

Views on the Pathophysiology of Aplastic Anaemia

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Abstract

Aplastic anaemia seems to be predominantly a defect of the stem cell rather than the stroma, though abnormalities of the microenvironment may co-exist. There is highly suggestive evidence that the stem cell is the target of an immune attack, though the main evidence remains the response to immunosuppression with antilymphocyte globulin and cyclosporin. The stem cell defect remains even after recovery of the peripheral blood counts and the AA marrow is a fertile environment for the emergence of abnormal clones, particularly PNH. However, it has recently become apparent that there is an overlap with the myelodysplastic syndromes and clones of monosomy 7 and trisomy 8 amongst others are not uncommon in aplastic anaemia. Recent work has suggested that the emergence of a clone of monosomy 7 cells carries a poor prognosis, whereas trisomy 8 has a good prognosis particularly in response to cyclosporin. However, the setting in which monosomy 7 arises may affect the phenotypic expression. The immune targeting of stem cells may be associated with increased apoptosis in aplastic anaemia, in part mediated by fas expression, but not exclusively. Understanding the pathophysiology of AA should help to improve and perhaps target therapy.

Acquired aplastic anaemia (AA) is a rare disorder affecting some 2-3/million of the population in Europe and North America, perhaps twice as many in Asia and the Far East, and probably in South America and Africa as well. AA is characterised by peripheral blood pancytopenia with a hypocellular marrow in which normal haemopoietic tissue is replaced by fat cells. The remaining peripheral blood cells are of normal morphology apart from some macrocytosis and toxic granulation in neutrophils. There is no increase in fibrosis and remaining cells appear normal. The condition may arise following exposure to drugs, including non-steroidal anti-inflammatory drugs, chloramphenicol, some sulphonamides and antithyroid drugs. These drugs are widely used and the risk of developing aplastic anaemia following exposure seems to be about 1:25-40,000 for chloramphenicol (one of the better drugs studied epidemiologically) and perhaps even less frequent for other drugs and chemicals. Viruses have also been implicated but no specific viruses have been clearly demonstrated to be a cause, the commonest indication of viral association being a preceding hepatic-like illness in about 5-15% of cases.

The natural history of the disease is not clear since therapeutic interventions including support obviously alter the outcome. The following observations need to be taken into account when considering the pathophysiology of AA.

1. There is a rare idiosyncrasy which leads to the bone marrow failure, of which genetic susceptibility seems to play only a small part.

2. There is wide variation in the degree of bone marrow failure (which has led to the classification of AA into severe (SAA), very severe (VSAA) and non-severe aplastic anaemia (NSAA)). Once established NSAA may remain stable for many years, may progress towards severe or may even recover spontaneously.

3. All precursor cells seem to be affected in a similar fashion and remaining haemopoiesis is usually but not exclusively polyclonal [1]. Although the majority of stem cells seem to be affected equally, there may be variation in the degree to which individual haemopoietic lineages are affected.

4. The emergence of abnormal clones, particularly those of paroxysmal nocturnal haemo-globinuria (PNH), is a

characteristic of AA [2].

5. Treatment of patients with antilymphocyte globulin (ALG) leads to at least partial recovery of marrow function in about two-thirds of patients so treated [3], but does not eliminate the risk of emergence of abnormal clones [4].

1. The Target in AA Stem Cell or Stroma?

As acquired AA became better defined and separated from other causes of pancytopenia, so the discussion arose as to whether the stem cell (seed) or microenvironment (soil) was the main target in the pathophysiology. Such discussions tacitly acknowledge the intimate relationship between stem cell proliferation and the microenvironment. Quite apart from the stromal cells which are essential for the maintenance of haemopoietic colonies in long-term culture, there is the role played by the extracellular matrix composed of fibronectins and heparinoids and other structural proteoglycans. This microenvironment is closely linked to the presentation of cytokines to stem cells and clearly is an essential part of the control of haemopoiesis. It is thus theoretically possible that perturbations in the microenvironment might be responsible for the failure of haemopoiesis.

Early work on clonogenic assays of late and early committed precursor cell colonies (CFU-GM, CFU-E, BFU-E and CFU mix) revealed a marked reduction in late colony formation. Such a reduction was seen not only at presentation but also following apparent complete remission. Later, cross-over studies with long-term culture demonstrated a marked defect in haemopoiesis which seemed to be mainly the result of abnormalities in the proliferative potential of early haemopoietic progenitor cells [5]. Thus stroma from aplastic marrows was capable of supporting haemopoietic colonies from normal bone marrow but not haemopoietic colonies from aplastic marrows. Stroma from normal marrows on the other hand was also capable of supporting normal haemopoietic cells but not aplastic progenitors. This suggested that the main defect was in the haemopoietic progenitor cells but did not give any indication of the nature of the damage nor how it came about. It should be noted however that it is not always possible to grow confluent stroma from the aplastic marrow and there seems to be considerable variation in the rate at which the stroma grows to confluence from different patients with AA [6]. It seems most likely that differences in the stromal kinetics are the consequence of the AA rather than pathogenesis.

2. Quantitative Defects in Haemopoietic Progenitor Cells in AA

In the investigation of haemopoiesis in the human marrow, the earliest identified stem cells are generally considered to be CD34⁺, CD38⁻, Lin⁻. Most work examining the quantity of stem cells in AA have used this immunophenotyping method for determining cell numbers. This number is then correlated with colony-

forming activity. The total percentage of CD34⁺ cells is markedly reduced in most AA marrows with median values about 0.5% [7], but with a wide range from 0 to the normal range. The severity of the bone marrow failure does not seem to correlate with the percentage of CD34⁺ cells present in the aplastic marrow. It may be that it is a population of the very earliest progenitor cells which are most affected in AA. Apart from the marked reduction in the number of CD34 positive cells, there is universally a reduction in the long-term culture initiating cells (LTCIC). As already mentioned, LTCIC remain depressed in number even following recovery of bone marrow function. Recent experiments suggest that in recovered aplastic marrow the number of LTCIC reflects the limiting capacity of the bone marrow for haemopoiesis in contrast to normal marrow where there is a considerable excess of LTCIC, many in a quiescent phase.

3. Qualitative stem cell defects in AA

As mentioned in the introduction, imbalance in the severity of the damage to different haemopoietic cell lineages is common in AA. At one extreme amegakaryocytic thrombocytopenia may progress slowly to more generalised bone marrow failure indicating preferential damage to the megakaryocytic lineage. In vitro, megakaryocyte colonies are greatly reduced compared to normal, but there are also abnormalities demonstrable at different levels of maturation of the megakaryocytic colonies. It is also clear that when CD34⁺ cell numbers from AA marrow are plated onto normal stroma in numbers equivalent to those obtained from normal marrow, colony formation is still grossly deficient, once again showing the qualitative defects as well as quantitative abnormalities in AA.

4. Clonal evolution in AA

In some 25-40% of patients who achieve transfusion independence following treatment with antilymphocyte globulin, transfusion requirements may return [4]. Sometimes this is undoubted relapse of the AA, but more commonly it is associated with the emergence of an abnormal clone. The most common clones are those associated with loss of the glycosyl phosphatidyl inositol (GPI) anchor, the molecular defect which gives rise to the PNH phenotype. The loss of the GPI anchor occurs at stem cell level, consequent on a somatic mutation of a gene on the X chromosome. GPI negative cells lack a wide range of proteins on the surface of the cell including CD55 (decay accelerating factor) and CD59 (membrane inhibitor of reactive lysis) which render red cells in particular sensitive to lysis by complement. It was proposed by Dacie and colleagues in the 1960s that the PNH clone had a growth advantage over the 'normal' stem cells in AA which is why the clone emerged [8]. More recent work has shown that the GPI negative stem cells do not have any growth advantage over normal GPI replete stem cells, but have a considerable

proliferative advantage over the GPI positive stem cells which remain in AA [9]. This growth advantage has been taken as evidence to support an immune basis for the stem cell damage, proposing that the cells which lack the GPI-anchored proteins escape from immune suppression mediated on the GPI positive cells, and hence repopulate the bone marrow [10,11]. It should be remembered however that there are two main and usually separate clinical situations that may arise. In the first case an aplastic marrow may be associated with a small clone of PNH cells which does not appear to have influenced the bone marrow suppression. On the other hand there is haemolytic PNH in which the marrow may appear cellular and the assumption is that the peripheral blood pancytopenia is associated with destruction of cells. Minor clones of PNH cells may also be detected in small quantities in normal marrow, often transient [12].

It has also been appreciated recently that abnormal cytogenetic clones may emerge in typical AA [13,14]. The clones may be detected at presentation, may occur during follow-up, and may be transient. In some but not all cases the presence of a cytogenetic abnormality may precede the emergence of a typical myelodysplastic picture. Trisomy 8 is one of the more common abnormalities found in AA and seems to be associated with a good response to antilymphocyte globulin and cyclosporin. Monosomy 7, another more common abnormality, on the other hand is associated more with a poor response and the emergence of myelodysplasia or acute leukaemia [15]. It is difficult to know whether the emergence of cytogenetically abnormal clones is related to a proliferative advantage over the aplastic marrow or whether it is a consequence of the damage to the stem cells in AA. In acquired AA there is an accelerated senescence of haemopoietic stem cells as indicated by an accelerated shortening of the mean terminal restriction fragment length (TRF) in the telomere region [16]. The amount of shortening seems to be related to disease duration, but the acceleration is halted when recovery occurs. The classic model of the role of telomeres in replicative senescence and immortalisation would predict that patients in whom the telomere length was approaching a critical point might be the higher risk of developing MDS. In one study, those AA patients with an acquired cytogenetic abnormality were amongst those with the shorter telomere length. It should be emphasised that in these studies the shortening of the TRF appeared to be a consequence of the AA rather than a cause.

5. Response of AA to ALG, Cyclosporin and Other Immunosuppressive Regimes

The introduction of ALG to the treatment of AA clearly demonstrated that improved remission and survival occurred following this therapy. Later, the addition of cyclosporin to the ALG regime seemed to be associated with faster recovery, though perhaps not a greater proportion of success. Undoubtedly some patients, per-

haps as many as a half, are cyclosporin dependent following recovery of the marrow. It is in this group of patients that the trisomy 8 group appear to have the best response [15]. This clinical observation strengthened the idea that AA may be an autoimmune disease. A variety of immunological phenomena have been identified in patients with AA which support this hypothesis, but so far no target area in the stem cell has been identified as the specific immunological trigger.

6. Role of cytokines

Erythropoietin, thrombopoietin, stem cell factor and most other cytokines seem to be appropriately elevated in response to the pancytopenia. Not surprisingly, AA does not respond to the introduction of epo or IL-6. An exception is the response to granulocyte colony stimulating factor (GCS-F). Most patients with AA, except those with the most severe bone marrow failure, show some response in the granulocytes to treatment with GCS-F. Clinical observations suggest that the addition of GCS-F to ALG and cyclosporin may also accelerate recovery of the marrow, though the formal trials have been methodologically flawed. In vitro, colony numbers from AA marrow are increased mainly by incubation with cytokine combinations which include GCS-F [17]. One of the actions of GCS-F in the normal marrow is to permit the survival of CFU-C by switching off apoptosis. Increased apoptosis may play a role in the diminution of colony numbers in AA [18]. In the normal marrow the percentage of CD34⁺ progenitors that show evidence of apoptosis is significantly less than for the CD34⁺ progenitors found in the peripheral blood. There seems to be a higher proportion of apoptotic cells in the CD34⁺ progenitors in AA patients compared to the normal bone marrows, and they appear less able to withstand GCS-F deprivation. CD34⁺ cells in AA bone marrow have a higher expression of the Fas antigen [17] than similar cells from normal donors. In both AA and normal marrow CD34⁺ Fas⁺ cells mostly show evidence of the process of apoptosis. In normal marrow CD34⁺ Fas⁻ cells are alive, whereas in the AA marrow CD34⁺ Fas⁻ cells also showed evidence of cell death in about half of the patients examined. This might suggest that mechanisms other than Fas/Fas ligand determined apoptosis are responsible for cell death in patients with AA.

7. Conclusions

AA provides an unusual opportunity to observe damage to haemopoietic stem cells in the human. Quantitative and qualitative defects may be observed. New techniques may allow the study of various pathways that are altered leading to cell death and proliferative abnormalities. It is probable that immune mechanisms play a major role at least in preventing the recovery of marrow, if not the original damage to stem cells. Follow-up studies of AA may also provide an opportunity to observe the mechanism by which abnormal clones,

including MDS and leukaemia, emerge within the bone marrow. In any event, the study of the marrow function in AA needs to go forward in parallel with careful clinical observation and management.

References

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