Senescent Sickle Erythrocytes and Endothelial Adhesion via Band 3 Peptides

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Abstract
The band 3 molecule is an anion channel for bicarbonate in the erythrocyte’s membrane that assumes a different role in senescent erythrocytes where it adopts a cluster configuration. These band 3 clusters expose previously hidden antigenic adhesive peptides which are recognized by natural band 3 antibodies that label their erythrocytes for reticuloendothelial elimination and prevent their endothelial adhesion. The sickle erythrocyte’s abnormal hemoglobin molecules cause them to experience premature senescence resulting in increased numbers of clusters, a portion of which remain uncloaked by antibodies and adhere to the vascular endothelium. The uncloaked clusters result from a deficiency of band 3 antibodies that are present in sickle cell anemia. The hypothesis presented is that the band 3 antibodies are insufficient in number to cloak all of the adhesive peptides present on cluster+ sickle erythrocytes and this is responsible for a portion of their adhesive pathology.

Keywords: Sickle; Endothelium; Adhesion; Band 3; Senescent; Erythrocyte; Falciparum

Abbreviations: B3: Band 3; Hgb: Hemoglobin; SCA: Sickle Cell Anemia

Introduction
First, consider the basic band 3 molecule (B3M), it is the third band seen when erythrocyte membrane proteins are examined by gel electrophoresis and it is a transmembrane protein important in the erythrocyte’s structure that functions as a channel for exchanging bicarbonate ions for chloride ions [1]. The B3Ms are normally dispersed on the erythrocytes surface but in senescent erythrocytes some of their Hemoglobin (Hgb) molecules have broken down and formed hemichromes that attach to the cytoplasmic portion of the erythrocyte’s membrane causing the band 3 molecules in the vicinity to cluster above them on the erythrocyte’s surface [2,3]. This clustering exposes previously hidden adhesive antigenic band 3 peptides which are recognized by natural band 3 antibodies that, in concert with a complement component [3], target these senescent erythrocytes for reticuloendothelial removal while blocking their endothelial adhesion.

In sickle cell anemia (SCA) the sickle erythrocytes experience premature senescence due to their mutant hemoglobin molecules and this causes band 3 clusters to form on their surface that are recognized by natural band 3 antibodies [4]. The significance of this regarding both anemia and endothelial adhesion in SCA is clear when you consider that in normal blood only 1% or less of erythrocytes have bound antibody compared to 30-40% in those with SCA [5]. The premature senescence of sickle erythrocytes also causes the apoptosis molecule, phosphatidylinserine, to appear on the erythrocytes surface and it also adheres to the vascular endothelium [6]. Aging has its consequences. There are other conditions that display these clusters such as hematological disorders like hemoglobin C anemia, glucose-6-phosphate dehydrogenase deficiency and thalassemia and by erythrocyte infections with malaria parasites. Patients with these cluster displaying conditions all have anemia but only SCA and erythrocytes infected by falciparum parasites, one of the four types of malaria, have erythrocytes that adhere to the endothelium via the adhesive band 3 peptides found on their band 3 clusters [3].

Documenting that band 3 peptides are agents of adhesion for these two disorders are several in vitro studies showing that band 3 antibodies and peptides can inhibit it [7-9]. As further proof, an in vivo study has shown that intravenous administration of band 3 peptides to monkeys infected by falciparum parasites is able to inhibit adhesion of their infected erythrocytes [8]. The reason for the endothelial adhesion in falciparum infections appears to be that the falciparum parasite is the only one of the four types of malaria parasites that have an additional adhesive molecule called the Plasmodium falciparum erythrocyte membrane protein 1[10]. The reason for the endothelial adhesion of band 3 peptides in SCA is a work in progress and a recent study suggests that one factor may be that some of the band 3 adhesive peptides are not cloaked by antibodies.

In this SCA study [11] the band 3 antibody levels were measured in control Hgb AA subjects, sickle trait, steady state SCA, and in sickle cell crisis.

Results
Hgb AA (9.64), Hgb AS (9.43), Steady state SCA (8.36) and sickle crisis (2.86)

The surprising findings were that the band 3 antibody levels present in conditions where erythrocytes contained sickle hemoglobin were lower than the control with a 13% drop in the steady state condition and a drastic 70% drop during a crisis. Normally antigen recognition by B cell clones would be expected to increase antibody levels and subsequent exposures would boost them even higher but band 3 antibodies are different in that they are natural antibodies whose B cell clones experience chronic exposure to the antigenic band 3 peptides. This suggests that there may be some type of biologic feedback control that limits their production and that in steady state SCA the cluster level exceeds this limit. The previously noted 30 fold increase in the presence of antibodies on sickle erythrocytes [5] could reasonably explain why...
band 3 antibodies may be unable to cloak all the senescent erythrocytes in SCA. This is not to say that antibody production does not increase in SCA, just that any increase is not sufficient to cloak all of the clusters present in this condition. Band 3 antibodies have not been measured in any other hematological conditions but they have been found to be elevated in falciparum endemic areas where chronic low grade parasitemia is present in survivors [12]. Though antibodies haven’t been measured in other hematological conditions the band 3 clusters have been semi-quantitated in hemoglobin C anemia where they were detected on intact erythrocytes [13]. In that study they found a few clusters on Hgb AA erythrocyte controls and many on the Hgb CC erythrocytes. The cluster count on the erythrocytes of hemoglobin C anemia was elevated but all of the cluster+ erythrocytes were cloaked by antibody since there is no endothelial adhesion present in this disease. The anemia in this disease is mild whereas it is severe in SCA which suggests that maximum band 3 antibody production probably occurs somewhere between hemoglobin C disease and SCA. The finding of Cluster+ erythrocytes in Hgb AA blood suggests that the clearance time of band 3 antibody cloaked erythrocytes is lengthy, which perhaps should not be unexpected since these antibodies are not lytic but target erythrocytes for macrophage elimination.

Discussion

The hypothesis presented is that the premature senescence of sickle erythrocytes may be the initiating factor that results in both the anemia and the endothelial adhesion seen in SCA. This premature sickle erythrocyte senescence results in increased numbers of band 3 clusters on the erythrocyte surface where newly exposed antigenic adhesive band 3 peptides are recognized by natural band 3 antibodies that, in conjunction with a complement component, target the red cell for reticuloendothelial elimination while cloaking the adhesive peptide. In SCA the inhibition of erythrocyte adhesion by band 3 peptides demonstrates that some of the cluster+ sickle erythrocytes are not cloaked by antibody. The numerous senescent sickle erythrocytes present in SCA have two effects: first, the reticuloendothelial removal of these erythrocytes exceeds new red cell production resulting in anemia and second, the band 3 antibodies are unable to cloak all the cluster+ erythrocytes causing them to adhere to the endothelium via their exposed adhesive peptides. The discovery that band 3 antibodies are reduced in SCA suggests an explanation for these uncloaked adhesive peptides. There are many agents involved in the endothelial adhesion that occurs in SCA but the presence of exposed adhesive band 3 peptides on sickle erythrocytes that cause those erythrocytes to adhere to endothelium and the in vitro studies showing that band 3 peptides and antibodies can block this adhesion suggest that the role they play is significant. There are therapeutic possibilities suggested by the cloaking ability of the band 3 peptides and antibodies and this is particularly so in a sickle cell crisis due to its characteristic temporary nature.

References