Heme:

Pancytopenia and the Hypocellular Bone Marrow

Roger Alan Fleischman, M.D., Ph.D.

Grand Rounds
Department of Internal Medicine
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INTRODUCTION

Not infrequently, the hematologist is asked to evaluate a patient with reduced numbers of erythrocytes, granulocytes, and platelets in the peripheral blood. This pancytopenia may be detected by routine blood counts when the patient is asymptomatic, but more often, particularly if the cytopenia is severe, the patient will present with symptoms secondary to marked anemia, neutropenia, or thrombocytopenia. The history may be acute or chronic, and the results of previous blood counts, if available, may range from a completely normal blood count a few months earlier to a stable or slowly progressive pancytopenia of many years' duration.

Although careful examination of the peripheral blood may reveal important clues to the etiology of the pancytopenia, the hematologist, with rare exceptions, must perform a bone marrow aspiration and biopsy at this stage in his evaluation of the disease process. The critical importance of the bone marrow is obvious when one remembers that all of the circulating erythrocytes, granulocytes, and platelets are produced in the bone marrow and must be rapidly replaced by new cell production. For example, the half-life of the approximately 20 trillion erythrocytes in the circulation of a 70 Kg man is about 60 days (Berlin and Berk, 1975), thus requiring the bone marrow to produce more than 600 billion new erythrocytes each day to maintain a normal hemoglobin and hematocrit. Furthermore, the half-lives of the granulocytes and platelets are even shorter, less than 24 hours and 4-6 days, respectively (Dresch et al, 1975; Paulus, 1971). Thus, complete bone marrow failure will result in agranulocytosis in one or two days, complete thrombocytopenia in one or two weeks, and severe anemia in a two or three months.

Examination of the bone marrow, therefore, may result in the hematologist making a diagnosis of pancytopenia, but with apparently normal bone marrow function. A frequent cause of this clinical picture, for example, is sequestration of the blood cells in a massively enlarged spleen. In this group, the bone marrow is normocellular and, in the absence of nutritional deficiencies, maturation of the hematopoietic precursors generally appears normal, as one would expect. Although in theory auto-antibodies to blood cells could also account for pancytopenia without affecting bone marrow precursors, the marked differences in the antigens on the surface of erythrocytes, granulocytes, and platelets presumably accounts for the apparent rarity of this etiology, particularly in view of the relatively frequent occurence of ITP or autoimmune hemolytic anemia. A small number of patients with Coombs positive hemolytic anemias do have co-existant thrombocytopenia and/or neutropenia, however, and may represent cases with rare cross-reactive antibodies or multiple antibodies (Evans and Duane, 1949;Evans et al, 1951). As we will see, pancytopenia in some cases may result from an autoimmune mechanism if the target cell is a precursor or stem cell for all three cell lineages, but in these cases the bone marrow is markedly abnormal.

Frequently, however, the hematologist will conclude that the patient has pancytopenia associated with an abnormal bone marrow. Traditionally, these patients have been divided into two further sub-groups: (1) pancytopenia with a cellular, albeit morphologically abnormal, bone marrow, and (2) pancytopenia with a hypocellular or aplastic bone marrow. Although this classification is by no means perfect, it remains a clinically useful way to separate these disorders and generally succeeds in discriminating cases in which maturation and cell differentiation persist to some extent, from cases in which maturation and hematopoietic cell differentiation appear to be completely blocked or absent. It should be noted here that a marrow aspirate alone is an unreliable means for determining cellularity, and the failure to obtain marrow at aspiration frequently results from infiltration of the marrow, rather than from decreased cellularlity. An adequate biopsy of the marrow is generally a reliable indicator of the true marrow cellularity; however, on occasion a sample may have been taken from an island of cellularity in an otherwise aplastic environment. In addition, a cellular marrow may precede the development of aplasia in certain cases, and aplasia may progress to recovery

of normal hematopoiesis or increased numbers of abnormal cells that herald the onset of acute leukemia. Clearly, repeat marrow aspirates and biopsies should be performed at regular intervals in these disorders, particularly if a change in clinical condition is observed.

While pancytopenia with a hypocellular marrow is the subject of today's discussion, a brief consideration of the differential diagnosis of pancytopenia with a cellular marrow is appropriate here. Obviously, megaloblastic maturation of the marrow precursors due to B12 or folate deficiency may cause pancytopenia in severe cases due to ineffective cell production, despite a marrow that usually remains relatively cellular. Other patients are distinguished by the accumulation of excess amounts of iron in the red cell precursors, giving rise to the "ringed" sideroblasts that are characteristic of the sideroblastic anemias, and may also manifest neutropenias and thrombocytopenia as well. In cases without sideroblasts, dyspoiesis of hematopoietic cell maturation is often present, occasionally accompanied by increased numbers of myeloblasts in the bone marrow. These "myelodysplastic" disorders, which not infrequently evolve to acute leukemia and may be associated with chromosome abnormalities, were the subject of an excellent grand rounds last year by Dr. R. Graham Smith, and anyone interested in this subject should refer to that presentation. Other causes of pancytopenia with a cellular marrow include marrow infiltration by lymphomas, myeloma, carcinoma, fibrosis, or frank leukemia, as well as granulomatous diseases, such as tuberculosis and histoplasmosis.

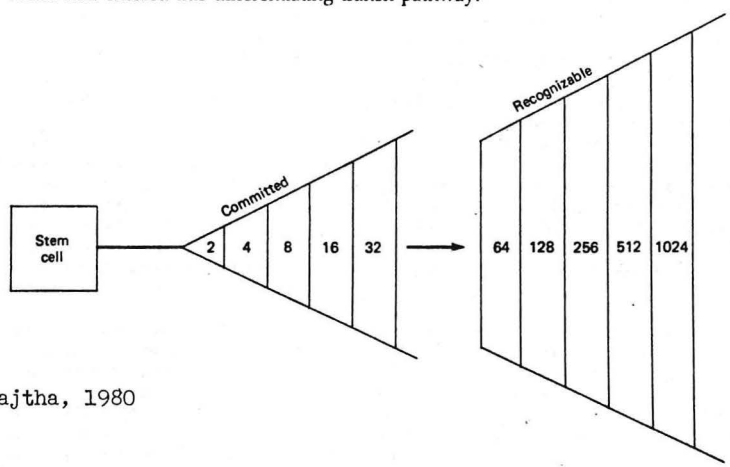
BONE MARROW APLASIA: A DISORDER OF THE HEMATOPOIETIC STEM CELL

Just as pancytopenia in the peripheral blood will result from a failure of the bone marrow to produce the mature blood cells, bone marrow aplasia will inevitably result from the failure of the hematopoietic stem cell (HSC) to produce the recognizable progenitors of the erythroid, granulocytic, and megakaryocytic cell lineages. Since approximately 1960, we have known that virtually none of the morphologically recognizable precursor cells in the bone marrow are self-maintaining, i.e. they are not capable of extensive regeneration (Lajtha and Oliver, 1960). For example, labeling of the earliest recognizable erythroid precursor, the proerythroblast, with radioactive iron demonstrated that these cells are capable of only an additional 4 or 5 cell divisions before maturation to the mature, non-dividing, erythrocyte. Furthermore, unlabeled proerythroblasts appear within a short time, suggesting the existence of a rare and unrecognized precursor cell for the proerythroblast. Thus, the bone marrow's extensive capacity for regeneration after radiation, chemical damage, or transplantation must reside in a very small number of morphologically unrecognizable "stem" cells.

The modern era of stem cell investigation, sometimes referred to as "hematology without the microscope", began a few years later with the demonstration by Till and McCulloch (1961) that approximately 1 out of 1000 bone marrow cells injected into lethally irradiated mice could form a macroscopic nodule in the recipient's spleen 8-12 days after transplantation. These spleen colonies were shown to result from the clonal proliferation of a single donor stem cell into as many as 10 million erythrocytes, granulocytes, or megakaryocytes (Wu et al, 1967). Furthermore, a small fraction of these spleen colonies contain several hundred additional spleen colony-forming cells (CFU-S), observed after transplantation into a second recipient (Siminovitch et al, 1963). This result suggested that the stem cell may be capable of extensive "self-renewal" or regeneration of stem cell numbers, as well as differentiation into mature progeny. Moreover, the fraction of spleen colonies that contained all three hematopoietic lineages, and thus were derived from a "pluripotent" stem cell, also contained the fraction of CFU-S that demonstrated significant self-renewal. Single lineage colonies, which may derive from a "committed" stem cell, contain little, if any, self-renewal capability in this system.

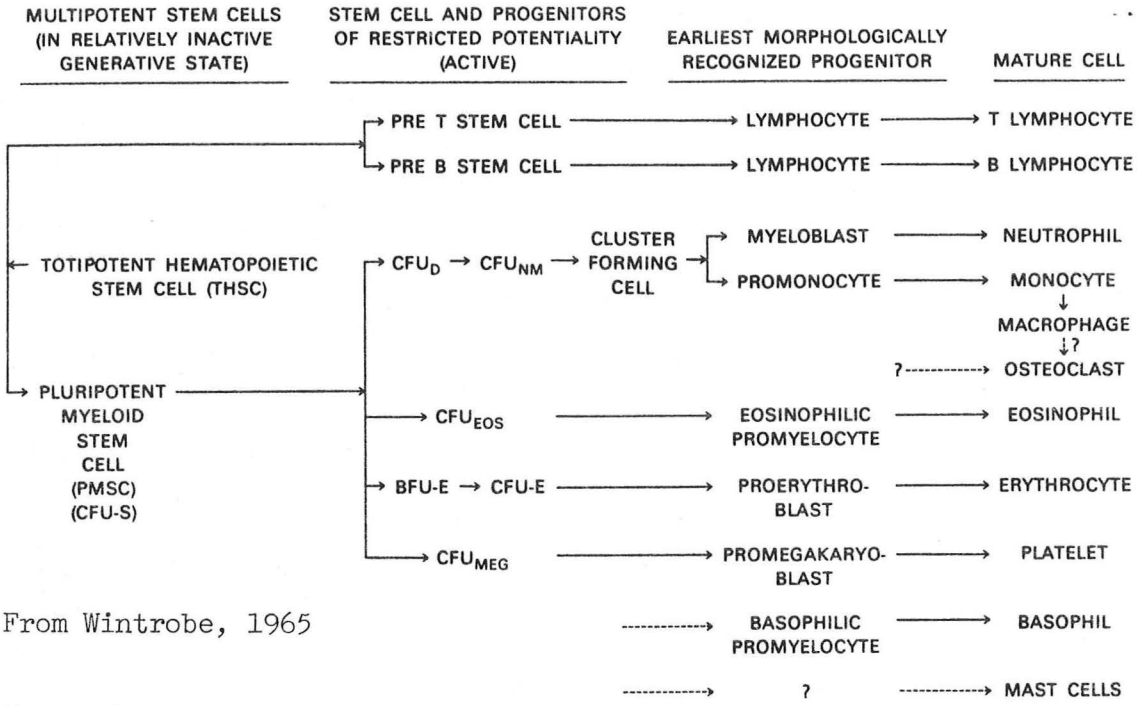
Although the spleen colony technique is not applicable to human studies, for obvious reasons, in vitro culture techniques were developed in the late 60's which permitted the growth of unilineage committed progenitors in soft agar media to produce granulocytic colonies (Bradley and Metcalf, 1966; Pluznik and Sachs, 1966), erythroid colonies (Axelrad et al, 1974), and, more recently, megakaryocytic colonies (Metcalf et al, 1975). The frequency of these progenitor cells was higher than that of the CFU-S, as one would expect, but the cells were still derived from the morphologically unrecognizable cell population in the bone marrow. Many of these committed colony-forming progenitors could also divide several times to produce additional colony-forming cells before final differentiation into the recognizable erythroid or granulocytic pathway. Thus, the committed cells also serve as amplifying populations, producing probably another 30 or so progeny proerythroblasts (5 cell divisons), which in turn undergo five cell divisions before becoming mature erythrocytes (Figure 1). Clearly, the proportion of stem cells in the bone marrow can be very small if sufficient amplifying cell divisions occur between committment of the stem cell and production of the mature progeny. Indeed, the pluripotent stem cells appear to be maintained in a non-cycling or G0 state for long periods of time, as judged by tritiated thymidine suicide experiments, and enter DNA synthesis and replication in large numbers only during periods of markedly increased demand or recovery from injury and aplasia (Lajtha et al, 1962, 1969). On the other hand, the majority of committed colony-forming cells are actively cycling and undergoing DNA synthesis, even in the normal animal. More recently, multi-lineage colonies, containing erythroid, granulocytic, and megakaryocytic cells, have also been successfully cultured in vitro and the pluripotent stem cells for these colonies appear to correspond to the CFU-S detected by spleen colonies (Fauser and Messner, 1979).

Figure 1 Diagram of amplification in transit populations during hematopoiesis. The first transit population consists of "committed" precursor cells, which, undergoing, e.g., five cell cycles, amplify by a factor of 32X. The second transit population, the "recognizable" cells (e.g., normoblasts), may also undergo five cell cycles, with a further 32X amplification resulting in an overall number of 1024 cells from the original stem cell which had entered this differentiating transit pathway.



From Lajtha, 1980

These studies of hematopoietic stem cells, then, have demonstrated that the extensive proliferative potential of the bone marrow is concentrated in a small number of stem cells which are pluripotent and differentiate into at least three different hematopoietic cell lineages (Figure 2). Additional evidence, however, suggests that an even more primitive stem cell exists which



From Wintrobe, 1965

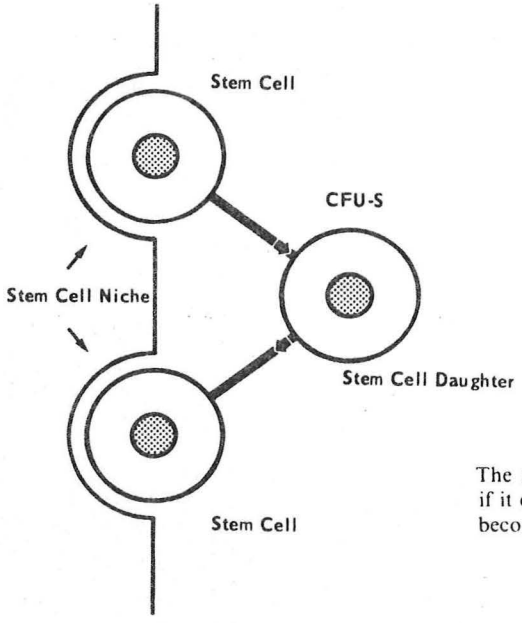
Figure 2 Hierarchy of the hematopoietic stem cell and progenitor systems. BFU, burst-forming unit; CFU, colony-forming unit; D, diffusion chamber; E, erythroid; EOS, eosinophil; MEG, megakaryocyte; NM, neutrophil-monocyte; S, stem cell.

is capable of differentiating into B and T lymphocytes as well as the pluripotent CFU-S stem cell (Abramson et al, 1977; Fleischman et al, 1982). For example, the observation of the Philadelphia chromosome in a proportion of the B cells in patients with chronic myelogenous leukemia suggests that the disease resulted from mutation of a primitive myeloid-lymphoid stem cell (Martin et al, 1980). A case of sideroblastic anemia in which all the the hematopoietic cells, including B and T lymphocytes, were derived from a single clone, has also been described in a patient who was heterozygous for the X-linked enzyme glucose-6-phosphate dehydrogenase (Prchal et al, 1978). It should be noted, though, that unlike the erythroid, granulocytic, and megakaryocytic cells, a proportion of the B and T lymphocytes are known to have a very long half-life in the circulation, ranging from months to years (Little et al, 1962). Thus, loss of the primitive stem cell population might not produce significant lymphopenia unless the patient were to survive for an extended period of time.

A number of very important questions about stem cell physiology remain unanswered, however, despite the progress that has been made in studying these cells. For example, the true proliferative capacity and life-span of the stem cell is unknown. Conceivably the stem cell under normal conditions may be immortal or capable of such extensive proliferative capacity that it is difficult to detect a decline in stem cell function in aged animals compared to young animals, as we shall see in more detail in a later section. A major stumbling block to progress is our inability to directly assay or purify the primitive stem cells that are capable of repopulating the bone marrow and subsequently sustaining long-lasting hematopoiesis. None of the colony assays as yet have been demonstrated to correlate with the true self-renewal potential of the bone marrow, as demonstrated by transplantation. Thus, when the proliferative capacity of the bone marrow is damaged by radiation, cytotoxic drugs, or even transplantation itself, subsequent regeneration of bone marrow

cellularity may be complete and the numbers of colony-forming cells, including CFU-S, may return to normal despite the fact that competition experiments with normal control bone marrow demonstrate very significant losses in the self-renewal ability of the treated bone marrow (Brecher et al, 1981; Harrison and Astle, 1982; Sonoda et al, 1985). A decrease in the numbers of the colony-forming cells thus appears to be a late event in the process of bone marrow failure and usually occurs at or near the onset of evident aplasia.

The mechanisms that regulate the activity of the normally quiescent stem cells, and balance the need for self-renewal of the stem cell pool with differentiation to produce the mature blood cells are also not well understood. One model proposes that cell-cell interactions between HSC and the stromal cell component or "microenvironment" of the bone marrow, presumably composed of fibroblasts, adipocytes, and possibly endothelial cells, maintains the apparently unlimited proliferative capacity of the normal bone marrow. The number of these sites or "niches" in the bone marrow is hypothesized to be limited, and after cell division, only one of the two daughter cells can be retained in the niche. The other cell will become a committed progenitor which will undergo further replication and maturation. If unoccupied niches exist, though, the daughter cell may be established in this niche and become a long-lived stem cell (Figure 3). Damage to the bone marrow microenvironment by radiation or cytotoxic drugs might destroy many of the stem cell "niches", thus preventing regeneration of the stem cell pool. This model also provides an explanation for the observation that successful bone marrow transplantation requires prior treatment of the host with radiation or cytotoxic drugs to deplete the endogenous HSC. Presumably, the donor HSC cannot gain access to the bone marrow "niches" if the host has normal numbers of stem cells.



From Schofield, 1978

Figure 3.

The hypothetical view of a stem cell niche. The stem cell daughter is a CFU-S. However, if it can find and occupy a niche it will itself become a stem cell

PURE RED CELL APLASIA: A DISEASE OF HEMATOPOIETIC PROGENITORS

An examination of the pathway for hematopoietic maturation and differentiation leads to the conclusion that acquired chronic pure red cell aplasia (PRCA), unlike bone marrow aplasia, cannot occur because of inadequate numbers of normal stem cells. By definition, the bone marrow in chronic PRCA is devoid of recognizable erythroid precursors but normal for the granulocytic and megakaryocytic cell types. Maintenance of the non-erythroid cell types is an immediate indication of the presence of functional pluripotent stem cells. Regeneration of the erythroid progenitors from this stem cell pool will inevitably take place if the agent leading to inhibition or continued destruction of the erythroid progenitors is removed or successfully treated. Bone marrow transplantation, even from identical twins, therefore, is not indicated in PRCA. Rarely, PRCA may occur in preleukemia and herald the eventual onset of acute leukemia. While the abnormality here is at the level of the pluripotent stem cell, it results from stem cells with a block in differentiation occurring as an early malignant change rather than from an inadequate number of stem cells.

Comparison of bone marrow aplasia with PRCA, then, may be useful as an indication of the pathophysiology and etiologic mechanisms involved in patients with abnormalities other than stem cell extinction. Not surprisingly, almost all cases of chronic acquired PRCA appear to be mediated by humoral antibody inhibitors of erythroid progenitors or erythroblasts, in many cases sparing the most primitive erythroid colony-forming cells (Krantz and Dessypris, 1985); or, in patients with CLL and other lymphoproliferative diseases, T cell mediated supression of erythroid progenitors (Mangan et al, 1982a; Lipton et al, 1983) (Figure 4). While it is not our intention to exhaustively review PRCA, comparisons with bone marrow aplasia will be made throughout this discussion where relevant.

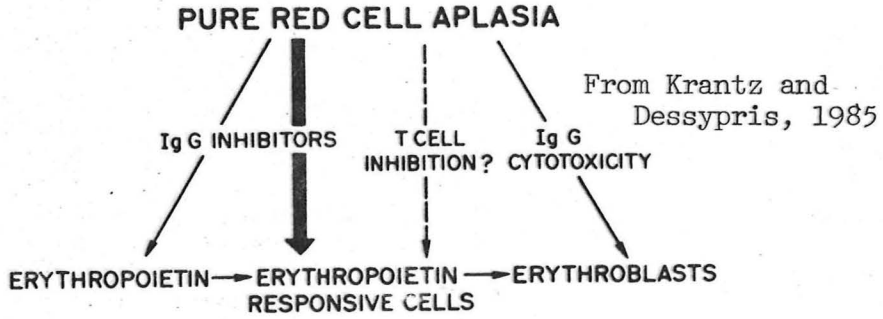


Figure 4. Different means by which erythropoiesis may be inhibited in PRCA.

Pure white cell aplasia, the analogous disorders of the granulocytic progenitor populations, has been reported in only a handful of cases (Cline and Golde, 1978; Bagby and Gabourel, 1979; Levitt et al, 1983). The literature on this disease also suggests that it may represent a maturation abnormality of the pluripotent stem cell preceding the onset of leukemia, or, more commonly, it has been associated with rheumatic diseases, thymoma, or in vitro evidence for antibody-mediated or T cell supression of granulopoiesis. Amegakaryocytic thrombocytopenia, an even rarer disorder, has been associated with systemic lupus erythematosis in several cases, and diffuse fasciitis in others (Hoffman et al, 1982; Hoffman, 1984).

MODELS OF BONE MARROW APLASIA

In conclusion, based on what is known about the pluripotent stem cell, one can envision at least four major pathophysiologic mechanisms to account for pancytopenia with a hypocelular or aplastic bone marrow. Obviously, the primitive stem cells may be normal initially and present in adequate numbers, but unable to differentiate into committed progenitors because of an immune-mediated supression of the stem cell pool. An additional possibility is the clonal dominance of the stem cell pool or marrow niches by the unregualted proliferation of an abnormal stem cell, possibly a preleukemic stem cell, with a growth advantage and impaired differentiation. Some of these mechanisms could lead to secondary losses of stem cell numbers as well, however. One would predict that these patients would not respond to infusion of syngeneic bone marrow alone, but would require radiation, cytotoxic therapy, or immunosupression to deplete abnormal stem cells or abnormal regulatory or supressor cell populations. Failure to completely eradicate the population of supressor cells or abnormal stem cells could lead to eventual relapse.

Alternatively, loss of the primitive stem cells in sufficient numbers or stem cell exhaustion could lead to bone marrow aplasia relatively rapidly. Of course, regeneration of the stem cell pool might also occur if sufficient reserve proliferative capacity remained. In theory, damage to the hematopoietic microenvironment, i.e. loss of stem cell niches, could also result in aplasia. One would predict that both of these groups would be unlikely to benefit from immunosupressive therapy. Because recent evidence suggests that the fibroblastic component of the hematopoietic microenvironment may also be transplanted by bone marrow injections (Singer et al, 1983), either group could potentially respond to bone marrow transplantation. Furthermore, if the bone marrow niches are unoccupied and accessible for seeding of transplanted stem cells or microenvironmental stromal cells, these patients should respond to simple infusion of syngeneic bone marrow, i.e. from an identical twin donor.

A limited amount of data, based on the experience reported with identical twins, is available to test these models (Champlin et al, 1984; Appelbaum et al, 1985). Of the 22 identical twins with aplastic anemia reported in the literature to date, 9 were cured by injection of twin bone marrow and one additional patient responded to a second infusion after failing the first attempt. This result demonstrates, then, that approximately half of the patients with aplastic anemia suffer from a lack of stem cells or other transplantable regulatory or microenvironmental cell populations. Of the remaining 12 patients, 9 received a second bone marrow transplant after undergoing a conditioning regimen of radiation and/or cytotoxic therapy and all 9 recovered normal hematopoiesis. Presumably, these cases represent patients with mechanisms for their aplasia other than simple failure or loss of pluripotent stem cells. Two of these patients relapsed 1-3 years later, a result that is consistent with an abnormality separate from the stem cell itself. One of these two subsequently responded to anti-lymphocyte serum and continues in remission 2 years later, albeit with mild thrombocytopenia (Figures 5,6).

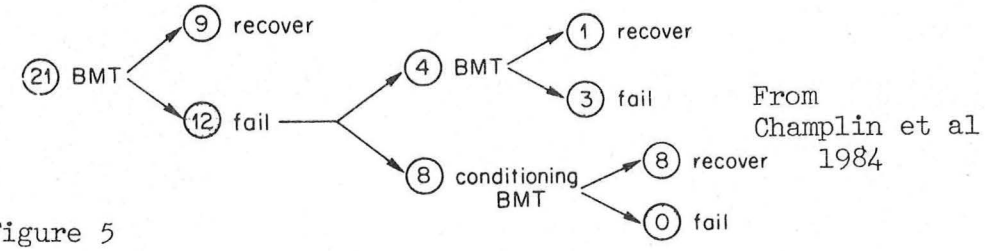


Figure 5

Outcome of bone marrow transplantation from identical twins in 21 evaluable patients with aplastic anaemia.

From Champlin et al, 1984

Figure 6
Bone marrow transplantation between identical twins with aplastic anaemia

Case	Age (yr)	Sex	Aetiology	Duration[a] (months)	Transplants (no.)	Marrow dose[b] (cells/10^8/kg)	Conditioning[c]	Response[d]	Survival (yr)	Reference
1	7	F	Drug	30	1	2·3	No	Complete	16	Robins & Noyes (1961)
2	3	M	Drug	4	2	2·4, 1·9	No	None	18+	Fernbach & Trentin (1962)
3	66	F	Drug	5	1	0·9	No	Complete	1[e]	Melvin & Davidson (1964)
4	9	F	Unknown	30	1	2·2	No	Complete	14+	Mills *et al* (1964)
5	9	F	Unknown	6	1	4·7	No	Complete	15+	Thomas *et al* (1964)
6	18	M	Mononucleosis	1	1	0·4	No	Complete	13+	Pillow *et al* (1966)
7	32	F	Unknown	4	3	0·6, 1·6, 2·6	No	None, transient	14+	Harvey & Firkin (1968)
8	18	M	PNH	9	1	2·6	No	Complete	4·5+	Fefer *et al* (1975)
9	15	F	Hepatitis	1	2	1·2, 3·3	Yes	Transient, complete	1·5+	Royal Marsden (1977)
10	18	F	Unknown	2	2	0·5, 0·8	No	Transient	8+	Harada *et al* (1978)
11	10	M	Unknown	60	2	3·5, 2·1	No	Transient	2+	Harada *et al* (1978)
12	19	F	Unknown	<1	1	2·2	No	Complete	5+	Appelbaum *et al* (1980)
13	4·5	F	Hepatitis	1	1	3·5	No	Complete	2+	Applebaum *et al* (1980)
14	53	M	Unknown	<1	2	1·3, 0·9	Yes	None, complete	1+	Appelbaum *et al* (1980)
15	44	M	PRCA	16	2	0·8, 1·8	Yes	None, transient	2+	Appelbaum *et al* (1980)
16	25	F	Drug	4	1	0·4	No	Complete	16+	Lu (1981)
17	14·5	M	Unknown	1	2	3·4	Yes	Complete	1+	Golombe *et al* (1979)
18	15	M	Unknown	15	2	2·1, 2·4	Yes	Transient, complete	7+	This report
19	13	M	Unknown	2	2	1·5, 1·7	Yes	None, complete	5+	This report
20	24	F	PNH	5	2	2·8, 3·9	Yes	Transient, complete	5+	This report
21	4	F	Unknown	2	2	3·5, 3·2	Yes	Transient, complete	1+	This report

PRCA = pure red cell aplasia; PNH = paroxysmal nocturnal haemoglobinuria.

[a] Diagnosis to transplant.

[b] Nucleated bone marrow cells $\times 10^8$/kg. Dose calculated from idealized age-adjusted weight. Different doses denote first, second, or third transplants.

[c] Conditioning given before the second transplant.

[d] Different responses denote, first, second or third transplants.

[e] Lost to follow-up.

HEMATOPOIESIS AND THE IMMUNE SYSTEM

Bearing in mind the model of pure red cell aplasia and the data from the identical twins, which suggests that half of the patients with aplasia will not respond to transplantation with normal bone marrow without conditioning therapy, we will now examine the data suggesting an immune etiology for some cases of aplastic anemia.

A. Normal Hematopoiesis

In recent years, increasing attention has been directed to the possible role of humoral regulators, possibly produced by lymphocytes or other cell types, in controlling normal hematopoiesis (Eaves and Eaves, 1984). Erythropoietin, for example, stimulates the proliferation of a committed erythroid progenitor cell which is capable of forming colonies of erythroid cells in vitro. Analogous stimulators of granulocytic colonies and megakaryocytic colonies have been described and purified, and cloned as well. None of these molecules, however, appears to stimulate the pluripotential stem cell. Thus, high levels of erythropoietin in vivo increase the proportion of late erythroid progenitors, called CFU-E, in cell cycle but do not affect the earliest erythroid progenitors, called BFU-E, or, for that matter, the pluripotent stem cells (Iscove, 1977; Wagemaker and Visser, 1980).

Because of this finding, much attention has been directed toward understanding the additional requirements, both cellular and humoral, for obtaining optimal growth of BFU-E *in vitro* (Eaves and Eaves, 1985) (Figure 7). Interestingly, BFU-E are found in significant numbers in the peripheral blood, as well as in the bone marrow. Studies of these circulating BFU-E appear to show that their growth *in vitro* is stimulated by a subset of T cells, the helper T cells, while supressor T cells, possibly including natural killer cells, supress colony growth (Torok-Storb et al, 1981; Mangan et al, 1982b,1984a; Levitt et al, 1984). Depletion of the helper lymphocytes with a specific monoclonal, for example, leads to decreased numbers of BFU-E colonies in cultures of peripheral blood mononuclear cells, while adding back these lymphocytes restores the colony growth. At least one group has presented evidence that these interactions are observed only if the erythroid progenitors and the stimulating T cells bear identical HLA antigens (Torok-Storb and Hansen, 1982).

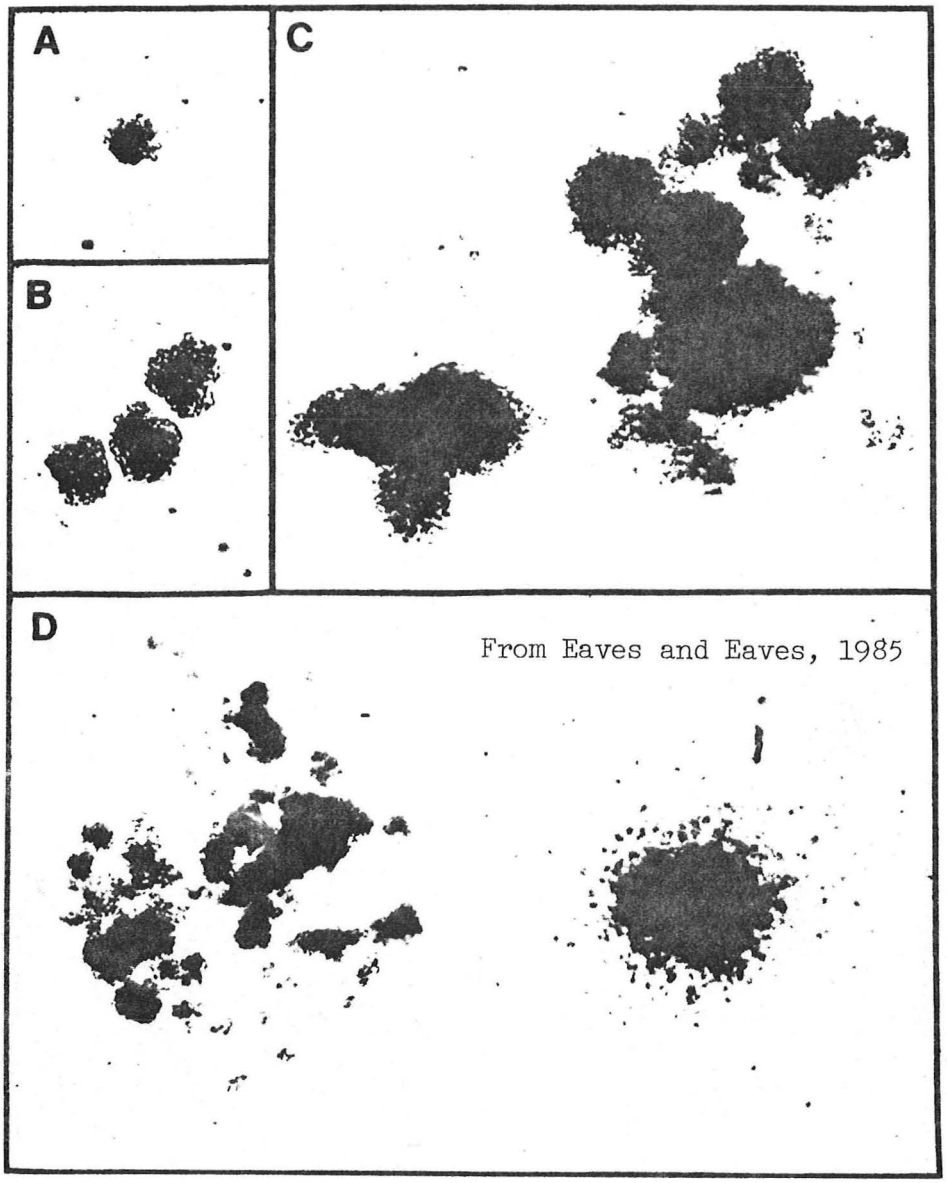


Figure 7 Photomicrographs of typical erythroid colonies of human origin. (A) a single cluster of erythroblasts derived from a CFU-E; (B) three clusters of erythroblasts derived from a mature BFU-E; (C) >eight clusters of erythroblasts derived from a primitive BFU-E; (D) lowpower view of a large multiclustered erythroid colony (*left*) similar to that shown in *c* by comparison to an adjacent granulocytic colony (*right*).

Other investigators, however, have either not been able to reproduce these findings or have found that the stimulation is in fact due to small numbers of monocytes that frequently contaminate preparations of pure "lymphocytes" (Zuckerman, 1981). Also unsettling is the finding that the BFU-E in the bone marrow, in contrast to the peripheral blood, seem to be independent of the the T cells for growth in vitro (Lipton et al, 1980). Thus, although alterations in normal regulatory cell populations clearly are a potential mechanism of stem cell failure, the relevance of these studies to in vivo control mechanisms is at best uncertain. Indeed, diseases associated with deficiencies of the immune cells are not usually characterized by pancytopenia or marrow hypoplasia. Peripheral blood counts are normal in congenital athymic states and congenital and acquired T cell deficiency syndromes (Jeunet and Good, 1968; Parkman, 1978; Aggio and Lozzio, 1979).

B. Hypoplastic Hematopoiesis

Although there is little evidence to implicate the immune system or lymphocytes in the regulation of normal hematopoiesis, a number of investigators have examined the possibility that bone marrow aplasia in some patients may result from supression of the stem cells by the patient's own lymphocytes (Zoumbos et al, 1984). This suggestion was strengthened by the not infrequent occurrence of pure red cell aplasia in patients with T-cell CLL, particularly those with proliferations of supressor (T gamma) or natural killer cell populations (Krantz and Dessypris, 1985).

Initially these experiments were similar to those described above for normal hematopoiesis. Thus, colony formation by bone marrow cells from patients with aplastic anemia was stimulated by removal of T cells, and normal bone marrow colony formation was inhibited by the addition of blood or bone marrow cells from aplastic patients (Hoffman et al, 1977; Kagan et al, 1979). Subsequent studies, however, have presented convincing evidence that these earlier findings resulted from sensitization of the patients by repeated blood transfusions and mismatching at the HLA locus between the patient and the normal bone marrows. When bone marrow cells from untransfused patients with aplastic anemia were cultured with HLA-matched bone marrow from normal siblings, only 3 of 16 cases exhibited inhibition of colony formation by the normal cells (Singer et al, 1979). In another study, blood from 12 of 34 transfused patients and none of 8 untransfused patients inhibited colony formation by normal bone marrow cells (Torok-Storb et al, 1980). Depletion of T cells eliminated the inhibition in 6 of the transfused patients but had no effect in the untransfused group. Even more discouraging was the finding that the liklihood of response to anti-thymocyte globulin did not correlate with enhanced colony formation in vitro after T cell depletion (Torok-Storb, 1983). Nevertheless, a small subset of untrasnfused patients with bone marrow aplasia may have a population of T lymphocytes which supress colony formation by hematopoietic progenitors in vitro. Furthermore, the subset of T cells responsible for the supression appears to be the T gamma cells, a supressor cell population also associated with pure red cell aplasia (Bacigalupo et al, 1980).

More recently, investigators have begun to utilize monoclonal antibodies to analyze in more detail the T cell subsets in aplastic anemia. Approximately one-third to one-half of patients with aplastic anemia have been found to have an abnormally low ratio of helper to supressor T cells in the peripheral blood despite a normal ratio in the bone marrow (Foon et al, 1984; Zoumbos et al, 1984a; Tuis-Arguellos et al, 1984; Falcao et al, 1984). The difficulty of interpreting these abnormalities is emphacized by the finding that, of four patients with low ratios who responded to ATG, two continued to manifest an abnormal ratio and two did not. Within the last few months, Young and his collaborators have gone on to show that 10 of 12 patients with aplastic anemia, in comparison to normals or multiply transfused controls, had significantly increased numbers of a particular sub-population of supressor T lymphocytes, those that express the HLA-DR antigen, a marker of T cell activation (Zoumbos et al, 1985a) (Figure 8). Five of these patients were also studied for

expression of the receptor for interleukin-2, a T cell growth factor and an independent indicator of T cell activation. In accord with the previous result, the patients with aplasia had receptor for interleukin-2 on 13-53% of their supressor T cell population, as compared to less than 1% in normal controls, 1-3% in multiply-transfused controls, and 8% in one patient who recovered from aplasia (Figure 9).

Figure 8
Cell Populations in 12 Patients with Aplastic Anemia and 7 Normal Controls, According to Monoclonal-Antibody Binding.*

Monoclonal Antibody	Target Cell	% Peripheral-Blood Lymphocytes Positive		P Value
		Aplastic anemia	Normal	
Leu-4$^+$	T lymphocytes	80.72±8.63	72.10±3.14	NS
Leu-3a$^+$	Helper T lymphocytes	43.82±11.57	43.37±6.19	NS
Leu-2a$^+$	Suppressor/cytotoxic T lymphocytes	37.69±11.61	28.29±7.47	NS (<0.1)
HLA-DR$^+$	B cells, monocytes, activated T cells	22.88±7.82	25.06±3.10	NS
Leu-M3$^+$	Monocytes/macrophages	6.54±1.25	10.27±3.15	NS
Leu-12$^+$	B lymphocytes	10.36±7.87	17.33±4.41	NS (<0.1)
Leu-15$^+$	30% of lymphocytes, monocytes	17.68±5.79	22.54±9.02	NS
Leu-3a$^+$–Leu 15$^+$	Subpopulation of helper T lymphocytes	3.94±2.80	2.65±2.18	NS
Leu-2a$^+$–Leu 15$^+$	Suppressor T lymphocytes	7.96±3.74	5.68±4.39	NS
HLA-DR$^+$–Leu 15$^+$	Heterogeneous population of activated lymphocytes	8.36±5.14	8.13±5.31	NS
Leu-3a$^+$–HLA-DR$^+$	Activated helper T lymphocytes	4.18±2.00	2.70±1.24	NS (<0.1)
Leu-2a$^+$–HLA-DR$^+$	Activated suppressor T lymphocytes	6.83±3.24	1.68±1.31	<0.001

*Values are expressed as means ±S.D. NS denotes not significant.

Figure 9
Distribution of Tac$^+$ T Cells in Patients with Aplastic Anemia and Control Groups.

Group/Subject No.	% of Tac$^+$ T Cells		
	in leu 1$^+$ cells	in leu 3$^+$ cells	in leu 2$^+$ cells
Patients with aplastic anemia			
1	46.04	79	53
2	23.14	22	20
9	42.25	65	25
10	23.15	24	13
11	56.33	68	43
Controls			
Patients given multiple transfusions			
1	10.36	37	3
2	13.45	32	3
3	7.16	15	1
Normal volunteers			
1	3.55	8	0.5
2	6.20	7	1
3	5.47	6	0.8
4	4.50	7.5	0.5
Patient who recovered from aplastic anemia			
1	14.52	31	8

The significance of this data is underscored by the earlier finding that the blood cells expressing the receptor for interleukin-2 are responsible for the release of gamma-interferon after lectin-stimulation of normal lymphocytes (Klimpel et al, 1982; Zoumbos et al, 1984b). In addition, the mediator of hematopoietic colony supression by aplastic anemia cells and stimulated normal lymphocytes appears to be gamma interferon. Addition of anti-sera to gamma interferon eliminates the inhibition. In an independent study (Zoumbos et al, 1985b), the same investigators have shown that 10 of 24 patients with aplastic anemia, but none of 16 normal controls and none of 18 multiply transfused controls, had increased levels of circulating interferon in vivo, at concentrations capable of inhibiting colony growth in vitro (Figure 10). Interferon levels in the bone marrow were detectable in normal controls, but were approximately 5 fold higher in the 8 patients with aplasia studied in this way (Figure 11).

Fig 10 Circulating interferon levels

	n	Interferon, IU/ml	No. > 10 IU/ml
Aplastic anemia patients	24	87 ± 30	10/24
Normal persons	16	<10	0/16
Multiply transfused patients with hereditary diseases	18	<10	0/18

Fig. 11 Interferon activity in bone marrow serum and blood serum

	Interferon, IU/ml	
	Bone marrow	Blood
Aplastic anemia patient		
3	250	50
4	227	90
6	250	ND
7	153	30
9	125	ND
11	153	10
12	270	90
13	200	50
Mean ± SD	203 ± 54*	53 ± 32
Normal individuals (n = 16)		
Mean ± SD	41 ± 23*	<10

Patient numbers 3–9 refer to Fig. 3. Patient 11 was partially recovered after anti-thymocyte globulin treatment. Patients 12 and 13 had been untreated.

Although this data is clearly very preliminary and needs to be confirmed in larger numbers of patients, as well as in other control groups such as patients with transient marrow aplasia secondary to chemotherapy, interferon is a substance that is known to have potent myelosupressive activity in vivo. Granulocytopenia and thrombocytopenia are a frequent side-effect in patients with cancer who are treated with recombinant alpha interferon (Gutterman et al, 1982). One patient with chronic lymphocytic leukemia developed severe aplastic anemia after treatment with interferon and subsequently achieved a partial remission with antithymocyte globulin (Mangan et al, 1984b). Thus, it may now be possible to prospectively examine the activated supressor T cells and interferon levels in patients with aplasia and begin to correlate these abnormalities with the response to immunosupressive therapy.

Many of these same immunologic abnormalities observed in patients with aplastic anemia are also present in patients with chronic viral infection. For example, gamma interferon is produced in certain chronic viral infections (Cunningham and Merigan, 1984), low T cell helper to supressor ratios occur in patients with HTLV-III related acquired immune deficiency syndrome and chronic herpes infections (Schupbach et al, 1984), production of interleukin-2 and increased expression of the receptor for interleukin-2 are observed in HTLV-I infection and the resultant adult T-cell leukemia (Hattori et al, 1981; Salahudin et al, 1984), and stimulation of supressor T lymphocytes is a feature of both retrovirus and herpes virus infections (Myburgh and Mitchison, 1976; Tosato et al, 1979). Because of these viral-induced abnormalities, the investigators whose studies of patients with aplasia are described above have speculated that an unknown virus may be the etiologic agent in many of their patients and is responsible for the immune abnormalities that they observed. A brief examination of the evidence linking viral infections to aplastic anemia will be considered at a later point in our discussion.

STEM CELL EXHAUSTION

The classic experiments of Hayflick (1965,1968) with fibroblasts in culture suggested that normal diploid cells have a proliferative capacity that is limited to approximately 50 cell divisions. Moreover, as the age of the cell donor increased, the proliferative capacity of the cells decreased (Martin et al, 1970), although the relevance of this in vitro data to stem cell function in vivo remains uncertain. A major unanswered question, therefore, in stem cell biology and the pathophysiology of aplastic anemia is whether the hematopoietic stem cell has infinite proliferative capacity under normal conditions, i.e. is immortal, or instead, is capable of very extensive but inherently limited proliferation. A related question concerns the possibility that the stem cells are not a homogeneous population with regard to proliferative capacity but instead have variable degrees of self-renewal potential. As indicated in an earlier brief discussion, none of the colony assays for hematopoietic progenitors appears to reflect the true potential of the bone marrow for regeneration (Harrison, 1980). Thus, much of the data to be reviewed here comes from animal studies of serial, i.e. repeated, bone marrow transplants. Although this approach may seem relatively cumbersome and unsophisticated, it does measure the fundamental capacity of the bone marrow to regenerate a stem-cell deficient recipient.

When bone marrow is transplanted into a recpient that has been lethally irradiated to deplete endogenous stem cells, it will repopulate the hematopoietic system and restore the blood counts, colony numbers, and marrow cellularity to normal within one or two months. If this bone marrow is used for a second transplant, however, marrow repopulation is less complete, and within three to five such serial transplants, the donor population fails entirely and is lost (Siminovitch et al, 1964; Cudkowicz et al, 1982). Increasing the interval between transplants, to allow additional time for complete regeneration of the stem cell pool to occur, has no discernible effect on the outcome of the serial transfers. The total proliferation of the donor marrow in these serial transfers, however, can be demonstrated to exceed the

lifetime production in the animal at least several fold. Treatment of the donor bone marrow with certain alkylating agents, such as busulfan, or with radiation prior to transplantation also produces a permanent loss of repopulating capacity that is dose-dependent (Botnick et al, 1976,1978; Harrison, 1982). Repeated treatments with hydroxyurea, a cycle-specific drug, reduce the number of CFU-S by over 70%, but do not result in any loss of repopulating capacity (Ross et al, 1982). Presumably the non-cycling primitive stem cell is protected from the toxic effect of this latter drug. One major criticism of these experiments, that the irradiation of the recipients damaged the hematopoietic microenvironment and hence secondarily led to loss of the donor stem cells, has been obviated by using a genetically stem-cell deficient mouse mutant. These animals accept histo-compatible bone marrow transplants without conditioning treatment, yet donor bone marrow transplanted serially in these hosts are similarly exhausted within three to five transfers (Harrison, 1972, 1973, 1975).

If these experiments are correct in suggesting that bone marrow stem cells are inherently limited in their potential for regeneration and proliferation, one would expect that aging itself, through the daily demands for differentiated cell progeny, would lead to at least a partial loss of stem cell numbers or capacity. Indeed, several investigators have directly compared bone marrow from aged mice with bone marrow from young adult mice by transplanting a single stem cell-deficient recipient with a mixture of the two cell populations (Ogden and Micklem, 1976; Harrison, 1984). Previous experiments had shown that this competitive repopulation system, in contrast to CFU-S assays, could demonstrate that once-transplanted bone marrow has a 20 fold reduction in erythropoietic repopulating capacity relative to control un-transplanted bone marrow (Harrison, 1982; Ross et al, 1982). Utilizing this sensitive technique, Harrison and his collaborators recently went on to demonstrate that equal numbers of cells from old bone marrow, young bone marrow, and even fetal liver (the site of hematopoiesis in utero) were equally effective in reconstituting young stem cell-deficient recipients. This result was not affected by subsequent repeated doses of sublethal irradiation at 3-6 month intervals (Figure 12) . These authors concluded, therefore, that hematopoietic stem cells have unlimited proliferative potential. Other investigators, using a similar technique, obtained similar results with bone marrow, but found that fetal liver was more effective than either young or old bone marrow (Micklem et al, 1972).

Figure 12
Competitive Repopulating Ability of B6 Donors in Immunologically Crippled or Intact W/W^v WBB6F$_1$ Recipients

Donor age, tissue	Percentage donor hemoglobin after:			
	83 d, 98 d	500 rad; 193 d, 196 d	500 rad; 298 d, 307 d	500 rad; 465 d, 495 d
In thymectomized, lethally irradiated recipients:				
Old marrow	75 ± 8 (6)	70 ± 10 (5)	71 ± 13 (5)	—
Young marrow	30 ± 4 (5)*	30 ± 7 (5)*	45 ± 9 (5)	—
Fetal liver	55 ± 6 (2)	43 ± 1 (2)	32 ± 13 (2)	—
In untreated W/W^v recipients:				
Old marrow	52 ± 3 (6)	57 ± 4 (6)	66 ± 8 (6)	59 ± 12 (5)
Young marrow	31 ± 5 (5)*	28 ± 5 (5)*	42 ± 11 (5)	42 ± 7 (5)
Fetal liver	43 ± 2 (2)	44 ± 9 (2)	41 ± 2 (2)	24 ± 13 (2)

At first glance, these results appear to contradict a number of older studies which indicate that hemoglobin levels and B and T cell functions decline with age in both mice and human subjects (Kishimoto et al, 1973; Albright and Makinodan, 1976; Tyan, 1977; Williams, 1983) . Convincing evidence suggests, however, that at least in mice, the disparity between young and old mice results from an extrinsic effect, i.e. aging of the hematopoietic microenvironment, rather than from an intrinsic defect in the stem cells.

Thus, the decreased responsiveness of aged mice to acute bleeding was not corrected by transplantation with young bone marrow (Tyan, 1982). Similarly, bone marrow from aged mice or young mice transplanted into young recipients responded equally well 3, 6, and 10 months after transplantation to immunization with sheep red blood cells, as judged by numbers of antibody producing cells in the spleen (Harrison et al, 1977).

More recently, Hellman and his associates compared the growth of bone marrow from young and old mice in long-term in vitro cultures (Mauch et al, 1982). The success of this technique requires that the bone marrow initially form an adherent cell layer on the surface of the culture flask, which is composed of fibroblasts, adipocytes, macrophages, and endothelial cells. Once formed, this stromal layer, apparently performing an analogous function to the bone marrow microenvironment, will support the proliferation of pluripotent CFU-S for several months. When the adherent layers were prepared from young mice, the hematopoietic cells from old mice and young mice produced equal numbers of progeny cells and CFU-S over the 3 month culture period (Figure 13a,b). In contrast, adherent layers from aged mice were less effective in supporting hematopoiesis from either young or old marrow donors.

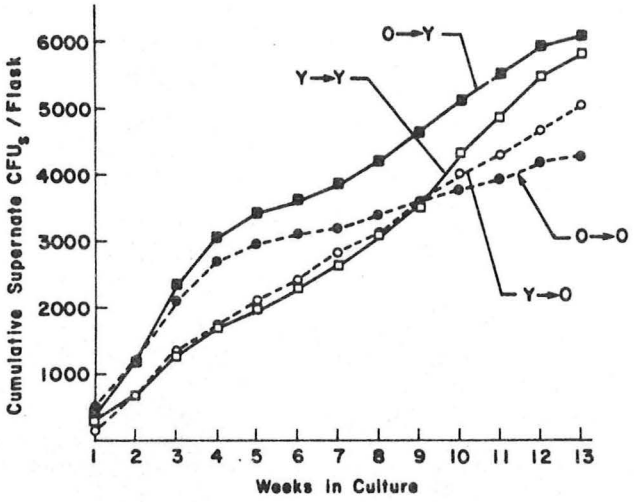
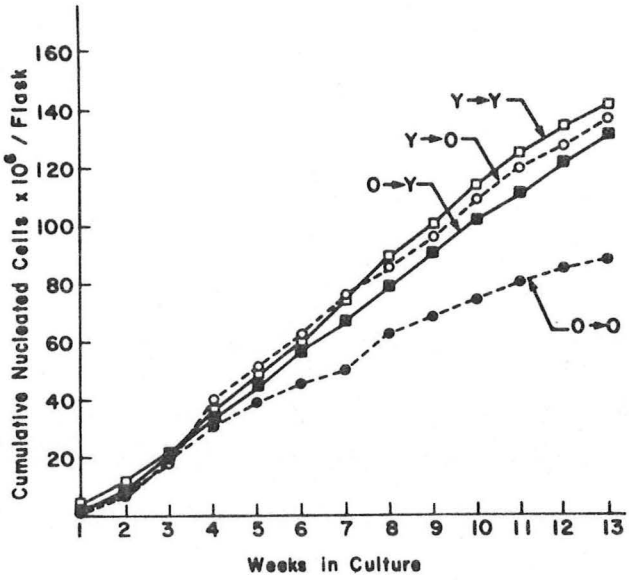


Fig. 13 **Cumulative nonadherent CFUs measurements per flask for old or young hematopoietic cells overlayed on old or young stroma and fed twice weekly.**



Cumulative nonadherent nucleated cell measurements per flask for old or young hematopoietic cells overlayed on old or young stroma and fed twice weekly.

While these studies of mice appear to demonstrate that aging does not lead to an intrinsic defect in hematopoietic stem cells, the short lifespan of a mouse may be inadequate to detect a decline in stem cell function. Lipschitz and his collaborators (1984) have recently reported their results with apparently healthy, elderly human subjects with mild anemia that remained unexplained after extensive clinical work-up and empiric trials of oral iron. Compared to young normal controls, these elderly anemic adults had normal numbers of BFU-E, the earliest erythroid progenitors, but significantly reduced numbers of CFU-E, the late erythroid progenitors, and CFU-C, the committed granulocytic progenitors (Figure 14). Interestingly, a group of non-anemic elderly subject had values for CFU-E and CFU-C intermediate between these two groups. The authors speculate that the defect in the late progenitors may be a reduced responsiveness to erythropoietin and the granulopoietin, GM-CSF. Extrapolating from the mouse data, one might also wonder if an "aged" microenvironment could account for these findings. In either case, this study also failed to find evidence for an age related decline in the numbers of early

progenitors most closely related to the pluripotent stem cell in humans.

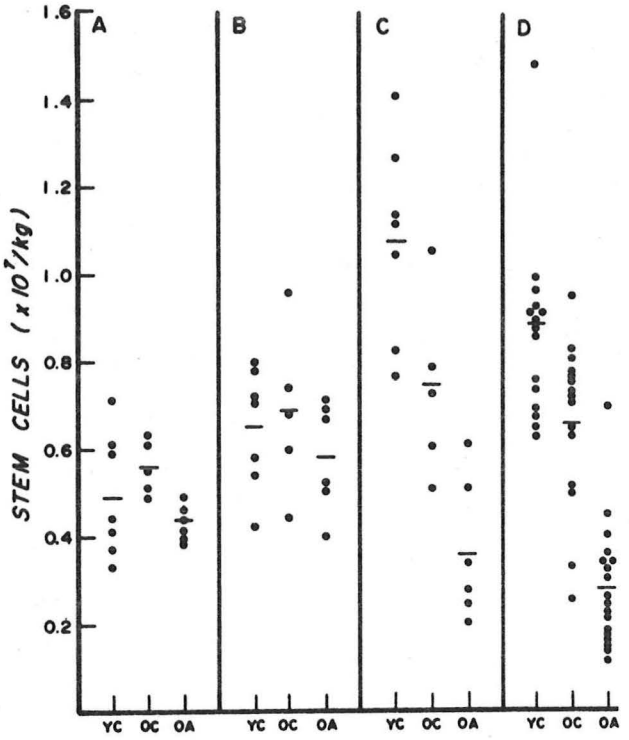


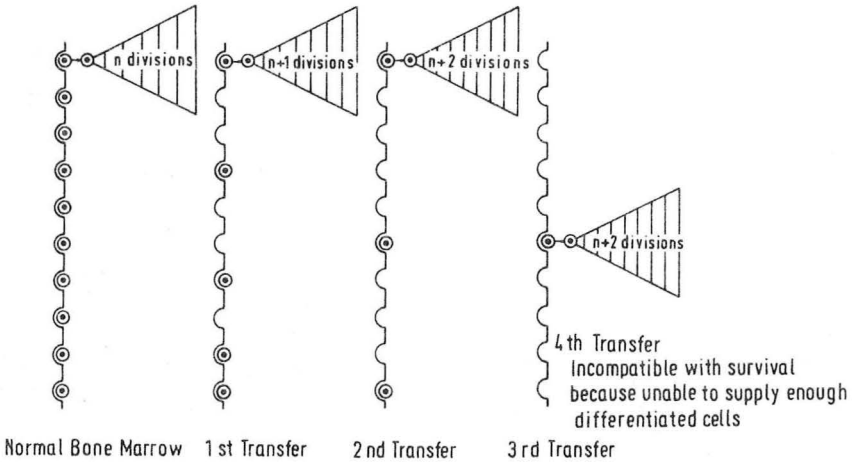
Fig. 14 **Quantitation of committed hematopoietic progenitor cells in young control (YC), old control (OC), and old anemic (OA) subjects. Quantitation of immature BFU-E (A), mature BFU-E (B), CFU-E (C) and CFU-C (D) are shown.**

Thus, under normal conditions, aging and the associated continued production of differentiated blood cells, does not seem to cause any detectable loss of stem cell numbers or renewal capacity. On the other hand, a single transplantation clearly causes a marked impairment in marrow reserve that is not corrected even after long recovery times. One explanation for this seeming paradox is that the number of amplifying cell divisions between the primitive stem cells and their mature progeny is so large that only rare stem cell divisions are required to satisfy normal homeostatic demands. Regeneration of stem cell numbers after a toxic insult, however, would require the surviving stem cells to undergo a large number of cell divisions, leading to a detectable decline in marrow reserve. Alternatively, one could imagine that the stem cell undergoes two different types of cell division: a symmetric cell division to produce two daughter, albeit diminished stem cells; and an asymmetric cell division which yields one commited progenitor cell and one stem cell but with undiminished renewal capacity. Although there is no evidence to support the concept of asymmetric cell division, this hypothesis would also resolve the apparent contradiction between the seemingly infinite capacity of the bone marrow to produce differentiated progeny under normal conditions and the finite capacity of the marrow to regenerate after toxic insults.

Another explanation that has been advanced is the "clonal succession" hypothesis. This model proposes first that the stem cells are heterogeneous for renewal capacity and second that the liklihood of committment to differentiation (and thus eventual extinction) by a given stem cell is directly related to this heterogeneity in proliferative ability. Thus, the stem cells are envisioned as contributing to normal homeostatic demands in a sequential fashion with the least capable stem cells differentiating first and the most capable stem cells differentiating last. During a normal lifetime, then, a small decline in stem cell numbers would occur rather than an overall decline in the renewal capacity of every stem cell. Moreover, the average

proliferative capacity of the stem cell pool would appear to increase with age because of selective utilization of the stem cells with the least renewal. Unfortunately, the absence of a quantitative assay for the most primitive stem cell pool has precluded experimentation to test the predictions of this hypothesis. Recent experiments in mice, however, have provided strong experimental support for the idea that the primitive hematopoietic stem cells capable of both myeloid and lymphoid differentiation are heterogeneous in their prolifertive capcity in vivo (Fleischman et al, 1982, Fleischman and Mintz, 1984; Mintz et al, 1984). Toxic damage to the bone marrow, therefore, would destroy stem cells randomly, including some with more extensive self-renewal ability, while aging would preferentially expend the stem cells with the least self-renewal (Figure 15).

Fig. 15 Serial transplantation From Schofield, 1978



The diagram illustrates that transfer of normal bone marrow into a lethally irradiated mouse results in incomplete occupation of the stem cell 'niches' by CFU-S, i.e., in reduction in the number of stem cells. To attempt to restore the CFU-S population on extra division is undergone. In the second and third transfers fewer niches are filled and hence the number of CFU-S produced falls with the result that the production of mature cells is seriously impaired

CLONAL DOMINANCE

In addition to immune-mediated supression and stem cell depletion, at least a proportion of the bone marrow aplasias appear to result from the unregulated proliferation of an abnormal stem cell clone leading to clonal dominance. In many cases, this clone retains some capacity for differentiation and produces a clinical picture that is characterized by pancytopenia with a cellular, though ineffective, bone marrow. These myelodysplastic disorders, which are frequently preleukemic, may present with hypercellular, normocellular, or hypocellualar bone marrows. Bone marrow aplasia in these cases, therefore, probably represents one end of a spectrum of impaired differentiation with a nearly complete block in stem cell maturation. An abnormal karyotype in the bone marrow cells, when demonstrable, provides suggestive evidence for a preleukemic state. Unfortunately, complete bone marrow aplasia often precludes cytogenetic studies.

Abkowitz and Adamson (1984) recently described a case of pancytopenia with a hypocellular bone marrow in a 14 girl who was heterozygous for the clonal marker glucose-6-phosphate dehydrogenase. All of the circulating erythr granulocytes, and platelets were characterized by a single enzyme typ were derived from the proliferation of a single stem cell clone. T lymphocytes appeared to be predominantly derived from the same clone,

the contamination of the B lymphocytes by monocytes, which have a high enzyme activity per cell, could have possibly accounted for this result. The patient was also lymphocytopenic with a reversed helper/supressor T cell ratio but analysis of the T cell sub-groups separated by cell sorting revealed equal activities of the two enzyme variants. Thus, the T cell abnormalities in this patient did not result from direct clonal expansion. Analysis of individual erythroid colonies revealed the persistence of some normal bone marrow progenitors. Repeated colony analysis appeared to demonstrate decreasing numbers of normal, i.e. non-clonal, progenitors on subsequent testing 3 and 6 months later.

Another disorder that appears to represent a clonal proliferation of an abnormal pluripotent stem cell that may occur in a setting of bone marrow aplasia is paroxysmal nocturnal hemoglobinuria (PNH) (Hartmann and Arnold, 1977; Rosse, 1978; Beutler, 1983). Although the abnormal complement sensitivity and the consequent clinical manifestations of PNH are most prominent in the erythroid lineage, the stem cell origin of the abnormal cells is suggested by the demonstration of increased sensitivity to complement in granulocytes and platelets as well. The frequent co-existence of the abnormal erythrocytes with complement-resistant or normal erythrocytes also is consistent with the clonal model. A direct demonstration of clonality in the abnormal population of complement sensitive erythrocytes was obtained in one patient who was heterozygous for electrophoretic variants of the X-linked enzyme, glucose-6-phosphate dehydrogenase (Oni et al, 1970). More recently, the BFU-E, CFU-E, and CFU-GM progenitor cells that produce erythroid and granulocytic colonies when cultured in vitro have also been shown to be abnormally sensitive to complement-induced lysis (Dessypris et al, 1983) (Figure 16).

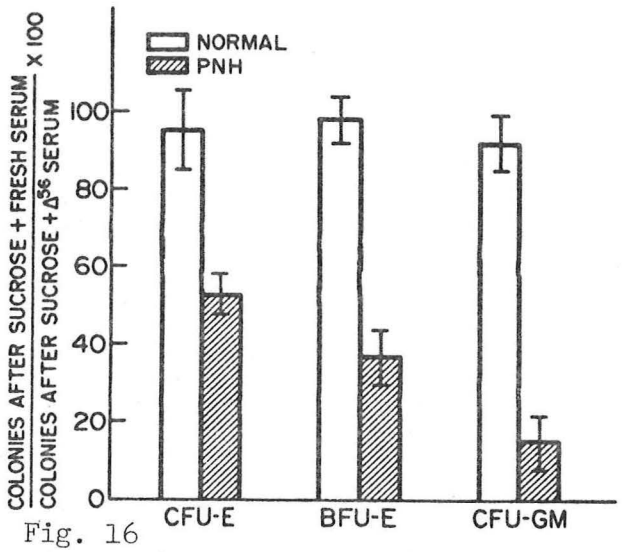


Fig. 16

Growth of Colonies (CFU–E, BFU–E, and CFU–GM) from Marrow Cells Preincubated in Sucrose and Fresh Serum, Expressed as the Percentage of Colonies Grown from Marrow Cells of the Same Subject That Were Preincubated in Sucrose and Heat-Inactivated (Δ^{56}) Serum.
Values (means ± S.E.M.) are for 10 normal controls and patients with paroxysmal nocturnal hemoglobinuria.

In many cases, PNH has been associated with evidence of myelodysplasia, chromosome aberrations, the development of acute and chronic leukemias, and myelofibrosis. The occurrence of PNH in this setting thus may reflect a

somatic mutation leading to increasing clonal dominance of a preleukemic stem cell. Conceivably the PNH defect confers a proliferative advantage on the stem cell. The degree of marrow aplasia and pancytopenia in these cases, in addition, would reflect the extent of impaired differentiation and clonal dominance associated with the particular premalignant stem cell. One would predict that aplasia could either precede or follow the onset of PNH, depending upon the evolution of the malignant phenotype. In fact, both patterns have been observed. Of course, many of the patients with PNH may die from the manifestations of their disease before the development of overt leukemia.

In other cases associated with marrow aplasia, however, the population of complement-sensitive erythrocytes is minor, sometimes present only transiently, and not associated with clinical symptoms of PNH (Lewis and Dacie, 1967; Ben-Bassat et al, 1975). Recovery from aplasia, both idiopathic and drug-related, seems to be most frequently associated with this subclinical abnormality. In the setting of stem cell depletion or stem cell regeneration, one could speculate that the presence of a minor proportion of complement sensitive cells may be a relatively frequent occurrence that does not necessarily reflect somatic mutation. Over time, the proportion of complement-sensitive cells may decline in some patients, and in a few, all evidence of the disease may disappear. Presumably, during the regeneration of the bone marrow from severe aplasia, the stem cell clones with the PNH phenotype may have an initial growth advantage that becomes less significant after partial or complete restoration of stem cell numbers. In this regard, it is interesting to note that one of the twins who was cured by simple marrow infusion had presented with idiopathic aplastic anemia subsequently complicated by mild manifestations of PNH (Fefer et al, 1975). Six months after the transplant, the pancytopenia had resolved although the Ham's test was still positive. Repeat testing a year later demonstrated a negative Ham's test. At least in this one patient, the PNH clone appeared to gradually disappear after correction of a presumed stem cell depletion. However, a second twin case with a diagnosis of PNH based on hemoglobinuria and a positive Ham's test, responded initially to marrow infusion but relapsed 6 months later with recurrent aplasia (Appelbaum et al, 1985). The second transplant was performed after conditioning with cyclophosphamide and radiation with a complete response maintained for more than three years at last report. Presumably the PNH clone in the second case had a proliferative advantage relative to the transplanted normal stem cells and achieved clonal dominance within a few months of the first infusion.

APLASIA AND THE BONE MARROW MICROENVIRONMENT

Studies of the fibroblastic cells in patients with aplastic anemia have found that the number and proliferative capacity of these presumed components of the hematopoietic microenvironment are not significantly different from normal controls (Gordon and Gordon-Smith, 1981; Kaneko et al, 1982). A major difficulty with investigations of the hematopoietic microenvironment is the ill-defined nature of the cellular components that comprise the marrow stroma. Indeed, Fialkow has presented data suggesting that some of the cells previously considered to be stromal may in fact be derived from the hematopoietic stem cell (Singer et al, 1983). High doses of irradiation will destroy the bone marrow support capacity, however, and infusions of normal bone marrow stem cells will not lead to the re-establishment of hematopoiesis in the irradiated bone (Knospe et al, 1966).

STEM CELL MODELS AND THE ETIOLOGY OF APLASIA

Although many patients with pancytopenia and an aplastic bone marrow have no obvious etiology for their disease, a number of etiologic agents have been implicated, including drugs, toxins, and radiation; viral infections; and

rheumatic and immunologic disorders. In general, though, the suggestion of a causative agent in these cases has not led to a clearer understanding of the underlying pathophysiologic mechanisms. A brief review of the known etiologies of bone marrow aplasia, bearing in mind the models of stem cell function and differentiation, is nevertheless appropriate at this point in the discussion (Figure 17).

Figure 17 Common Etiologic Agents in Acquired Aplastic Anemia

Drugs
- Antibiotics (chloramphenicol, penicillin, cephalosporins, sulfonamides)
- Anti-inflammatory agents (phenylbutazone, indomethacin, gold, penicillamine)
- Oral hypoglycemic drugs (chlorpropamide, tolbutamide)
- Antineoplastic cytotoxic drugs
- Antithyroid drugs, phenothiazines, antimalarials (quinacrine), diuretics (thiazides), antiepileptic drugs

Toxins
- Pesticides [gamma benzene hexachloride, chlorophenothane (DDT)]
- Aromatic hydrocarbon solvents and glues (benzene, toluene, xylene, naphthalene)
- Dyes, industrial toxins

Infections
- Hepatitis, Epstein-Barr virus, rubella, Venezuelan equine encephalitis, cytomegalovirus, brucellosis, tuberculosis, toxoplasmosis

Rheumatic and immunologic disorders
- Systemic lupus erythematosus, cryoglobulinemia, graft-versus-host disease

Paroxysmal nocturnal hemoglobinuria

Radiation

Thymoma

Pregnancy

From Champlin, 1985

A. Viral Infection

Aplastic anemia has developed concurrently with or following a variety of viral infections, including hepatitis, Epstein-Barr virus, cytomegalovirus, herpes varicella-zoster, rubella, and Venezuelan equine encephalitis (Young and Mortimer, 1984). The disease associated with hepatitis is apparently a unique clinical syndrome limited to young males and develops several months after recovery from non-A, non-B-induced liver inflammation (Foon et al, 1984). In contrast, infections with hepatitis B or hepatitis A virus have rarely been associated with aplastic anemia. The aplasia is particularly severe in these cases and the median survival is six months with fewer than 10% surviving for one year with conservative therapy. While most patients with idiophathic aplasias have normal numbers of lymphocytes and cellular immunity, hepatitis-associated disease is characterized by lymphocytopenia, particularly affecting T cells, reduced concentrations of serum IgG and IgM, and impaired mitogen reactivity and cutaneous hypersensitivity. More detailed characterization of the lymphocyte sub-classes in these patients has not yet been reported. One twin with apparent hepatitis-associated aplasia recovered with simple marrow infusion, although another required a conditioning regimen (Champlin et al, 1984). Both of these patients were female, however, and thus may represent a different disease.

It is tempting to speculate that some of these viruses may induce aplasia by direct infection of the hematopoietic precursors and stem cells. Indeed, this hypothesis appeared to be strengthened by the recent identification of parvovirus, a single-stranded DNA virus, as the etiologic agent in the "aplastic crises" observed in children with hemolytic anemias such as sickle cell disease and hereditary spherocytosis (Mortimer, 1984; Young et al, 1984a). Serologic studies have demonstrated that almost all of these cases, which actually represent an acute pure red cell aplasia, are linked to acute infection with parvovirus demonstrated by the presence of IgM antibody to the virus. Sera from these children markedly inhibited the growth of erythroid

colonies from bone marrow progenitors but had no effect on granulocytic progenitors (Mortimer et al, 1983). Furthermore, the virus could be directly demonstrated in the erythroid cells with virus specific monoclonal antibodies and shown to cause cell lysis and death. Fortunately, none of these children infected with parvovirus have developed lasting aplasia. A survey of patients with aplastic anemia demonstrated that the incidence of antibodies to parvovirus was identical to that seen in normal controls (Young et al, 1984b). Thus, parvovirus does not appear to be a cause of persistent marrow aplasia.

The demonstration that at least one virus can directly infect hematopoietic progenitor cells, however, suggests that other viruses may exist which induce aplasia by a direct cytopathic effect on the hematopoietic stem cell. One difficulty with this hypothesis is the fact that many viruses are unable to infect cells which are not actively replicating (Humphries and Temin, 1974; Varmus et al, 1977). Unlike the rapidly cycling progenitor cells, stem cells are predominantly in a G0 or non-cycling state. Parvovirus, for example, has an absolute requirement for rapidly cycling cells (Rose, 1975). Similarly, in mice a gene for resistance to infection with the Friend virus has been independently shown to control the basal rate of cycling by erythroid progenitors, resistance being associated with low rates of progenitor replication (Suzuki and Axelrad, 1980). Maintenance of the stem cell in G0, then, may be an important defense against viral infection of these critical cells.

One class of retrovirus, the slow viruses, which includes the sheep visna virus, appears to infect non-replicating cells and establish long-lasting latent infections (Harris et al, 1984). Recently, the structure of the viral agent of acquired immunodeficiency syndrome, HTLV-III, has been determined and shown to resemble most closely the visna virus and other slow viruses. Conceivably this property explains why this virus can cause such a profound aplasia of the lymphoid helper cell population by depletion of the lymphoid committed stem cells. Regeneration of lymphoid stem cells from the primitive stem cell pool in the bone marrow potentially could occur if this model is correct. Regeneration of T4 positive helper cells can require a year or more, however; for example, after allogeneic bone marrow transplantation (Witherspoon et al, 1984). Potential recovery of lymphoid cells could also be limited by depletion of the primitive stem cell pool. In this regard, aplastic anemia has been reported in one patient with AIDS (Sterry et al, 1983).

Despite these speculations regarding stem cell extinction as a mechanism for aplasia, additional data supports a viral-induced immune-mediated supressor etiology. For example, both AIDS and chronic Epstein-Barr infection have recently been associated with chronic pure red cell aplasia (Berner et al, 1983; Socinski et al, 1984), as well as aplastic anemia. As discussed previously, aplasia limited to this progenitor population cannot be caused by a simple lack of progenitor cells for erythropoiesis if the pluripotent stem cells are present. Maintenance of the granulocytic and megakaryocytic populations in pure red cell aplasia in fact confirms the continued functioning of the stem cell pool. In addition, pure red cell aplasia or aplastic anemia may be a consequence of infection in cats with the feline leukemia virus. Recently, cats heterozygous for the X-linked enzyme glucose-6-phosphate dehydrogenase have been analyzed after the development of pure red cell aplasia (Abkowitz et al, 1984). These animals had normal numbers of very early erythroid progenitors but absent progenitors for late erythroid colonies, placing the block within the erythroid progenitor maturation pathway. The early erythroid progenitors were not derived from a single stem cell clone, however, as judged by expression of the X-linked enzyme variants in individual colonies, ruling out an abnormal pluripotent stem cell as the etiology of the disease.

B. Drugs

The immediate or delayed onset of aplastic anemia has been associated with a variety of drugs, most frequently chloramphenicol and phenylbutazone (or

oxyphenbutazone). The actual risk of developing fatal aplastic anemia after being treated with chloramphenicol is low, about 1 in 20,000 (Smick et al, 1964; Modan et al, 1975), but more than ten times the risk of developing an idiopathic aplasia (Wallerstein et al, 1969). In many patients receiving the drug, a reversible supression of bone marrow progenitors is observed, apparently due to a dose-dependent inhibition of mitochondrial protein synthesis with decreased ferrochelatase activity (Yunis et al, 1970; Manyan et al, 1972). Associated with this effect is a nuclear vacuolization of the bone marrow cells and an increase in the serum iron (McCurdy, 1961; Weisberger, 1969). While this frequent and reversible marrow supression observed with chloramphenicol generally occurs during drug administration and is directly related to the dose and duration of therapy, the occurrence of irreversible aplasia appears to be unrelated to the length of drug exposure or the dose used, and typically occurs some time after the drug has been discontinued. Indeed, in many cases continuation of drug therapy after the onset of bone marrow supression has not been associated with adverse consequences (Waisbren et al, 1963). Nevertheless, it is obviously prudent to discontinue chloramphenicol or other drug therapy at the first sign of marrow injury or peripheral cytopenia whenever clinically possible.

The tendency for some of the drug-induced aplasias to occur in an idiosyncratic fashion has led to the suggestion that an underlying genetic or acquired stem cell hypersensitivity to the drug or a toxic metabolite of the drug is involved in the pathogenesis. Some studies have provided evidence that marrow from patients who have recovered from aplasia or from relatives of patients with chloramphenicol-induced aplasia is more sensitive in vitro to the inhibitory effects of the drug. Also, individual differences in the rate or extent of drug detoxification may play a role (For a review, see Vincent, 1984). In either case, it is important to note that the idosyncratic reactions observed with many of these drugs are distinct from the predictable, dose-related and reversible bone marrow aplasias induced by the anti-neoplastic cytotoxic drugs. Although pancytopenia has been observed in patients treated with multiple courses of chemotherapy, often some time after discontinuation of therapy, these cases are generally associated with a cellular marrow and often progress to preleukemia.

Reference to the data from identical twins demonstrates that at least some cases of drug-induced aplasia (chloamphenicol and an anti-convulsant) have responded to simple infusion of syngeneic marrow, indicating that the defect may have resulted from an inadequate number of stem cells. One case of apparent chloramphenicol-induced disease in a twin did not improve, however. In addition, it is useful to note that many of the drugws associated with aplasia of the bone marrow have also been linked to acute episodes of pure red cell aplasia. Chronic PRCA, on the other hand, has not occurred with any of these drugs without subsequent progression to complete aplasia. Since chronic PRCA is frequently caused by immune mechanisms, both humoral and cellular, the absence of drug-induced cases argues that similar immune mechanisms are not the primary event in the drug etiology of aplastic anemia. Pure white cell aplasia and amegakaryocytic thrombocytopenia have also not been associated with any of the drugs linked to complete marrow aplasia.

C. Toxins and Radiation

The aromatic hydrocarbon solvents, particularly benzene, and several pesticides, including DDT, have been associated with marrow aplasia. In many cases, however, the exposure leads to pancytopenia with a cellular, ineffective marrow, which may progress to acute leukemia. Presumably these agents are mutagenic as well as marrow toxic and exert their effects directly on the hematopoietic stem cells. The degree of marrow depression appears to be related to the duration and extent of exposure, rather than an idiosyncratic mechanism. Glue-sniffing, which exposes the user to toluene, has been associated with aplastic anemia in a number of reports.

Acute exposure to high doses of radiation will result in death due to marrow aplasia and pancytopenia, or intestinal necrosis and ulceration. As

with benzene, recovery from exposure has been associated with bone marrow hypoplasia in some cases, while in others pancytopenia occurs with a cellular, but ineffective marrow. Aplastic anemia was a relatively rare late sequelae to exposure to the atomic bomb radiation in Japan, particularly in comparison to the incidence of acute and chronic leukemia (Kirshbaum et al, 1971). Long-term exposure to low levels of radiation have been linked to the development of aplastic anemia but a definite cause and effect relationship has been difficult to establish.

D. Immunologic Diseases

The overlap between bone marrow aplasia and chronic pure red cell aplasia is perhaps most striking when one considers the known immunolgic diseases, such as systemic lupus erythematosis, thymoma, and, of course, pregnancy. Although the indicidence of complete aplasia, as compared to PRCA, is much lower in patients with thymoma (Rogers et al, 1968), and apparently higher in pregnancy (Fleming, 1973), both diseases have been described in association with each of these presumably immune disorders. Not only may the aplasia remit after termination of the pregnancy, but in three cases aplasia redeveloped with a second pregnancy.

Diffuse fasciitis is another disorder in which an autoimmune etiology is suspected and an association with aplastic anemia is described (Hoffman, 1984). The disease is characterized by deep, indurated plaque-like lesions of the skin and subcutaneous tissue. Peripheral blood eosinophilia and hypergammaglobulinemia are frequent, but antinuclear antibodies are usually absent and complement levels are normal. Nine cases of diffuse fasciitis with aplastic anemia have been described and an additional two patients have had autoimmune thrombocytopenic purpura. Amegakaryocytic thrombocytopenia occurred in at least one case, as well. Preliminary data suggests that the sera from some of these patients are inhibitory to hematopoietic progenitors, both heterologous and autologous.

TREATMENT OF BONE MARROW APLASIA

Patients with acquired bone marrow aplasia have been divided into prognostic groups based on the severity of their pancytopenia. Severe aplastic anemia (SAA), defined by a marked reduction in the numbers of at least two of the three cell lineages in the peripheral blood (granulocytes less than 500, platelets less that 20,000, absolute reticulocyte count less than 20,000) and a bone marrow cellularity less than 25% of normal, has a grave prognosis Camitta et al, 1976). Median survival for patients meeting these criteria is 3-6 months and 80-90% die within 1-2 years. Many survivors are transfusion dependent and fewer than 10% will spontaneously recover normal hematopoiesis.

Treatment of this life-threatening disease consists of the withdrawal of potential etiologic agents, if one can be identified, and supportive care with transfusions of red cells and platelets when necessary. Transfusions of blood products, particularly from family members, should be avoided, if possible, in patients who are a candidate for bone marrow transplantation. Obviously, infections are a major problem in these granulocytopenic patients and fever must be treated empirically with broad-spectrum antibiotics. Granulocyte transfusions should be reserved for patients with documented infections and severe granulocytopenia who fail to respond to a 2 or 3 day trial of appropriate antibiotics.

A number of agents have been used in the past in an attempt to restore normal hematopoiesis. Although occasional patients have responded after therapy with androgens or steroids, patients with severe aplasia appear unlikely to benefit from these agents (Camitta et al, 1979). In view of the low but significant spontaneous remission rate in severe aplasia, uncontrolled trials, often including patients with mild to moderate aplasia and reporting significant benefit from these agents, must be viewed with caution. Patients with mild to moderate aplasia may be more likely to improve after therapy with

androgens, although a several months of treatment may be necessary before a response can be observed (Shahidi, 1973).

More recently, controlled trials have demonstrated that treatment with preparations of anti-lymphocyte globulin or anti-thymocyte globulin leads to hematologic responses in approximately 40-60% of the patients with severe aplasia (Camitta et al, 1983). In addition, an improved survival was apparent at 2 years in comparison to controls receiving supportive care and androgen therapy (76% vs. 31%). In another controlled trial, 11 of 21 patients given ATG had a response as compared to 0 of 21 control patients during the three month period of the study (Champlin et al, 1983).

It should be emphacized, however, that the most of these responses are incomplete. Partial responses are defined by most investigators as an improvement in hematologic status so that the patient no longer qualifies as severe aplastic anemia and did not require transfusions of platelets or red cells. A small proportion of responders, about 10%, have also been reported to relapse after initial treatment. A determination of the true incidence of late relapses and long-term responders will clearly require longer periods of follow-up.

Although the apparent efficacy of anti-lymphocyte and anti-thymocyte preparations have generally been attributed to their immunosupressive activity, in accord with the data suggesting an immune-mediated mechanism in a proportion of patients with severe aplasia, ATG is not specific for lymphocytes and binds to B cells, granulocytes, platelets, and thymocytes. Recently, Young and his colleagues have presented evidence that ATG can act as a mitogen in vitro (Gascon et al, 1985). Supernatants from lymphocytes cultured in the presence of ATG produced lymphokines at levels comparable to lectin stimulated cells and secreted growth factors capable of supporting the growth of BFU-E in vitro. In view of the generally negative results that have been obtained with other forms of "immunosupressive" therapy, including T-cell specific monoclonal antibodies (Doney et al, 1984), these results suggest an alternate mechanism for the favorable results obtained with ATG.

Obviously, major efforts are being directed to the development of a reliable in vitro test that would predict those patients who would respond to ATG. Torok-Storb and collaborators (1984) have attempted to assay BFU-E in peripheral blood mononuclear cells after treatment with a variety of monoclonal antibodies. Although a weak statistical correlation between improved BFU-E growth and subsequent response to ATG was observed, the strength of the association decreased as the interval between diagnosis and treatment increased. Interestingly, a second test for BFU-E in the peripheral blood, performed within 48 hours after ATG administration to the patients, was a highly significant predictor of patient response.

Within the last week, Torok-Storb and her colleagues (1985) have examined lymphocyte subsets in the blood and correlated these findings with response to ATG. The helper T cell to supressor T cell ratio did not correlate with response (Figure 18), nor did the proportion of activated T cells. Unfortunately, the specific sub-group of activated supressor T cells, identified by other investigators as a consistent feature of aplastic anemia and a source of gamma interferon in their patients, was not specifically evaluated by Torok-Storb. An unexpected finding, however, was a highly significant association between recovery and the presence of cells staining with a monoclonal antibody 20.3, which normally reacts with nucleated erythroid cells (Figure 19). The precise nature of these cells, which resemble small lymphocytes morphologically, is still uncertain; possibly they are erythroid precursors released into the blood in response to anemia and aplasia. As yet, attempts to grow this new subset of cells in colony assays in vitro has not been described. Their numbers, which range from 0 to 2000 per cubic mm, suggest that they are not BFU-E.

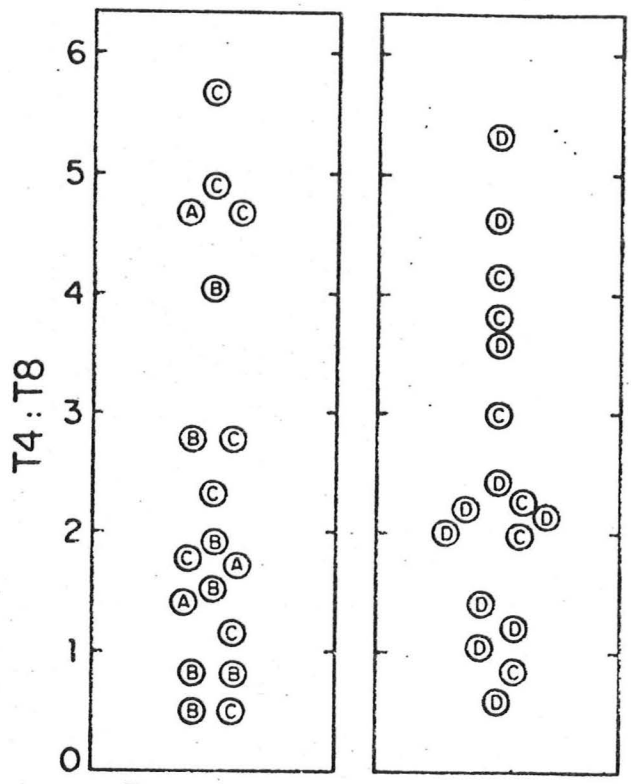


Fig. 18 The T4:T8 Ratios of Patients with Aplastic Anemia from Groups A to D, Shown in Relation to Clinical Course after Immunosuppressive Therapy.
There was no significant association between the T4:T8 ratio and the clinical response (Table 1).

Fig. 19 20.3 Scores in Relation to Clinical Recovery after Therapy with Antithymocyte Globulin for Aplastic Anemia.*

Recovery	20.3 Score			
	3	2	1	0
	no. of patients			
Yes	3	9	9	1
No	0	0	13	15

*The association between the 20.3 score and clinical recovery was significant ($P<0.0001$).

The immunosupressive drug cyclosporine has also been used successfully to treat both pure red cell aplasia, amegakaryocytic thrombocytopenia, and aplastic anemia (Totterman et al, 1984; Stryckmans et al, 1984; Hill and Landgraf, 1985). Further experience with this drug may help to delineate patients with an autoimmune basis to their disease.

Bone marrow transplantation, for patients under the age of 40 who have an HLA-identical sibling donor, offers a long-term cure rate of 70-80% when performed at centers with extensive experience with this technique. Chronic grat versus host disease (GVHD) that is not responsive to therapy is a major source of morbidity and mortality for approximately 10% of the successfully transplanted patients. Although some clinicians feel that marrow transplant is the treatment of choice for young patients with an appropriate donor (Thomas and Storb, 1984), the success achieved with ATG will require a re-evaluation of the indications for transplantation. Clearly, prospective trials comparing the two treatments will be necessary to clarify these issues (Speck et al, 1983).

REFERENCES

Abkowitz JL, Adamson JW: Clonal evolution in the pathogenesis of pancytopenia, in Young N, Levine A, Humphries RK (ed): Aplastic Anemia: Stem Cell Biology and Advances in Treatment. New York, NY, Alan R Liss, p 71, 1984

Abkowitz JL, Ott RL, Nakamura JM, Steinman L, Fialkow PJ, Adamson JW: Retrovirus-induced pure red cell aplasia (PRCA) in the cat: studies of disease progression and clonal evolution in a G-6-Pd heterozygote model. Blood, 64 [Suppl 1]:101a, 1984

Abramson S, Miller RG, Phillips RA: The identification in adult bone marrow of pluripotent and restricted stem cells of the myeloid and lymphoid systems. J Exp Med, 145:1567, 1977

Aggio M, Lozzio B: Hematopoiesis of hereditary asplenic-athymic (Lasat) mice. Exp Hematol, 7:197, 1979

Albright JF, Makinodan T: Decline in the growth potential of spleen-colonizing bone marrow cells of long-lived mice. J Exp Med, 144:1204, 1976

Appelbaum FR, Cheever MA, Fefer A, Storb R, Thomas ED: Recurrence of aplastic anemia following cyclophosphamide and syngeneic bone marrow transplantation: Evidence for two mechanisms of graft failure. Blood, 65:553, 1985

Bacigalupo A, Podesta M, Mingari MC, Moretta L, Van Lint MT, Marmont A: Immune suppression of hematopoiesis in aplastic anemia: activity of T gamma lymphocytes. J Immunol, 125:1449, 1980

Bagby GC, Gabourel JD: Neutropenia in three patients with rheumatic disorders. J Clin Invest, 64:72, 1979

Ben-Bassat I, Brok-Simoni F, Ramot B: Complement-sensitive red cells in aplastic anemia. Blood, 46:357, 1975

Berlin NI, Berk PD: The biological life of the red cell, in Surgenor D (ed): The Red Blood Cell. New York, NY, Academic, chapter 24, 1975

Berner YN, Berrebi A, Green L, Handzel AT, Bentwich Z: Erythroblastopenia in acquired immunodeficiency syndrome (AIDS). Acta Haemat, 70:273, 1983

Beutler E: Paroxysmal nocturnal hemoglobinuria, in Williams WJ, Beutler E, Erslev A, Lichtman M (ed): Hematology. New York, NY, McGraw-Hill, p 171, 1983

Botnick LE, Hannon EC, Hellman S: Limited proliferation of stem cells surviving alkylating agents. Nature, 262:68, 1976

Botnick LE, Hannon EC, Hellman S: Multisystem stem cell failure after apparent recovery from alkylating agents. Cnacer Res, 38:1942, 1978

BradleyTR, Metcalf D: The growth of mouse bone marrow cells in vitro. Aust J Exp Biol Med Sci, 44:287, 1966

Brecher G, Lawce H, Tijo JH: Bone marrow transplantation in previously irradiated, hematologically normal syngeneic mice. Proc Soc Exp Biol Med, 166:389, 1981

Camitta BM, Thomas ED, Nathan DG, Gale RP, Kopecky KJ, Rappeport JM, Santos, G, Gordon-Smith EC, Storb R: A prospective study of androgens and bone marrow transplantation for treatment of sever aplastic anemia. Blood, 53:504

Camitta BM, Thomas ED, Nathan DG, Santos G, Gordon-Smith EC, Gale RP, Rappeport JM, Storb R: Sever aplastic anemia: a prospective study of the effect of early marrow transplantation on acute mortality. Blood, 48:63, 1976

Champlin RE, Feig SA, Sparkes R, Gale RP: Bone marrow transplantation from identical twins in the treatment of aplastic anaemia: implication for the pathogenesis of the disease. Br J Haematol, 56:455, 1984

Champlin RE, Ho W, Gale RP: Antithymocyte globulin treatment in patients with aplastic anemia. A prospective randomized trial. N Eng J Med, 308:113, 1983

Cline MJ, Golde DW: Immune supression of hematopoiesis. Am J Med, 64:301, 1978

Cudkowicz G, Upton AC, Shearer GM, Hughes WL: Lymphocyte content and proliferative capacity of serially transplanted mouse bone marrow. Nature, 201:165, 1964

Cunningham AL, Merigan TC: Leu-3+ cells produce gamma-interferon in patients with recurrent herpes labialis. J Immunol, 132:197, 1984

Dessypris EN, Clark DA, McKee LC, Krantz SB: Increased sensitivity to complement of erythroid and myeloid progenitors in paroxysmal nocturnal hemoglobinuria. N Eng J Med, 309:690, 1983

Doney KC, Torok-Storb B, Dahlberg S, Buckner CD, Martin P, Hansen JA, Thomas ED, Storb R: Immunosuppressive therapy of sever aplastic anemia, in Young N, Levine A, Humphries RK, (ed): Aplastic Anemia: Stem Cell Biology and Advances in Treatment. New York NY, Alan R Liss, p 259, 1984

Dresch C, Najean Y, Bauchet J: Kinetic studies of 51Cr and DF32P labelled granulocytes. Br J Haematol 29:67, 1975

Eaves CJ, Eaves AC: Factors and hemopoiesis: Pandora's box revisited, in Young NS, Levine AS, Humphries RK (ed): Aplastic Anemia: Stem Cell Biology and Advances in Treatment, New York, NY, Alan R Liss, p 83, 1984

Eaves CJ, Eaves AC: Erythropoiesis, in Golde DW, Fumimaro T (ed): Hematopoietic Stem Cells, New York, NY, Marcel Dekker, p 19, 1985

Evans RS, Duane RT: Acquired hemolytic anemia. I. The relation of erythrocyte antibody production to activity of the disease. II. The significance of thrombocytopenia and leukopenia. Blood 4:1196, 1949

Evans RS, Takahashi K, Duane RT, Payne R, Lui CK: Primary thrombocytopenic purpura and acquired hemolytic anemia: Evidence for a common etiology. Arch Int Med 87:48, 1951

Falcao RP, Voltarelli JC, Bottura C: T-cell subsets in patients with aplastic anemia. Braz J Med Biol Res, 17:151, 1984

Fauser AA, Messner HA: Identification of megakaryocytes, macrophages and eosinophils in colonies of human bone marrow containing neutrophilic granulocytes and erythroblasts. Blood, 53:1023, 1979

Fefer A, Freeman H, Storb R, Hill J, Singer J, Edwards A, Thomas E: Paroxysmal nocturnal hemoglobinuria and marrow failure treated by infusion of marrow from an identical twin. Ann Int Med, 84:692, 1975

Fleischman RA, Custer RP, Mintz B: Totipotent hematopoietic stem cells: Normal self-renewal and differentiation after transplantation between mouse fetuses. Cell, 30:351, 1982

Fleischman RA, Mintz B: Development of adult bone marrow stem cells in H-2-compatible and-incompatible mouse fetuses. J Exp Med, 159:731, 1984

Fleming AF: Hypoplastic anaemia in pregnancy. Clin Haematol, 2:477, 1973

Foon KA, Mitsuyasu RT, Schroff RW, McIntyre RE, Champlin R, Gale RP:

Immunologic defects in young male patients with hepatitis-associated aplastic anemia. Ann Int Med, 100:657, 1984

Gascon P, Zoumbos NC, Scala G, Djeu JY, Moore JG, Young NS: Lymphokine abnormalities in aplastic anemia: implications for the mechanism of action of antithymocyte globulin. Blood, 65:407-413

Gordon MY, Gordon-Smith EC: Bone marrow fibroblastoid colony-forming cells (F-CFC) in aplastic anaemia: colony growth and stimulation of granulocyte macrophage colony-forming cells (GM-CFC). Br J Haematol, 49:465, 1981

Gutterman JU, Fine S, Quesada J, Horning SJ, Levine JF, Alexanian R, Bernhardt L, Kramer M, Spiegel H, Colburn W, Trown P, Merigan T, Dziewanowski Z: Recombinant leukocyte A interferon: pharmokinetics, single-dose tolerance, and biologic effects in cancer patients. Ann Int Med, 96:549, 1982

Harrison DE: Normal function of transplanted mouse erythrocyte precursors for 21 months beyond donor life span. Nature New Biol, 237:220, 1972

Harrison DE: Normal production of erythrocytes by mouse marrow continuous for 73 months. Proc Natl Acad Sci USA, 70:3184, 1973

Harrison DE: Normal function of tranplanted marrow cell lines from aged mice. J Gerontol, 30:279, 1975

Harrison DE: Competitive repopulation: a new assay for long-term stem cell functional capacity. Blood, 55:77, 1980

Harrison DE: Ultimate erythropoietic repopulating abilities of fetal, young adult, and old adult cells compared using repeated irradiation. J Exp Med, 160:759, 1984

Harrison DE, Astle CM: Loss of stem cell repopulating ability upon transplantation. Efects of donor age, cell number, and transplantation procedure. J Exp Med, 156:1767, 1982

Harrison DE, Astle CM, Doubleday JW: Stem cell lines from old immunodeficient donors give normal responses in young recipients. J Immunol, 118:1223, 1977

Harris JD, Blum H, Scott J, Traynor B, Ventura P, Haase A: Slow virus visna: Reproduction in vitro of virus from extrachromosomal DNA. Proc Natl Acad Sci USA, 81:7212, 1984

Hartmann RC, Arnold AB: Paroxysmal nocturnal hemoglobinuria (PNH) as a clonal disorder. Ann Rev Med, 28:187, 1977

Hattori T, Uchiyama T, Toibana T, Takatsuki I, Uchino H: Surface phenotype of Japanese adult T-cell leukemia cells characterized by monoclonal antibodies. Blood, 58:645, 1981

Hayflick L: The limited in vitro lifetime of human diploid cell strains. Exp Cell Res, 37:614, 1965

Hayflick L: Human cells and aging. Sci Am, 218:32, 1968

Hill W, Landgrad R: Successful treatment of amegakaryocytic thromcocytopenic purpura with cyclosporine. N Eng J Med, 312: , 1985

Hoffman R: Hematological sequelae of diffuse fasciitis, in Young NS, Levine AS, Humphries RK (ed): Aplastic Anemia: Stem Cell Biology and Advances in Treatment, New York, NY, Alan R Liss, p 185, 1984

Hoffman R, Bruno E, Elwell J, Mazur E, Gewirtz AM, Dekker P, Denes AE: Acquired amegakaryocytic thrombocytopenic purpura: A syndrome of diverse etiologies.

Blood, 60:1173, 1982

Hoffman R, Zanjani ED, Lutton JD, Zalusky R, Wasserman LR: Supression of erythroid-colony formation by lymphocytes from patients with aplastic anemia. N Eng J Med, 296:10, 1977

Humphries EH, Temin HM: Requirement for cell division for initiation of transcription of Rous sarcoma virus RNA. J Virol, 14:531, 1974

Iscove NN: The role of erythropoietin in regulation of population size and cell cycling of early and late erythroid precursors in mouse bone marrow. Cell Tiss Kinet, 10:323, 1977

Jeunet FS, Good RA: Thymoma, immunologic deficiencies and hematological abnormalities. Birth Def Orig Art Ser, 4:192, 1968

Kagan WA, Ascensao JL, Fialk MA, Coleman M, Valera EB, Good RA: Studies on the pathogenesis of aplastic anemia. Am J Med, 66:444, 1979

Kaneko S, Motomura S, Ibayashi H: Differentiation of human bone marrow-derived fibroblastoid colony forming cells (CFU-F) and their role in haemopoiesis in vitro. Br J Haematol, 51:217, 1982

Kirshbaum JD, Matsno T, Sato K, Ischimarn M, Tsucchmoto T, Ishimarn T: A study of aplastic anemia in an autopsy series with special reference to atomic bomb survivors in Hiroshima and Nagasaki. Blood, 38:17, 1971

Kishimoto S, Shigemoto S, Yamamura Y: Immune response in aged mice: change of cell-mediated immunity with aging. Iransplantation, 15:455, 1973

Klimpel GR, Fleischmann WR, Klimpel KD: Gamma interferon and interferon alpha/beta suppress murine myeloid colony formation: magnitude of suppression is dependent upon level of colony-stimulating factor. J Immunol, 129:76, 1982

Knospe WH, Blom J, Crosby NH: Regeneration of locally irradiated bone marrow. I. Dose dependent long-term changes in the rat, with particular emphasis upon vascular and stromal reaction. Blood, 28:398, 1966

Krantz SB, Dessypris EN: Pure red cell aplasia, in Golde DW, Fumimaro T (ed): Hematopoietic Stem Cells. New York NY, Marcel Dekker, p 229, 1985

Lajtha LG, Oliver R: Studies on the kinetics of erythropoiesis: a model of the erythron. Ciba Foundation Symposium on Haemopoiesis, 289, 1960

Lajtha LG, Oliver R, Gurney CW: Kinetic model of a bone marrow stem cell population. Br J Haematol, 8:442, 1962

Lajtha LG, Pozzi LV, Schofield R, Fox M: Kinetic properties of haemopoietic stem cells. Cell Tissue Kinet, 2:39, 1969

Lajtha LG: The common ancestral cell, in Wintrobe, WM (ed): Blood, Pure and Eloquent. New York, NY, McGraw-Hill, p 81, 1980

Levitt LJ, Kansas G, Foung S, Engleman E: Effects of purified immunoregulatory T-cell subsets on human erythroipoiesis. Blood, 64 [Suppl 1]:115a, 1984

Levitt LJ, Ries CA, Greenberg PL: Pure white-cell aplasia--antibody-mediated autoimmune inhibition of granulopoiesis. N Eng J Med, 308:1141. 1983

Lewis SM, Dacie JV: The aplastic anaemia-paroxysmal nocturnal haemoglobinuria syndrome. Br J Haematol, 13:236, 1967

Lipschitz DA, Udupa KB, Milton KY, Thompson CO: Effect of age on hematopoiesis in man. Blood, 63:502, 1984

Lipton JM, Nadler LM, Canellos GP, Kudisch M, Reiss CS, Nathan DG: Evidendce for genetic restriction in the suppression of erythropoiesis by a unique subset of T-lymphocytes. J Clin Invest, 72:694, 1983

Lipton J, Reinherz E, Kudisch M, Jackson P, Schlossman S, Nathan D: Mature bone marrow erythroid burst forming units do not require T cells for induction of erythropoietin-dependent differenitation. J Exp Med, 152:350, 1980

Little JR, Brecher G, Bradley TR, Rose S: Determination of lymphocyte turnover by continuous infusion of 3H-thymidine. Blood, 26:236, 1962

Mangan KF, Chikkappa G, Bieler LZ, Scharfman WB, Parkinson DR: Regulation of human blood erythroid burst-forming (BFU-E) proliferation by T-lymphocyte subpopulations defined by Fc receptors and monoclonal antibodies. Blood, 59:990, 1982b

Mangan KF, Chikkappa G, Farley P: T gamma cells suppress growth of erythroid colony-forming units in vitro in the pure red cell aplasia of B-cell chronic lymphocytic leukemia. J Clin Invest, 70:1148, 1982a

Mangan KF, Hartnett ME, Matis SA, Winkelstein A, Abo T: Natural killer cells suppress human erythroid stem cell proliferation in vitro. Blood, 63:260, 1984a

Mangan KF, Zidar B, Zieglar Z, Winkelstein A, Shadduck RK: Interferon induced aplasia: role of suppressor T cells and recovery after treatment with horse anti-human thymocyte globulin. Clin Res, 32:314a, 1984b

Manyan DR, Arimura GK, Yunis AA: Chloramphenicol-induced erythroid suppression and bone marrow ferrochelatase activity in dogs. J Lab Clin Med, 79:137, 1972

Martin GM, Sprague CA, Epstein EJ: Replicative lifespan of cultivated human cells: effectws of donor's age, tissue and genotype. Lab Invest, 23:86, 1970

Martin PJ, Najfeld V, Hansen JA, Penfold GK, Jacobson RJ, Fialkow PJ: Involvement of the B-lymphoid system in chronic myelogenous leukemia. Nature, 287:49, 1980

Mauch PM, Botnick LE, Hannon EC, Obbagy J, Hellman S: Decline in bone marrow proliferative capacity as a function of age. Blood, 60:245, 1982

McCurdy PR: Chloramphenicol bone marrow toxicity. JAMA, 176:588, 1961

Metcalf D, MacDonald HR, Odartchenko N, Sordat B: Growth of mouse megakaryocyte colonies in vitro. Proc Natl Acad Sci USA, 72:1744, 1975

Micklem HS, Ford CE, Evans EP, Ogden DA, Papworth DS: Competitive in vivo proliferation of foetal and adult haematopoietic cells in lethally irradiated mice. J Cell Physiol, 79:293, 1972

Mintz B, Anthony K, Litwin S: Monoclonal derivation of mouse myeloid and lymphoid lineages from totipotent hematopoietic stem cells experimentally engrafted in fetal hosts. Proc Natl Acad Sci USA, 81:7835, 1984

Modan B, Segal S, Shani M, Sheba C: Aplastic anemia in Israel: Evaluation of the etiologic role of chloramphenicol on a community-wide basis. Am J Med Sci, 270:441, 1975

Mortimer PP: A virological perspective on bone marrow failure, in Young N, Levine A, Humphries RK (ed): Aplastic Anemia: Stem Cell Biology and Advances in Treatment. New York, NY, Alan R Liss, p 121, 1984

Mortimer PP, Humphries RK, Moore JG, Purcell RH, Young NS: A human parvovirus-like virus inhibits haematopoietic colony formation in vitro. Nature, 302:426, 1983

Myburgh JA, Mitchison NA: Supressor mechanisms in neonatally acquired tolerance to a Gross virus-induced lymphoma in rats. Transplantation, 22:236, 1976

Ogen DA, Micklem HS: The fate of serially transplanted bone marrow cell populations from young and old donors. Transplantation, 22:287, 1976

Oni SB, Osunokaya BO, Luzzatto L: PNH: evidence for monoclonal origin of abnormal red cells. Blood, 36:145, 1970

Parkman R: The immunopathology of marrow failure. Clin Haematol, 7:475, 1978

Paulus JM: Measuring mean platelet life-span, mean age and variants of longevity in platelets, in Paulus JM (ed): Platelet Kinetics. Amsterdam, North-Holland, p 60, 1971

Pluznik DH, Sachs L: The induction of clones of normal mast cells by a substance from conditioned medium. Exp Cell Res, 43:553, 1966

Prchal JT, Throckmorton DW, Carroll AJ, Fuson EW, Gams RA, Prchal JF: A common progenitor for human myeloid and lymphoid cells. Nature, 274:590, 1978

Rogers BH, Manaligod JR, Blazek WV: Thymoma associated with pancytopenia and hypogammaglobulinemia; report of a case and review of the literature. Am J Med, 44:154, 1968

Rose JA: Parvovirus reproduction, in Fraenkel-Conrat H, Wagner R (ed): DNA Animal Viruses, Comprehensive Virology. New York, NY, Plenum, vol 3, 1975

Ross EA, Anderson N, Micklem HS: Serial depletion and regeneration of the murine hematopoietic system. Implications for hematopoietic organization and the study of cellular aging. J Exp Med, 155:432, 1982

Rosse WF: Paroxysmal nocturnal hemoglobinuria in aplastic anemia. Clin Hematol, 7:541, 1978

Ruiz-Arguelles GJ, Katzmann JA, Greipp PR, Marin-Lopez A, Gonzalez-Llaven J, Cano-Castellanos R: Lymphocyte subsets in patients with aplastic anemia. Am J Hematol, 16:267, 1984

Salahuddin SZ, Markham PD, Lindner SG: Lymphokine production by cultured human T cells transformed by human T-cell leukemia-lymphoma virus-I. Science, 223:703, 1984

Schofield R: The relationship between the spleen colony-forming cell and the haemopoietic stem cell. Blood Cells, 4:7, 1978

Schofield R: The pluripotent stem eell. Clin in Hematol, 8:221, 1979

Schupbach J, Popovic M Gilden RV, Gonda MA, Sarngadharan MG, Gallo RC: Serological analysis of a subgroup of human T-lymphotropic retroviruses (HTLV-III) associated with AIDS. Science, 224:503, 1984

Shahidi NT: Androgens and erythropoiesis. N Eng J Med, 289:72, 1973

Siminovitch L, McCulloch EA, Till JE: The distribution of colony-forming cells among spleen colonies. J Cell Comp Physiol, 62:327, 1963

Singer J, Doney K, Thomas ED: Coculture studies of 16 untransfused patients with aplastic anemia. Blood, 54:180, 1979

Singer JW, Keating A, Fialkow PJ: Evidence suggesting a common progenitor for hematopoietic and marrow stromal cells. Exp Hematol, 11 [Suppl 14]:4, 1983

Smick K, Condit PK, Proctor RL, Sutcher V: Fatal aplastic anemia: An

epidemiological study of its relationship to the drug chloramphenicol. J Chronic Dis, 17:899, 1964

Socinski MA, Ershler WB, Tosato G, Blaese RM: Pure red blood cell aplasia associated with chronic Epstein-*barr virus infection: Evidence for T cell-mediated suppression of erythroid colony forming units. J Lab Clin Med, 104:995, 1984

Sonoda T, Hayashi C, Seike J, Nakayama H, Terasaka K, Morioka T, Nakano T, Kitamura Y: Extensive proliferation of subsequently injected marrow cells in parental-to-F1 hematopoietic chimeras that restored normal stem cell concentration after initial transplantation. Exp Hematol, 13:143, 1985

Speck B, Gratwohl A, Nissen C, Osterwalder B, Signer E, Jeannet M: Bone marrow graft versus ALG in patients with aplastic anaemia. Biomed Pharmacother, 37:139, 1983

Sterry W, Marmor M, Konrads A, Steigleder GK: Kaposi's sarcoma, aplastic pancytopenia, and multiple infections in a homosexual (Cologne, 1976). Lancet, 1:1663, 1983

Stryckmans Pa, Dumont JP, Velu T, Debusscher L: Cyclosporine in refractory severe aplastic anemia. N Eng J Med, 310:655, 1984

Suzuki S, Axelrad AA: Ev=2 locus controls the proportion of erythropoietic progenitor cells (BFU-E) synthesizing DNA in normal mice. Cell, 19:225, 1980

Till JE, McCulloch EA: Direct measurement of the radiation sensitivity of normal mouse bone marrow cells. Radiation Res, 14:213, 1961

Thomas ED, Storb R: Acquired severe aplastic anemia: progress and perplexity. Blood, 64:325, 1984

Torok-Storb B: T cell effects on in vitro erythropoiesis: Immune regulation and immune reactivity, in Young N, Levine A, Humphries RK (ed): Aplastic Anemia: Stem Cell Biology and New Clinical Concepts, New York, NY, Alan R Liss, 1983

Torok-Storb B, Doney K, Brown SL, Prentice RL: Correlation of two in vitro tests with clinical response to immunosuppressive therapy in 54 patients with severe aplastic anemia. Blood, 63:349, 1984

Torok-Storb B, Doney K, Sale G, Thomas ED, Storb R: Subsets of patients with aplastic anemia identified by flow microfluorometry. N Eng J Med, 312:1015, 1985

Torok-Storb B, Hansen JA: Modulation of in vitro BFU-E growth by normal Ia-positive T cells is restricted by HLA-DR. Nature, 298:473, 1982

Torok-Storb B, Hansen JA, Martin PJ: Regulation of in vitro erythropoiesis by normal T cells: Evidence for two T cell subsets with opposing function. Blood, 58:171, 1981

Torok-Storb B, Sieff C, Storb R, Adamson J, Thomas ED: In vitro tests for distinguishing possible immune mediated aplastic anemia from transfusion induced sensitization. Blood, 55:211, 1980

Tosato G, Magrath I, Koski I, Dooley N, Blaese M: Activation of suppressor T cells during Epstein-Barr-virus-induced infectious mononucleosis. N Eng J Med, 301:1133, 1979

Totterman TH, Nisell J, Killander A, Gahrton C, Lonnqvist B: Successful treatment of pure red-cell aplasia with Cyclosporin. Lancet, 2:693, 1984

Trentin JJ: Influence of hematopoietic organstroma (Hematopoietic Inductive

Microenvironments) on stem cell differentiation, in Gordon AS (ed): Regulation of Hematopoieis. New York, NY, Appleton-Century-Crofts, p. 161, 1970

Tyan ML: Age-related decrease in mouse T-cell progenitors. J Immunol, 118:846, 1977

Tyan ML: Old mice: marrow response to endotoxin or bleeding. Proc Soc Exp Biol Med, 169:295, 1982

Varmus HE, Padgett T, Heasley S, Simon G, Bishop JM: Cellular functions are required for the synthesis and integration of avian sarcoma virus-specific DNA. Cell, 11:307, 1977

Vincent PC: In vitro evidence of drug action in aplastic anemia. Blut, 49:3, 1984

Wagemaker G, Visser TP: Erythropoietin-indlependent regeneration of erythroid progenitor cells folowing multiple injections of hydroxyurea. Cell Iiss Kinet, 13:505, 1980

Waisbren BA, Smiski C, Change PL: Administration of maximum doses of chloramphenicol. Am J Med Sci, 245:35, 1963

Wallerstein RO Condit PK, Kasper CK, Brown JW, Morrison FR: Statewide study of chloramphenicol therapy of fatal aplastic anemia. JAMA, 208:2045, 1969

Weisberger AS: Mechanisms of action of chloramphenicol. JAMA, 209:97, 1969

Williams WJ: Hematology in the aged, in Williams WJ, Beutler, E, Erslev AJ, Lichtman MA (ed): Hematology, New York, NY, McGraw-Hill, p 47, 1983

Wintrobe MM, Lee, GR, Boggs DR, Bithell TC, Foerster J, Athens JW, Lukens JN: Clinical Hematology. Philadelphia, Lea and Febiger, p 43, 1965

Witherspoon RP, Lum LG, Storb R: Immunologic reconstitution after human marrow grafting. Sem in Hematol, 21:2, 1984

Wu AM, Till JE, Siminovitch L, McCulloch EA: A cytological study of the capacity for differentiation of normal haemopoietic colony forming cells. J Cell Physiol, 69:177, 1967

Young NS, Moore JG, Humphries RK: The human parvovirus and in vitro hematopoietic colony formation, in Young N, Levine A, Humphries RK (ed): Aplastic Anemia: Stem Cell Biology and Advances in Treatment. New York, NY, Alan R Liss, p 129, 1984

Young NS, Mortimer PP: Viruses and bone marrow failure. Blood, 63:729, 1984

Young NS, Mortimer PP, Moore JG, Humphries RK: Characterization of a virus that causes transient aplastic crisis. J Clin Invest, 73:224, 1984

Yunis AA, Smith US, Restrepo A: Reversible bone marrow suppression from chloramphenicol: A consequence of mitochondrial injury. Arch Int Med, 126:272, 1970

Zoumbos N, Djeu J, Young N: J Immunol, 133:769, 1984b

Zoumbos NC, Ferris WO, Hsu SM, Goodman S, Griffith P, Sharrow SO, Humphries RK, Nienhuis AW, Young N: Analysis of lymphocyte subsets in patients with aplastic anemia. Br J Haematol, 58:95, 1984a

Zoumbos NC, Gascon P, Djeu JY, Trost SR, Young NS: Circulating activated supressor T lymphocytes in aplastic anemia. N Eng J Med, 312:257, 1985a

Zoumbos NC, Gascon P, Djeu JY, Young NS: Interferon is a mediator of hematopoietic suppression in aplastic anemia in vitro and possibly in vivo. Proc Natl Acad Sci USA, 82:188, 1985b

Zoumbos N, Gascon P, Young N: The function of lymphocytes in normal and suppressed hematopoiesis. Blut, 48:1, 1984

Zuckerman K: Human erythroid burst forming units. Growth in vitro is dependent on monocytes, but not T lymphocytes. J Clin Invest, 67:702, 1981