Mast Cell Therapy for Sickel Cell Anaemia

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ABSTRACT
Some sickle cell anaemia (SCA) patients suffer significantly worse phenotypes than others. Causes of such disparities are incompletely understood. Comorbid chronic inflammation likely is a factor. Recently, mast cell (MC) activation (creating an inflammatory state) was found to be a significant factor in sickle pathobiology and pain in a murine SCA model. Also, a new realm of relatively non cytoproliferative MC disease termed MC activation syndrome (MCAS) has been identified recently. MCAS has not previously been described in SCA. Some SCA patients experience pain patterns and other morbidities more congruent with MCAS than traditional SCA pathobiology (eg, vasoocclusion). Presented here are 32 poor-phenotype SCA patients who met MCAS diagnostic criteria; all improved with MCAS-targeted therapy. As hydroxyurea benefits some MCAS patients (particularly SCA-like pain), its benefit in SCA may be partly attributable to treatment of unrecognized MCAS. Further study will better characterize MCAS in SCA and identify optimal therapy.

Key words: Mast cell (MC), Sickel cell anaemia (SCA), Mast cell activation syndrome.

INTRODUCTION
A mast cell (also known as a mastocyte or a labrocyte) is derived from the myeloid stem cell. It is part of both the immune and neuroimmune systems and contains many granules rich in histamine and heparin. Although best known for their role in allergy and anaphylaxis, mast cells play an important protective role as well, being intimately involved in wound healing, angiogenesis, immunetolerance, defense against pathogens, and blood–brain barrier function. The mast cell is very similar in both appearance and function to the basophil, another type of white blood cell. They differ in that mast cells are tissue resident, e.g., mucosal tissues, while basophils are found in the blood. Mast cells were first described by Paul Ehrlich in his 1878 doctoral thesis on the basis of their unique staining characteristics and large granules. These granules also led him to the incorrect belief that they existed to nourish the surrounding tissue, so the named them Mastzellen (from German Mast, meaning "fattening", as of animals). Mast cells are fascinating, multifunctional, tissue-dwelling cells that have been traditionally associated with the allergic response. However, recent studies suggest these cells may be capable of regulating inflammation, host defence, and innate immunity.

Mast cell Development and Differentiation:
Mast cells develop from progenitor cells that in turn arise from uncommitted hematopoietic stem cells in the bone marrow. These cells express the receptor for stem cell factor (SCF receptor or c-kit) that binds to SCF, the latter being a major growth factor for mast cells. Researchers have described a CD34+, c-kit+, and CD13- precursor that develops into mast cells in the presence of specific growth factors. Mast cell progenitors also have been described in peripheral blood by others, which may suggest the presence of a distinct pool of cells separate from leukocytes or mononuclear cells. The interactions between SCF and c-kit and the subsequent signalling that follows are crucial for the growth and development of mast cells. In humans, studies have demonstrated that mutations of c-kit and elevated levels of the c-kit proto oncogene are associated with the development of the syndrome of mastocytosis, a condition characterized by mast cell infiltration of skin and other tissues. SCF has multiple biological effects on mast cells, including modulating differentiation and homing,

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preformed granule-associated mediators; newly

Mast Cell Mediators:

Human mast cells and basophils express the receptor for IgE, FceRI. [10] FceRI (in contrast to the other receptor for IgE, FceRII) binds IgE with high affinity. [11] The other receptor for IgE, FceRII, has been detected on eosinophils, mononuclear cells, lymphocytes, and platelets. FceRI is a multimeric complex composed of four chains, designated as (which has the IgE-binding domain), , and the two disulfide linked chains. [12] Typically, multivalent antigen binds to IgE, which in turn binds by the Fc portion to the α-chain of FceRI, leading subsequently to receptor aggregation and internalization and culminating in receptor-mediated signalling. The β and γ chains of FceRI possess the immune receptor tyrosine-based activation motifs, which are considered pivotal to signal transduction. [13] The bridging of two IgE molecules by multivalent antigen or by univalent antigen in presence of a carrier molecule results in activation of Lyn kinase, which then phosphorylates the and chains. The absence of Lyn has been associated with defective mast cell signalling in mice. [14] Syk kinase then becomes activated sequentially, followed by involvement of phospholipase C γ, mitogen-activated protein kinases , and phosphoinositol-3 kinase. [15] The generation of inositol triphosphate and of diacylglycerol and other second messengers leads to release of calcium intracellularly as well as protein kinase C activation, events culminating in FceRI-mediated secretion. Degranulation appears to be associated with activation of G proteins that cause actin polymerization and relocalization. These events also are accompanied by the transcription of several cytokine genes, leading to further evolution of the inflammatory cascade.

Mast Cell Mediators:

Mast cells produce three main classes of mediator: preformed granule-associated mediators; newly generated lipid mediators; and a wide variety of cytokines and chemokines. Of the granule-associated mediators, proteases are important constituents, including enzymes with tryptase-, chymase- or carboxy peptidase-like activities, and these have been shown to have various roles in tissue remodelling and cellular recruitment. [16] Vasoactive amines, such as histamine, are another type of mediator found in mast-cell granules. They have potent effects on vascular permeability and have been implicated in many of the symptoms of acute allergic disease. Mast-cell granules also contain proteoglycan, either heparin or one of several other related, highly sulphated structures, [17] which might have an important role as a depot for chemokines and growth factors. Of the lipid mediators that are produced by mast cells, the leukotrienes LTC4 and LTB4 have been the most extensively studied in the context of host defence. LTC4 is not only a potent Bronchoconstrictor but also increases vascular permeability and aids in the recruitment of eosinophils, whereas LTB4 promotes neutrophil recruitment. The rapid degranulation and lipid-mediated responses that follow mast-cell exposure to some pathogens provide important signals for the initiation of vascular changes, as well as for the mobilization and recruitment of effector cells. Cytokines and chemokines that are produced by mast cells include classical pro-inflammatory mediators, such as tumour-necrosis factor (TNF) and IL-1β, as well as cytokines that are associated with anti-inflammatory or immunomodulatory effects, such as IL-10 and transforming growth factor-β. Although frequently described as a source of T helper 2 (TH2)-type cytokines, including IL-4, IL-5 and IL-13, mast cells can also produce TH1-type cytokines, such as interferon-γ (IFN-γ), IL-12 and IL-18. Mast cells can also be an important source of several chemokines, including those associated with TH2-type responses, such as CC-CHEMOKINE ligand 5 (CCL5), and TH1-type responses, such as CXC-CHEMOKINE ligand 8 (CXCL8; also known as IL-8) and CXCL10.

Recently, mast cells have also been shown to produce several antimicrobial peptides, such as the human cathelicidin known as LL37 or the mouse cathelicidin-related antimicrobial peptide (CRAMP). [18]

Role of mast cells in inflammation:

Mast cell activation and mediator release can independently, as well as in concert with other immune cells, induce much of the pathology observed in allergic inflammatory conditions. Mast cell mediators such as histamine, leukotrienes and prostaglandins contribute to eosinophil recruitment, increase vascular permeability and smooth muscle contraction. Proteases can activate fibroblasts thereby promoting collagen deposition and fibrosis. [19] Mast cell-derived cytokines have numerous effects on other cells of the immune system as well as endothelial cells. For example, mast cell-derived cytokines can cause B cells to class switch to synthesize IgE, induce basophil histamine release, recruit neutrophils and eosinophils, and

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promote the development of T cells into a T helper 2 (Th2) phenotype.[20] Mast cell products may both induce an immediate reaction and contribute to a late phase reaction. The immediate phase reaction occurs within minutes of FcεRI cross linking and its consequences are referred to as an immediate hypersensitivity reaction. Pre-formed granule associated and newly generated mediators released during this phase include histamine, proteases and lipid-derived mediators. Late-phase reactions peak 6–12 h following antigen challenge and are associated with cytokine and chemokine production and release in part from eosinophils, neutrophils and basophils that have entered the inflammatory site following the immediate reaction. Mast cells are also involved in chronic allergic inflammation where symptoms relapse and remit over time, of which asthma is a classical example.

The mast cell in allergic diseases:-

Asthma:- The role of the mast cell in asthma is of renewed interest due to reports that mast cell numbers are increased within the airway smooth muscle bundles of asthmatic patients.[21] This has led to a re-evaluation of the mast cell as a crucial effector cell in the pathogenesis of asthma, especially asthma with an allergic basis.

Allergic rhinitis:- Allergic rhinitis (AR) is the most common allergic disease in the United States. It affects up to an estimated 40% of children and 25% of adults. The pathophysiology of AR shares many similarities to allergic asthma and the two diseases are often considered manifestations of ‘one airway, one disease’, [22].

Atopic dermatitis:- Mast cells are increased in a variety of chronic inflammatory skin disorders, including atopic dermatitis (AD)[23]. Biopsies of AD lesions demonstrate an increase in mast cell numbers as compared with uninvolved sites [24]. The precise contribution of this mast cell presence to the pathophysiology of AD is not, however, understood.

Anaphylaxis:- Anaphylaxis is an acute, severe, systemic reaction to a foreign stimulus that is often thought to be associated with mast cell activation. The strongest evidence of a role for mast cells in anaphylaxis comes from assessments of serum tryptase levels during anaphylaxis [25]. Serum levels of tryptase, which predominantly arise from mast cell degranulation, peaks 1–2 hr following the onset of IgE-mediated anaphylaxis [26].

Functions of Mast Cells in Physiological and Pathological States:-

Vascular Disease:

Mast cells are uniquely positioned around capillary vessels and may thus play crucial roles in vascular injury and atherosclerosis.[27] Mast cell granule components, released upon activation, could have both anticoagulant and thrombogenic functions [28].

Host Defence:

Mast cells may play crucial roles in host defense by modulating both innate and adaptive immune responses[29]. Various functions of mast cells make them crucial players in host defense.

Tissue Remodeling/Fibrosis:

Mast cells are increased in numbers in many fibrotic diseases and may play a crucial role in the development of fibrosis[30]. The percentages of mast cells in bronchoalveolar lavage fluid from patients with sarcoidosis or interstitial fibrosis are greater than from control individuals and patients with idiopathic interstitial pulmonary fibrosis show evidence of mast cell degranulation and elevated mast cell numbers[31].

Systemic Mastocytosis and Malignancy:

A disorder characterized by excessive numbers of mast cells and tissue infiltration by these cells is systemic mastocytosis. In this condition, mutations of c-kit (Asp 816 Val mutation) occur [32], and a subsequent pathological infiltration of affected tissue by mast cells may be seen, resulting in many of the manifestations [33].

HIV and Rheumatological Disease:

A probable role for mast cells and IgE-mediated pathology has been reported in HIV infection. The chemokine receptor, CCR3 is expressed on mast cells and may provide one explanation for the chemotactic effects of tat protein on mast cells [34].

SICKEL CELL ANAEMIA

Sickle cell disease (SCD) is an inherited, lifelong condition. The sickle mutation consists a single nucleotide change (GAT->GTT) in the sixth codon of exon 1 of the β–globin gene coding for the β-globin polypeptide of hemoglobin (Hb) (α2β2). This change results in replacement of the wild type glutamic acid residue by a valine residue in β-globin chain and the formation of the sickle Hb (HbS) in homozygotes for this mutation. Heterozygotes live a normal life. In SCD patients, sickle erythrocytes are rigid with decreased deformability and reduced life span resulting in hemolysis, vaso-occlusive disease, vasculopathy.
and subsequent inflammation and end organ damage. Sickle cell disease affects millions of people worldwide. Today, with proper health care, many SCD patients have a good quality of life (QoL) and are in fairly good health most of the time. These people can live up to their forties or fifties or longer. Patients with SCD are at high risk for developing multisystem acute and chronic complications associated with significant morbidity and mortality.

**Acute Complications of Sickle Cell Disease:**

**Vaso-occlusive crisis (VOC) or sickle cell crisis:**
A VOC is the hallmark acute complication for SCD and manifests as acute severe pain. The sickled erythrocytes block the flow of blood through the small blood vessels (capillaries) resulting in ischemia. Sudden episodes of pain throughout the body are a common symptom of SCD.

**Fever Infection:**
Patients with SCD have an increased risk for severe bacterial infection, resulting primarily from reduced or absent splenic function. The result is an extremely high risk of septicemia and meningitis, primarily due to Streptococcus pneumoniae. The risk of such infections continues throughout childhood and to a lesser extent in adults.

**Acute Renal Failure:**
Acute renal failure is defined as a rapid reduction in renal function manifested by a rise in serum creatinine and reduction in glomerular filtration rate (GFR), with or without a decline in urine output.

**Hepatobiliary complications:**
Biliary tract abnormalities are common in SCD patients. These abnormalities include cholelithiasis, acute cholecystitis, biliary sludge, and acute choledocholithiasis. Hemolysis of any etiology results in increased secreted unconjugated bilirubin that tends to precipitate and leads to gallstones and sludge.

**Acute anemia:**
Acute anemia, defined as a decline in hemoglobin concentration by 2.0 g/dL or more below the patient’s baseline value, may have diverse etiologies. Therefore, splenic sequestration in a child or an aplastic episode at any age may require urgent evaluation and therapy.

**Splenic sequestration:**
Splenic sequestration is defined as sudden enlargement of the spleen and reduction in hemoglobin concentration by at least 2 g/dL below the baseline value. It is a major cause of acute anemia and it may present acutely accompanied by severe anemia and hypovolemic shock. The reticulocyte count and circulating nucleated red blood cells are usually elevated.

**Acute chest syndrome (ACS):**
Acute chest syndrome is a life-threatening condition for SCD patients. It is the second most frequent reason for hospitalization in children and adults with SCD and the most common cause of death. It's similar to pneumonia and is caused by an infection or by sickle cells trapped in the lungs. Patients with this condition usually have chest pain, fever, and an abnormal chest x ray. Over time, lung damage may lead to pulmonary hypertension.

**Acute stroke:**
Stroke is one of the most common and devastating complications of SCD. Sickle-shaped red blood cells may stick to the walls of the tiny blood vessels in the brain. This type of stroke occurs mainly in children. This complication presents as sudden onset of weakness, aphasia, and sometimes seizures or coma and results in adverse motor and cognitive sequelae. In the absence of secondary prevention measures such as a chronic transfusion program or hematopoietic stem cell transplantation, recurrence rates have been shown to range between 46 and 90 percent in children with SCD. Brain hemorrhage occurs more often in adult patient.

**Priapism:**
Males with sickle cell disease may have painful and unwanted erections lasting about 4 hours, called priapism. This happens because the sickle cells stop blood flow out of an erect penis. Priapism is a common complication of SCD, affecting 35 percent of male patients. Over time, priapism can damage the penis and lead to impotence.

**Multisystem organ failure:**
Multisystem organ failure is a severe, rare and life-threatening complication usually associated with a VOC and characterized by failure of the lungs, liver, and/or kidneys. Symptoms linked to this complication are fever and changes in mental status such as sudden tiredness and loss of interest in their surroundings.

**Chronic Complications of Sickle Cell Disease:**

**Chronic pain:**
In SCD, pain is considered chronic if it persists more than 3 months. Chronic pain may be an extension of recurrent acute painful episodes or in a specific tissue or organ, such as avascular necrosis of the hips, or leg ulcers. Chronic pain is often associated with other conditions that enhance its chronicity. These include psychosocial factors such as depression, anxiety, feelings of despair, insomnia, loneliness, helplessness and dependence on pain medications\[42\]. Chronic pain can be hard and may lead in mental draining.

**Leg ulcers:**
Leg ulcers are a common complication of SCD\[43\]. Sickle cell ulcers usually begin as small sores on the lower third of the leg. Leg ulcers occur more often in males than in females and usually appear between the ages of 10 and 50. The cause of leg ulcers is not clear. Some heal rapidly, but others persist for year.

**Pulmonary Hypertension:**
Pulmonary hypertension (PH) is defined as an elevation of the resting mean pulmonary arterial pressure (>25 mmHg) as determined by right heart catheterization (RHC). PH can occur in chronic hemolytic anemia and in the setting of chronic lung disease, chronic thromboembolic disease, or can be due to unclear and multiple mechanisms. Initial testing for PH has been done with an echocardiography assessment to estimate pulmonary artery pressure using tricuspid regurgitant jet velocity (TRV), but diagnosis requires right heart catheterization and direct measurement of the pulmonary arterial pressure and vaso-reactivity of the vessels\[44\]. Excessive shortness of breath is an important symptom of PH.

**Renal complications:**
Identification of early renal disease in people with SCD is important, as these patients hyper secrete creatinine through the proximal tubules, thus masking significant renal impairment before the serum creatinine rise\[45\]. Microalbuminuria is most often the first manifestation of chronic kidney disease in SCD. Proteinuria due to glomerular injury is also common, but both microalbuminuria and macroalbuminuria are typically asymptomatic. The most common renal complication in people with SCD is hyposthenuria, or the inability to concentrate the urine, which is progressive with age\[46\].

**Ophthalmologic complications:**
Chronic ophthalmological complications of SCD include proliferative sickle retinopathy and vitreous hemorrhage. They occur in up to 50 percent of patients with SCD and are associated with significant visual loss\[47\].

**Approaches for mast cell therapy for sickle cell anemia:**

**Symptomatic for painful crises:**
Eight patients with the symptomatic crises of sickle cell anemia were treated by limited exchange transfusion. Buffy coat-free, packed erythrocytes were used in therapy. On the first day 250 ml red cells were given followed by phlebotomy of 500 ml whole blood and an infusion of a second 250 ml of red cells. The second day's program resembled the first except that two 250-ml units of red cells succeeded the phlebotomy. The method is not accompanied by untoward reactions, it rapidly ameliorates fever and bone pain; interrupts the cycle of hypoxia, vasocclusion, and organ injury by diluting autologous circulating erythrocytes containing sickle hemoglobin with those holding hemoglobin A; and raises the oxygen-carrying capacity of the blood with only a minor increase in total blood volume. The average length of hospital stay has been reduced from 7 to 3 days. This program probably should supplant other types of treatment that do not directly influence the dynamic changes involved in the pathogenesis of symptomatic crises in sickle cell anemia.\[48\]

**Hydroxyurea for painful crises:**
A major therapeutic approach to sickle cell anemia has been to try to shift haemoglobin production from sickle haemoglobin to fetal hemoglobin, by changing marrow-proliferation kinetics to favor F-cell production. To evaluate this concept, hydroxyurea, a cytotoxic drug that had already been in use for decades to reduce the abnormally high hematocrit and platelet count in patients with polycythemia vera, was initially tested in anemics baboons\[49\]. The first patients treated with hydroxyurea had a response within 72 hours after therapy, with a burst of young F cells, and ultimately with an elevated level of fetal haemoglobin\[50\]. Hydroxyurea is the most successful drug therapy for SCD.

**Red cell transfusion:**
Transfusions are not needed for the usual anemia or episodes of pain associated with SCD. Urgent replacement of blood is often required for sudden, severe anemia due to acute splenic sequestration, parvovirus B19 infection, or hyperhemolytic crises. Transfusion is helpful in acute chest syndrome, perioperatively, and during pregnancy.
Acute red cell exchange transfusion is indicated in the following situations:

- Acute infarctive stroke
- Severe acute chest syndrome
- Multiorgan failure syndromes
- Right upper quadrant syndrome
- Priapism that does not resolve after adequate hydration and analgesia

Transfusion-related complications include alloimmunization, infection, and iron overload. Treatment of iron overload is becoming easier with the new oral chelators. Alloimmunization is a common problem that arises from the differences in certain minor red cell antigens found in the predominantly black patient population and the mostly white blood donors. Matching for C, E, Kell, JKB (Kidd), and Fya (Duffy) antigens can significantly reduce alloimmunization.

**Bone marrow transplantation:**
Bone marrow transplants have proven to be effective in children. At present it is only available curative therapy for sickle cell anaemia. While the survival rate from this procedure is roughly 91% and the cure rate is 82%. This option is currently limited primarily to children under 16 years of age with severe, pre-existing complications. There is difficulty in finding suitable donor for the vast majority of patients; it is estimated that only 14% of patients have a human leukocyte antigen (HLA)-matched sibling donor. There are so many short and long-term complications after transplantation including intracerebral haemorrhage, graft-versus-host disease (GVHD), seizure, and gonadal dysfunction.

**Prophylactic therapy:**
Three prophylactic measures have become widely accepted in the management of SCD; penicillin prophylaxis, immunization against pneumococcal infection and folate administration. The mortality rate due to *Streptococcus pneumoniae* pneumonia, sepsis, and meningitis was historically very high prior to the age of 6 years in children with SCD. This rate has been lowered tremendously by three maneuvers. The first is diagnostic screening for SCD in neonates, with immediate initiation of penicillin VK 125 mg twice daily, increased at the age of 3 years to 250 mg twice daily and continued until 5 years old. The second is immunization with heptavalent pneumococcal-conjugated vaccine at 2, 4, 6, and 12 months of age. The third is immunization with 23-valent pneumococcal polysaccharide vaccine at 2 and 5 years of age. Due to the increased metabolic requirement for folate, this is frequently provided as a supplement at a dose of 1 mg daily, although the adequate dietary intake in the United States appears not to mandate such supplementation.

**Future Therapy for Sickle Cell Disease:**
New treatments for SCD are directed at ameliorating the secondary events related to sickling as well as finding new fetal Hb-modulating agents. Clinical trials are in progress or planned to moderate red blood cell dehydration by blocking the Gardos channel, Clotrimazole inhibits the Gardos channel and magnesium retards potassium efflux, preventing erythrocyte dehydration and thereby reducing sickling.

**Globin gene therapy:**
The current status of gene transfer experiments indicates that it is possible to provide safe and efficient retroviral packaging lines for gene transfer; and vectors containing the human beta-globin genes and selectable marker genes which can be transmitted into erythroid cells and are appropriately expressed. In animal autologous bone marrow transplantation experiments, stable and high-level expression of retroviral vectors containing human beta-globin genes has not yet been achieved. New retroviral vectors are being tested that contain different components of the retrovirus as well as the newly described enhancer elements 5' to the epsilon gene and surrounding the beta-globin gene. The long-term goal of human gene therapy for sickle cell disease then consists of constructing optimally safe and efficient retroviral packaging lines as well as retroviral vectors containing the human beta-globin gene and selectable markers such as the neo gene. One would then remove bone marrow cells from patients with sickle cell disease, transfer the retroviral vectors into the bone marrow cells, and subject the cells to G418 selection in vitro. Next, one would ablate the host bone marrow and autotransplant the manipulated bone marrow bearing the retroviral vector.

**REFERENCE**


