A novel etiology of aplastic anemia: the uncontrolled adipogenic differentiation of mesenchymal stem cells in bone marrow induced by an abnormal immunological reaction

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[Abstract] Aplastic anemia (AA) is a clinical syndrome manifested by cytopenia and the fatty replacement of the marrow with a near absence of hematopoietic precursor cells. The present review explored the pathological roles of adipocytes in AA. The original articles and critical reviews selected were relevant to aplastic anemia, mesenchymal stem cells and adipocytes. The following well-documented evidence demonstrates that over adipogenesis has negative effects on aplastic anemia. 1) Increasing evidence indicates that toxins and toxic drugs that can frequently lead to AA can concomitantly induce mesenchymal stem cells (MSCs) to undergo adipogenesis. 2) The response time of immunosuppressive therapy for AA is significantly longer than that of other immuno-related cytopenia disorders, such as immune thrombocytopenia, indicating that not only hematopoietic cells but also possibly MSCs are targeted by abnormal immune status in AA patients. 3) Both the immunosuppressive and androgen therapies used for AA can decrease the adipogenesis of MSCs in the marrow. 4) A number of myelosuppressive cytokines, including tumor necrosis factor (TNF-α), interleukin 6 (IL-6) and interferon (IFN-γ), can be produced by adipose tissue. Exorbitant adipogenesis can directly inhibit hematopoiesis in animal models. This novel etiological model is worthy of further corroboration via experiments. If this finding is valid, the therapeutic modality would undoubtedly shift from immune suppression to the combination of immune suppression and the interference of the pathway involved in the adipogenesis of MSCs. The findings suggest the uncontrolled adipogenic differentiation of mesenchymal stem cells in bone marrow induced by an abnormal immunological reaction, which is a novel etiology of aplastic anemia.

[Key words] anemia, aplastic; mesenchymal stem cells; adipocyte

再生障碍性贫血病因学的新探索：异常免疫诱导骨髓间充质干细胞的过度脂肪化

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[摘要] 再生障碍性贫血(简称再障)是一种以血细胞减少为表现的疾病。骨髓脂肪过多且造血前体细胞减少是其显著骨髓病理学特征。本文解读和分析了与再障和脂肪细胞相关的论著和关键综述，旨在对骨髓内间充质干细胞向脂肪细胞分化的作用进行初步探索。这些文献提示，再障相关性毒素和毒性药物，一般也具有诱导骨髓间充质干细胞向脂肪细胞分化的作用。再障的免疫治疗反应时间要远远长于典型免疫相关性血细胞减少症，表明异常免疫的靶细胞不仅仅是造血干细胞，骨髓间充质干细胞也可能是靶细胞。免疫抑制剂和雄激素治疗再障有效，二者也具有抑制骨髓间充质干细胞向脂肪细胞分化的作用。脂肪细胞分泌的很多细胞因子均有抑制骨髓造血作用，包括肿瘤坏死因子(TNF-α)，白细胞介素6(IL-6)和干扰素γ(IFN-γ)。动物模型也证实，骨髓过度脂肪化可抑制造血。另外，再生性再生障碍性贫血一般被视作一种免疫介导的疾病，包括免疫细胞和分子介导造血干细胞破坏导致骨髓造血衰竭。综上，骨髓间充质干细胞向脂肪细胞过度分化可能是再障发病的新的病理学机制之一。

[关键词] 贫血，再生障碍性；间质干细胞；脂肪细胞

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It has generally been accepted that the defects of hematopoietic stem cells/hematopoietic progenitors (HSCs/HPCs), which are immune system disorders and abnormalities of the bone marrow microenvironment, are concomitant in acquired aplastic anemia (AA). Currently, most investigative efforts have concentrated on the elucidation of the immune-mediated mechanisms of hematopoietic cell destruction. Although the replacement of hematopoietic active marrow with fat cells is another characteristic feature of AA, the fat cells themselves have received little attention, and the mechanisms and underlying significance of fatty marrow replacement remain unclear. When discussing the replacement of hematopoietic active marrow by fat cells in AA, it appears that an apparent fatty marrow infiltration has been considered a secondary phenomenon.

It is generally accepted that the bone marrow microenvironment consists of adipocytes, fibroblasts, osteoblasts, osteoclasts, and endothelial cells that are derived from mesenchymal stem cells (MSCs). MSCs support hematopoiesis and regulate the function of many immune cells. Thus, abnormal MSCs affect hematopoiesis. When MSCs abnormally differentiate to fibroblasts, osteoblasts, and osteoclasts, this can cause anemia with myelofibrosis, osteoporosis, and osteopenia, respectively. AA is characterized by fatty replacement in bone marrow (BM) that results in pancytopenia. As with myelofibrosis, osteoporosis, and osteopenia, AA appears to share this mechanism of abnormal MSC differentiation.

Effective AA treatments, such as cyclosporine, androgens, lithium chloride, and Bojungbangdangtang, inhibit the differentiation of MSCs to adipocytes, but this characteristic is often overlooked. The same is true for the pathogenic factors related to acquired AA. The infrequently used antibiotic chloramphenicol can cause acquired AA and can also induce MSC adipogenesis. Auto-active T cells can induce both the apoptosis of HSCs/HPCs and adipogenesis differentiation of MSCs. Androgens, such as oxymetholone, were used extensively in the treatment of acquired AA for decades and could also inhibit the differentiation of human MSCs (as well as preadipocytes) to adipocytes.

It is crucial to clarify the cause of fat cell accumulation in acquired AA, which may offer protective/therapeutic effects in acquired AA.

1 Drugs and a series of therapy and adipogenesis

1.1 Toxins and toxicity drugs: inducing AA via increased adipogenesis Many toxins and toxicity drugs are potential causes of acquired AA, and some of these agents can induce MSCs to differentiate into adipocytes. Chloramphenicol is the most notorious drug known to cause acquired AA. The risk of developing acquired AA in patients treated with chloramphenicol is approximately one in 20,000 or 10- to 50-fold that of the general population. There is no direct evidence of the myelosuppressive effect of this drug within a normal dose range; however, there is evidence of this effect at very high doses. Though lacking robust evidence, this sensitivity is also believed to produce immunologic marrow suppression because the affected patients responded to immunosuppressive therapy. Again, there is lack of direct evidence for toxicities against HSCs/HPCs from chloramphenicol. More recently, a series of studies failed to produce a chronic aplastic anemia mouse model using chloramphenicol succinate. The studies also indicated that chloramphenicol may cause acquired AA in humans through other time-cost avenues (such as the adipogenesis of MSCs) instead of impairing HSCs/HPCs or immune stirring.

Chloramphenicol can damage mitochondria; this is considered to be another pathological avenue for inducing acquired AA. Although there is close relationship between mitochondrial defects and acquired AA, the mechanism of mitochondrial damage and acquired AA is unclear. Recently, Vankoningslo et al. found that chloramphenicol could induce triglyceride accumulation in 3T3-L1 preadipocytes and could also increase the differentiation of adipocytes from preadipocytes; this may be the underlying mechanism of chloramphenicol-related acquired AA. Chloramphenicol may induce the MSCs to preferentially differentiate to adipocytes in AA patients. Furthermore, the HSCs/HPCs lost hematopoietic support from the MSCs, and finally pancytopenia arose. In refractory acquired AA in which stem cell transplantation failed to recover normal hematopoiesis, MSCs infusion could salvage the graft failure. This finding indicated that normal MSCs warrant normal hematopoiesis recovery from AA and that defect MSCs, such as over adipogenesis, impair normal hematopoiesis.

1.2 Effective therapy for acquired AA may increase hematopoiesis by inhibiting adipogenesis in bone marrow in a time-consuming manner In addition to stem cell transplantation, immunosuppressive therapy (IST) and androgens are the two most frequently used treatments for acquired AA. IST was thought to inhibit T cell toxicities to stem/progenitor cells; if this were true, hematopoiesis should shortly recover after the depletion of T cell toxicities, just as in the treatment of immune thrombocytopenia (ITP). However, this is not true in AA clinical practice due to the recovery time of hematopoiesis.

1.3 Immunosuppressive therapeutic reagents inhibit adipogenesis Cyclosporine is a standard immunosuppressive therapeutic reagent (IST) for acquired AA, though other IST types, such as sirolimus, also have therapeutic effects against this disease. The overall survival rate after IST for acquired AA is currently approximately 75% at 5 years. The relapse rate after immunosuppressive
therapy was approximately 30%\textsuperscript{[24]}. Patients are at risk for later clonal disease, myelodysplastic syndrome (MDS)/acute myeloid leukemia (AML, 8%), hemolytic paroxysmal nocturnal hemoglobinuria (PNH, 10%) and solid tumors (11%) at 11 years, respectively\textsuperscript{[26]}. These results warrant exploring other effective and safe methods that have the benefits of IST without its toxic side effects.

IST was also found to decrease both the adipocyte numbers and cell mass in animals and patients taking IST. Adipogenesis decreased both in the bone marrow and throughout the body. When rats were given sirolimus 1.0mg/kg three times per week for 12 weeks, both the body mass index and adipocyte diameters were lower than those of the control group (356g vs 507g, \(P<0.01\), 25\(\mu\)m vs 36\(\mu\)m, \(P=0.009\))\textsuperscript{[21]}. After kidney transplantation, the recipients took cyclosporine. Two years later, the body mass indexes of the patients decreased significantly\textsuperscript{[21]}. Cyclosporine and other ISTs could decrease adipogenesis, and this may have underlying significance in its pharmacodynamics. Nuclear factor of activated T cells (NFAT) is a family of transcription factors that are present in 3T3-L1 adipocytes and MSCs, and also participates in adipocyte differentiation\textsuperscript{[23]}. Cyclosporine A could prevent NFAT nuclear localization and thus inhibit cell differentiation. These results demonstrated that, with the exception of its immune inhibition effect, cyclosporine A could also inhibit the differentiation of fat cells; this may play an important role in the treatment of acquired AA.

1.4 Inhibitive effects of androgens on adipogenesis An association between androgens and erythropoiesis has been acknowledged for decades. Oxymetholone was used extensively in the treatment of acquired AA. In some patients, oxymetholone can stimulate erythropoiesis in particular but sometimes can produce a trilineage response. Oxymetholone in combination with IST more significantly increases this response compared with IST alone\textsuperscript{[24-26]}. The mechanism of how androgens stimulate hematopoiesis is poorly understood. It has been thought that the stimulation of erythropoietin release and increases bone marrow activity\textsuperscript{[26]}. An anecdotal use of hHuEpo in acquired AA has shown that it is ineffective, which is not surprising in view of the demonstration of markedly elevated serum erythropoietin levels in the majority of patients with acquired AA\textsuperscript{[27]}. Thus, androgens may stimulate hematopoiesis through other mechanisms instead of the EPO pathway.

Recently, Gupta et al\textsuperscript{[28]} found that androgens could inhibit the differentiation of human mesenchymal stem cells and preadipocytes to adipocytes. In this study, dihydrotestosterone (DHT) (0–30nmol/L) downregulated the expression of adipocyte differentiation genes, including aP2, leptin, and PPAR\(\gamma\) mRNAs, in a dose-dependent manner.

This suggested that androgens may reverse normal hematopoiesis by inhibiting MSC adipogenesis.

1.5 Response time of AA is significantly longer than that of immune-related cytopenia disorders Immune inhibitors require significantly more time to recover hematopoiesis in acquired AA than immune-related cytopenia such as ITP. Acquired AA responses to ATG and cyclosporine are delayed, and the response usually does not begin before 3–4 months of treatment. For ITP, which is considered a typical immune disorder-related, platelet-destroying disease, 4 weeks or less are usually required to recover normal platelet counts\textsuperscript{[16]}. This recovery time is significantly longer than that of neutrophils; and platelet after stem cell transplantation are approximately 28 days\textsuperscript{[18]}, which is also the length of time that it takes for hematopoiesis to recover (without other disturbances).

Not surprisingly, the platelet count recovery time after effective IST treatment is the same as that of stem cell transplantation; this may be the time course of platelet production. In acquired AA, the scenario may be significantly more complex because a longer recovery time is required after IST treatment.

In summary, the response time of IST in the treatment of acquired AA is significantly longer than that of IST in the treatment of ITP. There must be an additional contributor to cytopenia in acquired AA (in addition to direct toxicities against hematopoiesis by T lymphocytes). Over adipocytosis of the MSCs in bone marrow requires time and may account for this.

2 Cell-mediated immunity and adipogenesis

2.1 Abnormal immunity may increase adipogenesis in bone marrow Although the replacement of hematopoietic marrow with fat cells is the primary characteristic feature of acquired AA, the fat cells themselves have received little attention, and the mechanisms of fatty marrow replacement remain unclear. Study results have shown that abnormal T lymphocytes may increase the adipogenesis differentiation of MSCs by excreting cytokines such as IFN-\(\gamma\) and TNF-\(\alpha\). In a non-random controlled clinical trial including seven patients with AA and nine normal age-matched controls, Hara et al\textsuperscript{[24]} measured T-cell-derived intracellular cytokine production levels in the peripheral blood and bone marrow of patients with AA. The results demonstrated that BM lymphocytes in patients with AA produced significantly larger amounts of IFN-\(\gamma\) compared with controls.

It has been demonstrated that auto reactive T lymphocytes can induce adipogenesis from MSC. A variety of cytokines, including IFN-\(\gamma\) and TNF-\(\alpha\), have been confirmed as the key mediators of hematopoietic suppression and could also cause MSCs to differentiate to adipocytes. The transcription factor GATA-2 may play an important role in the balance between hematopoiesis
and adipogenesis in bone marrow. GATA-2 is specifically expressed not only in hematopoietic tissues but also in preadipocytes, and it is known to be an important adipogenic regulator.[30]

Xu et al.[30] found that both the protein and mRNA levels of GATA-2 were lower in the marrow MSCs from AA patients than those in normal subjects. They further verified that incubation with interferon-γ induced the downregulation of GATA-2 levels in MSCs in normal subjects; this increased the differentiation of MSCs to adipocytes. These results showed that autoactive T lymphocytes may increase adipogenesis in marrow by secreting cytokines such as IFN-γ. Other cytokines from T lymphocytes, such as IL-15, have similar effects in adipogenesis.[31]

2.2 Over adipogenesis decreases B lymphocytes in AA Bone marrow failure has been considered to be related to the strong immunologic function of T lymphocytes in a scenario of concurrently reduced B lymphocyte levels. Li et al.[32] found that there are fewer CD19+ B lymphocytes in the bone marrow of AA patients than that of healthy controls (P = 0.002). It appears that the relative decrease in B lymphocytes could not be due to the proliferation of T lymphocytes in AA because NK cells, which are another of the three main lymphocyte subsets, did not obviously decrease in AA. It appears likely, therefore, that a reduction in (CD34+/CD19+) B lymphocyte progenitors explains the B lymphocyte decrease observed in AA in the course of the disease, whereas the number of adult B lymphocytes is significantly decreased. Unfortunately, it remains unknown why the earliest B cell progenitors, CD34+/CD19+ B lymphocyte progenitors, decreased in AA. It appears that adipocytes may negatively regulate the production of B lymphocytes in AA.

Many adipocyte products, including type 1 IFN, PGs, leptin, and sex steroids, are known modulators of lymphohematopoiesis. Adiponectin is an abundant protein made exclusively by adipocytes. Hematopoetic cells and the microenvironment that supports their differentiation are also adiponectin targets. Yokota et al.[33] used long bone marrow cultures to investigate the effects of adiponectin on lymphohematopoietic cell differentiation. They found that recombinant adiponectin strongly inhibited B lymphopoiesis in long-term bone marrow cultures. These results indicate that adipocytes in bone marrow can contribute to the regulation of B lymphocyte formation.

2.3 Over adipogenesis may decrease the T-cell suppression effect of MSCs Bone marrow MSCs have immunosuppressive activity both in vitro and in vivo.[33-36] It is generally accepted that abnormal immunity is the primary factor mediating the pathogenesis of acquired AA. This abnormal immunity may be the result of the decreased suppression effect against T cells by MSCs after their adipogenesis differentiation. In a clinical experiment of 23 severe AA cases and 19 healthy controls, Bacigalupo et al.[37] compared the suppressive effect of MSCs (derived from the two patient groups) on T-cell activation. They found that the abnormalities of MSCs from severe AA patients included 1) a significantly lower suppression of T-cell proliferation induced by alloantigens; 2) an impaired capacity to suppress CD38 expression on PHA-primed T cells; 3) an impaired ability to suppress IFN-γ production in PHA cultures. The ability of MSCs to downregulate T-cell priming, proliferation, and cytokine release is deficient in patients with SAA. In another study, Liu et al.[38] and Li et al.[39] found that MSCs lost their immune regulation effect after differentiating to adipocytes. Thus, we could deduce that the inhibition of MSC differentiation to adipogenesis (restoring the T-cell suppression of MSCs) may be beneficial in recovering normal hematopoiesis in acquired AA.

3 Over adipogenesis in marrow and hematopoiesis

3.1 Over adipogenesis of MSCs and the excretion of hematopoietic inhibitors During aging, hematopoietic bone marrow is increasingly replaced by adipose tissue[40]; this may at least in part explain the high rate of anemia in the aging population. This phenomenon can also be observed in hematopoiesis diseases and especially in AA. Adipose tissue produces a number of cytokines including tumor necrosis factor (TNF)-α, interleukin (IL)-6, IFN-γ and others.[31-45]. Present data indicate that IL-6, IFN-γ and TNF-α[46] belong to myelosuppressive cytokines. IL-6, IFN-γ and TNF-α could induce the death of hematopoietic progenitor cells by increased apoptosis at very low cytokine concentrations[45-47]. Adipocytes may exert their inhibitory effects on hematopoiesis by excreting these negative cytokines in AA.

3.2 The increased adipogenesis of MSCs decreases normal hematopoiesis It is well known that MSCs support hematopoiesis and that they are impaired in acquired AA, especially in scenarios of over adipogenesis. Recently, Wu et al.[48] directly verified this via the co-transplantation of MSCs following hematopoietic stem cell transplantation in a severe AA patient; this treatment increased the reconstitution of normal hematopoiesis. Over adipogenesis of MSCs can have negative effects on normal hematopoiesis via the reduced production of hematopoietic supporting factors and the excessive excretion of hematopoietic inhibitors (Figure 1); these could retard the recovery of normal hematopoiesis after hematopoietic stem cell transplantation or radiation damage.

To explore if adipocytes influence hematopoiesis or if they simply fill the marrow space as a secondary result after radiation, Naveiras et al.[49] used a "fatless" mice model and found that hematopoiesis in fatless marrow engraftments after irradiation was
accelerated compared with that of fatty marrow. This indicated that over adipogenesis participated at least in part with the origin of acquired AA. It also indicated that an increased adipocyte level is an initiating and not a secondary phenomenon in acquired AA. These data showed that antagonizing marrow over adipogenesis may enhance normal hematopoietic recovery in the over adipogenesis of marrow observed in AA.

Although acquired AA is a heterous cytopenia syndrome, most cases share the same pathological characteristics of over adipogenesis in bone marrow. This abnormal adipogenesis may be both the stirrer and result of abnormal immunity. This cycle of abnormal immunity and over adipogenesis may account for the cytopenia in most acquired AA patients (Figure 1). This finding warrants further exploration for new target drugs against adipogenesis in the treatment of acquired AA.

Fig 1. Mesenchymal stem cells (MSCs) are the primary components of the hematopoietic niche in bone marrow. In a homeostatic condition, hematopoiesis is maintained via support from MSCs. When bone marrow is attacked by acquired AA pathogenic factors (such as abnormal immune reactions, chemicals, virus infections, radiation, etc.), however, over adipogenesis happened and adipocytes predominantly suppress hematopoiesis. (→ increase; ← inhibit)

Hematopoietic stem cells/hematopoietic progenitors

Hematopoietic cytokines, including TPO, SCF, IL-11, G-CSF, GM-CSF

Adipokines and myelosuppressive cytokines, including TNF-α, IL-6, IFN-γ and others

Acquired AA pathogenic factors (abnormal immune reactions, chemicals, virus infections, radiations, etc.)

Homeostatic condition | Aplastic anemia

Mesenchymal stem cells

Mesenchymal stem cells and adipocytes

[References]


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