Placental umbilical cord whole blood transfusion to combat anemia in the background of advanced rheumatoid arthritis and emaciation and its potential role as immunoadjuvant therapy

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Summary

Rheumatoid arthritis is the commonest form of inflammatory arthritis and affects about 1-3% of the population in the West and even more in the developing world due to the compounded factors of late detection and inadequate treatment in the overall background of poverty, deprivation, and improper macro and micronutrients in the diet in a sizeable segment of the population. Nearly 90% of patients with aggressive disease will become clinically disabled within 20 years. Furthermore, in patients with severe disease or extra-articular symptoms, mortality is equal to that for patients with triple artery coronary artery disease or Stage IV Hodgkin's lymphoma. Anemia is a very common comorbidity of rheumatoid arthritis.

Anemia in rheumatoid arthritis is caused by various factors, for instance, cytokine impact of the advanced arthritic process on the host, or lack of proper nutrition and essential micronutrients in the diet, or coexistent helminthiasis, and/or impact of antiarthritic drugs on the host system, i.e., high steroid induced gastritis or ulcerations in gastric mucosa or subclinical or clinical hepatitis due to methotrexate or salazopyrin effects on bone marrow, only to name a few. Other pre-existing or compounding gastrointestinal problems, which alter the available iron stores or cause bone marrow dysfunction, may also help in adding to an anemic condition. If the anemia is 8 g/dl or less, blood transfusion or erythropoietin injection with adequate hematinic reserve is effective in normal situations, but is not that effective in anemia with a chronic disease background like rheumatoid arthritis.

Cord blood, because of its rich mix of fetal and adult hemoglobin, high platelet and white blood cell (WBC) counts, and a plasma filled with cytokine and growth factors, as well as its hypo antigenic nature and altered metabolic profile, has all the potential of a real and safe alternative to adult blood transfusion.

Seventy-eight units (42 ml -136 ml mean 80.6 ml ± 3.6 ml SD, median 82.4 ml, mean packed cell volume 48.2 ± 2.1 SD, mean percent hemoglobin concentration 16.4 g/dl ± 1.5 g/dl SD) of placental umbilical cord whole blood was transfused (from 1 April 1999 to April 2005) after lower uterine cesarean section (LUCS) from consenting mothers to 28 informed consenting patients with advanced rheumatoid arthritis who had plasma hemoglobin of 8 g/dl or less. After collection, the blood was immediately transfused following the standard adult blood transfusion protocol. Each case was passed through the institutional ethical committee. The patients received two to six units of freshly collected placental umbilical cord blood without encountering any clinical, immunological or non-immunological reactions. Three days after completion of the transfusion of placental umbilical cord blood, the peripheral blood hematopoietic stem cell (CD34) estimation revealed a rise from the pretransfusion base level (.09%), varying from 2.03 to 23%, which returned to base level in most of the cases at the three-month CD34 re-estimation, without provoking any clinical graft *vs* host reaction in any of the patients.

Key words: Safe placental umbilical cord blood transfusion; Rheumatoid arthritis.

Introduction

Anemia is a common comorbidity in individuals with rheumatoid arthritis (RA). In fact, anemia of the type characterized by low serum iron concentrations in conjunction with adequate iron stores is frequently associated with RA and has served as a model for anemia in chronic disease. Investigators have suggested that patients with RA who have anemia are likely to have more severe joint disease, but if the anemia is successfully treated, the joint disease is likely to respond to treatment as well [1]. Antigen-activated CD4+ T cells stimulate monocytes, macrophages, and synovial fibroblasts to produce the cytokine interleukin-1, interleukin-6, and TNF-α and to secrete matrix metalloproteinases through cell-surface signaling by means of CD69 and CD118 as well as through the release of soluble mediators such as interferon-γ and interleukin-17. Interleukin-1, interleukin-6, and TNF-α are the key cytokines that drive inflammation in rheumatoid arthritis. Activated CD4+ T cells also stimulate B cells through cell-surface contact and through the binding of $\alpha_L\beta_2$ integrin, CD154 (CD40 ligand), and CD28 to produce immunoglobulins, including rheumatoid factor.

Patients with RA are considered to be at nutritional risk for many reasons. One cause of poor nutritional status in this patient population is thought to be the result of the

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weight loss and cachexia linked to cytokine production [2]. In patients experiencing chronic inflammation, the production of cytokines, such as interleukin-1 and tumor necrosis factor, increases resting metabolic rate and protein breakdown. The patient is then faced with the challenge of increasing both calorie and protein intake to meet the nutritional requirements of the increased metabolic rate. This is frequently difficult partly because of the pain and swelling associated with RA, which make food preparation and purchasing difficult for those who live alone or have limited resources. In the Indian subcontinent, malnutrition and anemia, weakness and an emaciated look with arthritis is a quite typical presentation in patients reporting to state government hospitals for free treatment, in the rural and semi-urban areas.

Blood transfusions are widely used as a rapid and effective therapeutic intervention. Transfusions are particularly helpful in the context of either severe anemia (in which the hemoglobin is less than 8.0 g/dl) or life-threatening anemia (in which the hemoglobin is less than 6.5 g/dl), particularly when the condition is aggravated by complications that involve bleeding. Blood transfusions are widely used as a rapid and effective therapeutic intervention and have been associated with increased survival rates in anemic patients.

Our team of doctors has been successful in transfusing placental cord whole blood, which is rich in fetal hemoglobin content as well as cytokine and growth factors, as an alternative emergency source of blood transfusion in the background of anemia and emaciation of any etiology.

The placenta, or the afterbirth, is discarded routinely everywhere in the world (in India alone, there are more than 20 million placentas produced as afterbirth every year), and is actually a cause of environmental pollution in many parts of the developing world because it attracts natural scavengers and spreads infection, unless aseptically treated or incinerated. The centers of excellence in the Western developed world have been working on the use of a tiny microscopic fraction of cord blood, i.e., CD34 stem cells only (.01% of the nucleated cells of placental blood). Whether fetal hemoglobin rich placental umbilical cord whole blood (which has the potential to carry more oxygen to the tissue vol/vol than adult blood because of its fetal hemoglobin component, if collected aseptically after the birth of a healthy newborn at or near term) could be an emergency and safe substitute for adult whole blood in cases of rheumatoid arthritis victims with a hemoglobin concentration of less than 8 g/dl, who cannot afford to buy erythropoietin injections or even arrange for fresh packed cells for transfusion, was the main idea behind the present study.

Materials and Methods

The problem of treatment of rheumatoid arthritis is somewhat different in developing countries, due to the poor socio-economic and educational backgrounds in the majority of patients. Here, for the most part, non-compliance with the suggested drug starts as soon as there is some relief. We frequently come across poor patients with intractable pain due to the progression of rheumatoid arthritis, with involvement of the inflammatory and neuropathic components of the disease. The ultimate goal in the therapy of rheumatoid arthritis is primarily reduction and relief of the pain and inflammation, and secondarily, maintenance of function and protection of articular structures, and systemic involvement.

We followed the American College of Rheumatology's (ACR) revised criteria for inclusion of rheumatoid arthritis patients in the present study who had anemia (8 g/dl or less) [3]. While being well meaning and helpful in treatment guidance, strictly speaking, the criteria are not optimal in distinguishing early rheumatoid arthritis from undifferentiated polyarthritis and systemic lupus erythematosus. As per the suggestions of the ACR, one to three years of the disease process is considered as early disease in this government hospital-based study conducted in Calcutta (India), where most poor patients are admitted to receive free treatment.

The patients in this study included marginalized persons, i.e., homeless people, alcoholics, migrants, drug abusers, landless laborers and the poor from any strata of society. We enrolled patients from our hospital who were suffering from anemia, rheumatoid arthritis, emaciation and who could not buy erythropoietin or arrange for fresh whole blood or concentrated red blood cells (RBC) for cord blood transfusion. All enrolled patients gave proper informed consent and the institution based ethical committee approved each case.

Seventy-eight units of human placental umbilical cord blood were collected from consenting mothers aseptically after lower uterine cesarean section (LUCS) under general or regional anesthesia and the same was transfused (42 ml-136 ml mean 80.6 ml ± 3.6 ml SD, median 82.4 ml, mean packed cell volume 48.2 ± 2.1 SD, mean percent hemoglobin concentration of 16.4 g/dl ± 1.5 g/dl SD) from 1 April 1999 to April 2005 to 28 informed consenting patients with advanced rheumatoid arthritis who had plasma hemoglobin 8 g/dl or less. In case of gross prematurely or dysmaturity, or if the projected weight of the fetus was less than 2 kg, or if there was any specific disease affecting the mother like hepatitis or HIV, etc., the cord blood collection was abandoned. Cord blood was collected from only informed, healthy mothers with their consent after the birth of their healthy babies. The collection process started only after the baby was safely removed from the operation field and the anesthetist verified the stable physical condition of the mother. It was only then that the obstetrician took the decision to proceed with the umbilical cord blood collection. Immediately, the cord was disinfected by spirit/Betadine solution at the site of the proposed puncture of the umbilical vein and a 16 g needle was attached to a standard pediatric collection bag (containing 14 ml anticoagulant citrate phosphate dextrose adenine solution), which was used for the purpose of collection. When the collection was complete, the blood bag tubing was closed, sealed, and stored at 1-4°C, after putting necessary identification markings. Another sample of the cord blood collected from the placenta was immediately tested for blood group (Rh and ABO), HIV (1 and 2), hepatitis B and C, VDRL, malaria, fungus and bacterial study, as per standard blood transfusion protocol, which we have reported on earlier [4-7]. The collected cord whole blood was transfused immediately or at the most, within three days of collection to a patient with anemia, after grouping, cross-matching and following the standard adult blood transfusion WHO guidelines for screening and transfusion, strictly adhering to the institutional ethical committee's instructions and the patient consent protocol. Pretransfusion and three days after the transfusion, blood was drawn from the consenting patients for peripheral blood hematopoietic stem cell estimation (CD34) by flow analysis cytometry as per standard protocol at the Ranbaxy Laboratory.

Result and Analysis

In the present series 28 patients with arthritis volunteered and were included (as per the American College of Rheumatology (ACR) revised criteria for inclusion) for the cord blood transfusion protocol to combat anemia. The background pre-transfusion hemoglobin varied from 5.6 to 7.9 g/dl. Each patient received two units to six units of cord blood within a span of 15 days depending on availability and need. The age of the patients varied from four years to 62 years. Eighteen were females and the rest (10 patients) were males. Type O (Rh+) was the commonest blood group (11 cases), followed by A(Rh+) in seven cases. Blood group B (Rh +) was present in six cases and the remaining four cases belonged to the AB (Rh +) group. A total of 78 units of freshly collected cord blood were transfused to the arthritic volunteers. The blood was transfused as soon as it was collected from consenting mothers and the screening, grouping and cross-matching was completed. The volume of cord blood varied from 42 ml-136 ml, mean 80.6 ml ± 3.6 ml SD and median 82.4 ml, mean packed cell volume 48.2 ± 2.1 SD, and mean percent hemoglobin concentration 16.4 g/dl ± 1.5 g/dl SD. The study, which began in April 1999, was followed up till April 2005. Not a single post-transfusion patient suffered from any immediate or late complication of blood transfusion, i.e., immunological or non-immunological reaction. There was a rise in body weight of three to five pounds in 75% of the patients. A sense of well being, both subjective and objective, as well as an improvement in appetite was present in all patients.

We performed a peripheral blood CD34 study by flow analysis cytometry before cord blood transfusion and 72 hours after transfusion and it was repeated again after three months. Three days after completion of the transfusion, the peripheral blood hematopoietic stem cell (CD34) estimation revealed a rise from the pre transfusion base level (.09%), varying from 2.03 to 23%. The flow analysis report of a case is shown 72 hours after two units of cord blood were transfused in Figure 1. The report shows the increase of peripheral blood CD34 to 5.3%. This returned to base level in most cases at the three-month CD34 re-estimation, without provoking any clinical graft *vs* host reaction in any of the patients.

Anemia in rheumatoid arthritis is a very complex phenomenon of cytokine interregulation and belongs to a specific subgroup of anemia known as anemia in chronic disease and is the second most prevalent cause of anemia. The first important cause is dietary iron deficiency. In cases of anemia in chronic disease there is acute or chronic immune activation of a specific cytokine system, which helps in shifting the iron from its normal route. The condition has also been termed as "anemia of inflammation" [8]. This condition is immune driven; the cytokines and cells of the reticuloendothelial system induce changes in iron homeostasis, the proliferation of erythroid progenitor cells, the production of erythropoietin, and the life span of red cells, all of which contribute to the pathogenesis of anemia. Erythropoiesis can be affected by disease underlying anemia in chronic disease. Moreover, it can be due to pro-inflammatory cytokines and free radicals that damage erythroid progenitor cells. Bleeding episodes, vitamin deficiencies (e.g., of cobalamin and folic acid), hypersplenism and autoimmune hemolysis, may also contribute to the anemic process affecting diseases like rheumatoid arthritis. Freshly collected cord blood, rich in hemoglobin and growth factors, may have a positive impact on anemia in chronic disease.

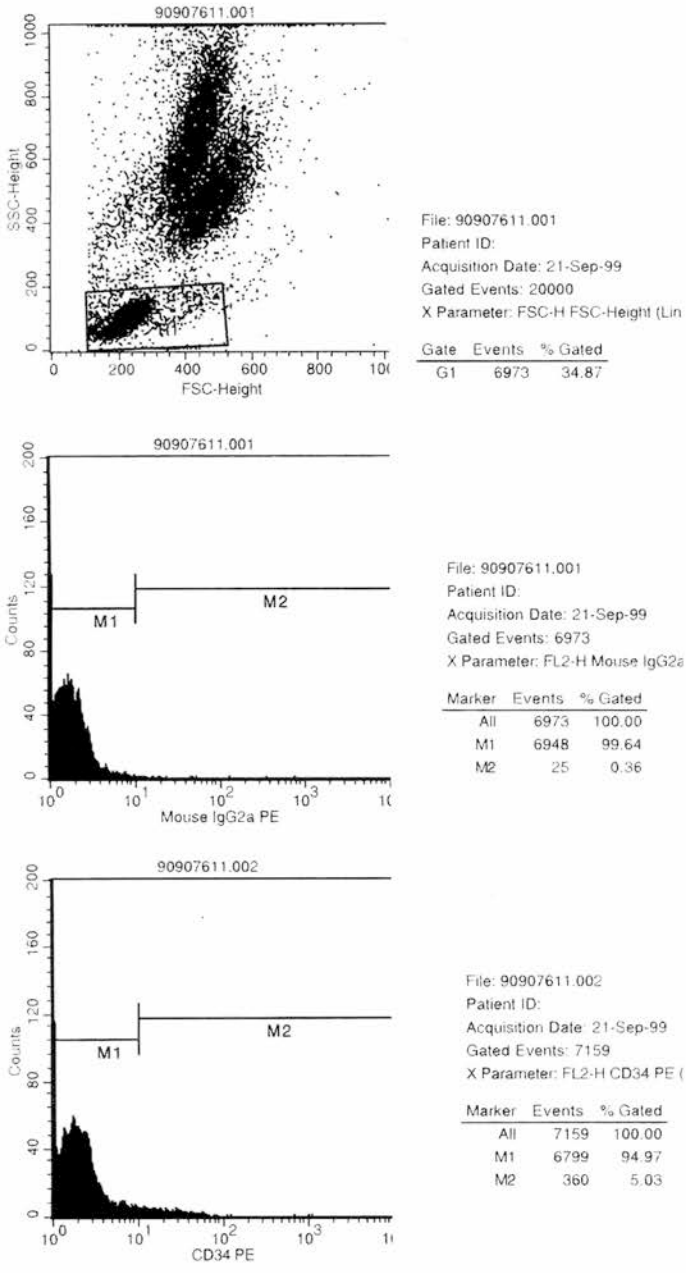

Figure 1. — Flow analysis cytometry report of the peripheral blood CD34 level (5.03% in the peripheral blood) 72 hours after the ABO/Rh group cross-matched cord blood transfusion.

Discussion

Rheumatoid arthritis is the commonest form of inflammatory arthritis and it affects about 1 to 3% of the population in the Western hemisphere. The clinical presentation is heterogeneous with a wide variation in age at onset, degree of joint involvement, and severity. Most patients with aggressive disease will become clinically disabled within 20 years. Furthermore, in patients with severe disease or extra-articular symptoms, mortality is equal to that for patients with triple artery coronary artery disease or Stage IV Hodgkin's lymphoma [9]. Although rheumatoid arthritis predominantly affects peripheral joints, discovertebral joints of the cervical spine are often affected. As arthritis is more prevalent in overweight adults, it accounts for 7.4% of admissions [10]. The long-term prognosis in this disease is very poor [11]. Recent advances in rheumatoid arthritis provide an insight into new therapeutic updates. However, these therapies appear to be suited for well-to-do patients with medical insurance, especially those who live in Western, developed countries. The problem of rheumatoid arthritis is compounded in countries with inadequate resources, as a result of economic constraints, malnutrition, and a limited number of specialists [12]. Most affected patients initially go to a primary care physician in developing countries like India. If the patient's condition is mixed with compounding complications such as bone destruction, severe pain, and the development of fibrous or bony ankylosis [13], or the progress of vasculitis, activation of subclinical tuberculosis, restrictive lung disease, renal parenchyma disease, hypothyroidism, altered glucose tolerance, frank diabetes, and cardiomyopathy, the scenario which emerges is typical of a government hospital arthritis patient in Calcutta (India), and its probable presentation.

Anemia is one of the essential comorbidities frequently associated with arthritic processes, more so in under-resourced patients where the cost of treatment is unaffordable for many. As a result, many patients frequently discontinue the treatment with slight remission. Diet may play a role in the management of RA, particularly in alleviating the symptoms of the disease, combating the side-effects of therapy and reducing the risk of complications. Proper antioxidant nutrients (vitamin A, vitamin C, selenium) may provide an important defense against increased oxidant stress and a supplementation of folate and vitamin B12 in patients treated with methotrexate (MTX), can reduce the incidence of side-effects, and offset the elevation in plasma homocysteine, which is frequent in these patients. Calcium and vitamin D in patients treated with corticosteroids can reduce bone loss, while a simple supplementation with iron may not always prevent anemia. However, such a balanced diet containing all the micro-nutrients and protein is not affordable to under-resourced and marginalized people who report to government hospitals for free treatment. The cause behind anemia in arthritis is also not so simple that a properly balanced diet can fully alleviate the problem. Neither iron or folic acid, nor B12 supplements can effectively reverse the condition of anemia in arthritis.

Recent studies have given us some clues on the causation and perpetuation of anemia in arthritis and other chronic diseases. Hepcidin, an iron-regulated acute-phase protein that is composed of 25 amino acids, has helped to shed light on the relationship of the immune response to iron homeostasis and anemia in chronic disease. Hepcidin expression is induced by lipopolysaccharide and interleukin-6 and is inhibited by TNF-α [14]. Transgenic or constitutive over-expression of hepcidin results in severe iron-deficiency anemia in mice [15].

Another option to tackle anemia is to inject erythropoietin provided there is no dearth of iron or B12 stores. However, there is little data currently available on the possible effects of erythropoietin on the course of underlying disease, particularly since erythropoietin can exert additional biologic effects, including interference with the signal-transduction cascade of cytokines [16].

A hallmark of anemia in chronic disease is the development of disturbances in iron homeostasis, with increased uptake and retention of iron within the cells of the reticuloendothelial system. This leads to a diversion of iron from the circulation into storage sites of the reticuloendothelial system, subsequent limitation of the availability of iron for erythroid progenitor cells, and iron-restricted erythropoiesis. In mice that are injected with the pro-inflammatory cytokine interleukin-1 and tumor necrosis factor (TNF-α) [17], both hypoferremia and anemia develop; this combination of conditions has been linked to cytokine-inducible synthesis of ferritin, the major protein associated with iron storage, by macrophages and hepatocytes [18]. In chronic inflammation, the acquisition of iron by macrophages most prominently takes place through erythrophagocytosis [19] and the transmembrane import of ferrous iron by the protein divalent metal transporter 1 (DMT1) [20]. Interferon-γ, lipopolysaccharide, and TNF-α up-regulate the expression of DMT1, with an increased uptake of iron into activated macrophages. These pro-inflammatory stimuli also induce the retention of iron in macrophages by down-regulating the expression of ferroportin, thus blocking the release of iron from these cells. Ferroportin is a transmembrane exporter of iron, a process that is believed to be responsible for the transfer of absorbed ferrous iron from the duodenal site [21].

One Western study has suggested that the incidence of anemia was high: 49%, 46%, and 35% in RA, SLE (systemic lupus erythematosus), and PsA (psoratic arthropathy), respectively. Low levels of serum B12 were also frequent (24%), with an almost similar occurrence in the three disease groups [22]. During the active phase of the disease, elevated plasma concentrations of inflammatory cytokines, such as interleukin-6 (IL-6), interleukin-1beta (IL-1beta), tumor necrosis factor-alpha (TNF-α) and acute-phase proteins not only cause anemia but also lead to a reduction of fat-free body mass (FFM) with a mean loss of 15% of cell body mass (CM) and a consequent reduction of muscle strength [23]. The common anemic form in arthritis is mainly normochromic or hypochromic, normocytic; it can be even microcytic where iron defi-

ciency is common. There is also associated thrombocytosis, raised ferritin, low serum iron and iron binding capacity [24]. The effect of erythropoietin on such anemia is controversial, and evidence exists that cytokines may affect hemopoiesis, possibly by affecting sensitivity to erythropoietin [25].

In the present series, the patients enrolled for cord blood transfusion were not able to afford injections of erythropoietin or fresh packed cells to combat their anemia in the background of chronic arthritis. In India more than 20 million registered births take place per year, and there is potentially an abundant supply of placental cord blood. In our hospital, we used the freshly available placental blood, rich with cytokine and growth factors, from our own in-patient department, and it worked well. The question is why is this so? Why was there not a single case of immunological or non immunological reaction and why did the patients who received the cord blood gain weight? The answer may be found in the hypoantigenecity of the fetal system. Our team of doctors have been working on fetal cell/tissue transplant in adult degenerative diseases and we have published several reports on our experience [26-30]. The basic reason for non-rejection of the conceptus is the fact that pregnancy and neoplasm are two outstanding examples of natural tolerance to homografts. In order to avoid maternal HLA system recognition, there are non-cytopathic antibodies inside the placenta apart from hypoantigenic fetal cells. At or near term, however, a slow bidirectional traffic of cells at the fetal-maternal interface slowly develops. Moreover, fetal progenitor cells have been found to persist in maternal peripheral blood for decades after childbirth. Progenitor cells can differentiate into mature immune-competent cells. Chimerism is used to indicate a body that contains cell populations derived from different individuals; microchimerism indicates low levels of chimerism. Male DNA, of presumed fetal origin, can be detected in the maternal circulation decades after delivery and is referred to as fetal microchimerism (FM) [31]. Lymphohemopoietic cytokines are now recognized to be central participants in the cellular communication events underlying the complex and dynamic remodeling processes required to accommodate the semi-allogeneic conceptus during mammalian reproduction. Cytokines are identified to be of particular importance in mediating communications between the conceptus and maternal cells, particularly uterine epithelium and infiltrating leukocytes, both prior to implantation and as the placenta develops.

What is intriguing is the rise of peripheral blood CD34 levels after cord blood transfusion in cases of non-pregnant recipients, as seen in the flow analysis cytometry report. The reason for the transient rise of hematopoietic stem cells as seen in the peripheral blood in HLA randomized recipients without any immunosuppressive support and without provoking clinical graft vs host reaction, still remains a mystery.

However, we can venture some probable explanations. The placenta has a unique microenvironment and its sensitization impact on cord blood cells may have a role in the transient transplantation impact on the host system. One very important factor, apart from intrinsic differences, is the fact that hematopoietic stem cells (HBCs) in umbilical cord blood (UCB) cells have had a different set of microenvironmental exposures compared to those of adult marrow or peripheral blood stem (PBS) cells. An example of differences between sources are some of the observed changes in the HSC cycle status, gene expression and the adhesive and invasive properties induced by mobilization procedures used to generate PBS cells, e.g., G-CSF (granulocyte colony stimulation factor). The placenta is a complex organ that regulates maternofetal interactions [32]. This placental environmental exposure of cord blood cells along with immune suppression mosaic cells in the host, either due to drugs, the chronic nature of the disease, malnutrition with helminthiasis, or other associated factors like the impact of growth factors or selective cytokine impact of the cord blood on the bone marrow of the recipient, may help in the transient rise of CD34 in the host. Our preliminary bone marrow study also suggested a positive impact on the host bone marrow cellularity in those patients.

Conclusion

Rheumatoid arthritis is the commonest disorder of connective tissue and is an important cause of disability, morbidity, and mortality, and life expectancy is reduced by four years in men and by ten years in women. The overall incidence of female: male involvement is 3:1. It is associated with serious infections, vasculitis (leg ulcers, mononeuritis), anemia, thrombocytopenia, and lymphadenopathy. Extra-articular manifestations of rheumatoid arthritis include vasculitis, pulmonary involvement with alveolitis, eventually leading to varying degrees of fibrosis of the lung. Cardiac involvement includes pericarditis, which is common, apart from conduction defects, mitral valve disease and varying degrees of cardiomyopathy. Cutaneous involvement implies vasculitis, palmer erythema and pyoderma gangrenosum. Little is known about the primary cause of RA. Although the primacy of T-cell-related events early in the disease remains debated, strong evidence indicates that autoantigen recognition by specific T cells is crucial to the pathophysiology of rheumatoid synovitis [33]. The massive influx of T cells into arthritic joints is accompanied by the energizing of over 90% of T cells in this compartment – which further substantiates the concept of the RA attractor within the self-regulating immune system. Thereby, the RA-attracted immune system is not able to completely down-regulate the inflammation and local tissue damage/repair. Thus, the immune system is permanently stimulated and suddenly by chance shifts to a stable state different from the healthy system - reaching the wide fields of rheumatoid arthritis which in itself is as self-sustaining as the healthy state before disease onset [34]. Anemia in chronic diseases like arthritis is a complex process and its dysregulation in inflammation-induced cytokine interplay is poorly understood.

The under-resourced world has under-resourced

patients and doctors who have very few options or for that matter, material resources. This stimulates clinical research for alternatives for those who cannot afford to buy the prescribed medicine and the needed support. We have seen that cord blood collected aseptically at birth, which is rich in cytokine and growth factors along with high fetal hemoglobin plasma, can be used in the case of those desperately ill patients who cannot buy or arrange for erythropoietin or fresh blood for their transfusion requirements. The rise in CD34 after cord blood transfusion is an unique phenomenon in HLA-randomized recipients without specific immunosuppressive support. Further studies on its use in cell therapy and its role in bone marrow rejuvenation are ongoing.

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