery in AA following immune suppression.

Aplastic anemia: clonal evolution.

- **Bone marrow failure is a risk factor for clonal evolution: cells resistant to apoptotic cues, are adaptively selected from the stem cell pool for survival.**
- **10-20 % of AA survivors will develop within a decade a clonal disease: PNH, MDS, AML (Young, Bagby)**
- **“ In a stressful hematop. Micro-ecosystem a new genotype in adapted clones will result in an emergent phenotype of BM cells, more “fit” than their non adapted progenitors.”**

Aplastic anemia: clonal evolution.



Aplastic anemia: Therapy

- **1. Young patients who have an HLA identical and MLC compatible donor should definitely undergo BMT or HSCT.**
- **That is probably the only chance for complete cure, by destroying also the adaptive, resistant clone.**
- **Transplant should occur within less than 80 days and if possible within weeks.**
- **Transfusions, especially from prospective donor must be avoided.**

Aplastic anemia: Therapy

- A. Bagicalupo et al: 1567 pts. Allografted from 1991-2002:
- Favorable predictors for survival were:
- Transplant after 1997,
- Matched sibling donor,
- Age younger than 16 yrs.
- Interval from diagnosis to transplant less than 83 days.
- Conditioning regimen without radiation: CTX 50 mg/kg/d. X 4 days, survival 91%.

Aplastic anemia: Therapy

- HSCT from PB is fraught with chronic GVHD, which is only harmful in AA.
- Cyclosporin A and Methotrexate are used to prevent and treat GVHD
- HLA identical BMT for pts. Older than 30 yrs. – conditioning regimen of CTX 300 mg./M^2, Fludarabine 30 mg./M^2 X 4 days +/- ATG – mortality 30%.

Aplastic anemia: Therapy

- **Matched unrelated donor transplants (MUD):**
- **European Registry: 318 donor transplants from 1988-98:**
- **Rejection – 15%,**
- **GVHD II-IV 48%,**
- **5-year survival 39%**
- **Conditioning regimen: CTX, ATG, minimal radiation.**

Aplastic anemia: Therapy

- **Immune Suppression: for patients who don’t have a donor and for older patients.**
- **Cyclosporin and ATG**
- **This treatment will not prevent later clonal evolution.**
- **Relapses occur. Treatment may be repeated.**

Aplastic anemia: Immune suppressive therapy

- There is a real risk of progression to clonal disorders following immune suppressive therapy:
- PNH, MDS, AML
- European Registry: 10 year incidence of malignancy in 860 pts. 19 % = 5.2 X the overall cancer risk in age matched non AA, non immune suppressed population.
- Monosomy 7 and trisomy 8. are frequent.
- MDS and AML evolve sooner in pts. Treated with G-CSF.

Aplastic anemia: Neoplastic complications

- **Post Transplant Lymphoproliferative disorder (PTLD) due to EBV – may be polyclonal or monoclonal.**
- **Polyclonal PTLD may remit spontaneously in the absence of immune suppression.**
- **Monoclonal PTLD has to be treated like an aggressive lymphoma.**
- **Congenital AA-s (esp. Fanconi), have a much higher incidence of solid tumors due to chromosomal breakages.**

Aplastic anemia(AA): Summary.

1. AA is a rare hematological disease, characterized by Pancytopenia, empty bone marrow, absence of hepato-splenomegaly.

A. Moderate AA: marrow cell. < 30%, depression of at least 2/3 blood elem.

B. Severe AA: marrow cell. < 25 % - Retics., < 40,000, PMN- < 500, Plat. < 20,000

C. Very severe AA : PMN < 200

Aplastic Anemia (AA): Summary

- 2. AA may be congenital or acquired.
- 3. Cong. AA is often associated with skeletal and organ anomalies and have a tendency to develop neoplasias both hematological and solid tumors.
- 4. Acquired AA are sometimes due to toxic or drug induced marrow injury.
- Most of them are immune mediated.
- 5. Acquired AA may evolve into clonal disease: PNH, MDS, AML.

Aplastic Anemia (AA): Summary

- 6. AA is best treated in young patients by HLA identical BMT with only CTX conditioning and no or minimal preceding transfusions.
- 7. CR is normalization of blood counts.
- PR is independence of transfusions.
- 8. AA in older pts or absence of donors should be treated with immune suppression: ATG + CyA
- 9. Clonal evolution after immune suppression is quite likely.