CORRESPONDENCE

Visualization of IgG-Binding Loci on the Surface of Sickle Erythrocytes

To the Editor:

Sickle cell disease (SCD) results from the defect in genes coding the for β globin polypeptide (ie, codon 6, GAG to GTG transition) that produces the substitution of valine for glutamic acid in position 6. The product, sickle hemoglobin (HbS), has the capacity to polymerize upon deoxygenation and to participate in pathogenic interactions with the red blood cell (RBC) membrane. The extensive number of RBC abnormalities attributed to these interactions could play significant roles in SCD pathophysiology. Petz et al. using a sensitive complement fixing-antibody consumption test, showed that sickle RBC abnormalities include the capacity to bind autologous IgG antibody in vivo. Our studies, and those from other investigators, used a variety of techniques, including phagocytosis assays, to detect IgG bound in vivo to sickle RBCs. Studies on density-separated sickle cells showed that the most-dense irreversibly sickled cell (ISC)-enriched RBCs bound comparatively high levels of IgG in vivo. Relationships between HbS-membrane interactions and IgG binding to sickle RBCs have been shown. Fluorescence assays determined that autologous IgG binds to band 3 coclustered with Heinz bodies in sickle RBCs. However, direct visualization of IgG antibody bound to the surface of ISC has eluded detection. Thus, it remains to be established that the IgG detected in previous studies is actually bound to specific cell surface loci. We therefore present our study on sickle RBC surface ultrastructure that is designed to determine whether autologous IgG indeed binds to loci on the intact sickle cell.

We have used scanning electron microscopy and employed the highly specific backscattered electron imaging technique to analyze the RBC surface ultrastructure and detect IgG-binding loci on the surface of sickle erythrocytes. Density-separated sickle erythrocytes were isolated from nontransfused HbS subjects, as described previously. The cells were mildly fixed and then sequentially labeled with antihuman IgG F(ab’), andgold-conjugated (30 nm) antimouse IgG. Scanning electron microscopy and backscattered electron imaging were performed using procedures already described. Control RBCs included Coomb’s RBCs similarly labeled and sickle RBCs either unlabeled or exposed only to the gold conjugate. The results presented represent the mean observations from the analyses of greater than 100 ISCs per HbS specimen (n = 10).

The electron micrographs shown in Fig 1 compare the IgG-binding by dense ISCs isolated from two representative HbS patients. Our study analyzed sequential segments along the vertical axis of the ISCs (Fig 1). The backscattered-electron images in Fig 1A (e, f, and g) and 1B (b, c, and d) show that autologous IgG binds to specific loci on the intact cell surface. Analyses of ISCs obtained from different HbS specimens showed a consistent random distribution of IgG-binding loci on the cell body. In some ISCs, IgG-binding loci were also detected on membrane domains that appear to be retracted after vesiculation (Fig 1B:d). Micrographs in Fig 2 illustrate the detection of IgG-binding loci on the surface of low-density discoid sickle (HbS) RBCs. Images shown in Fig 2c and e, show that IgG-binding loci on low-density HbS discoid RBCs are greatly diminished in quantity and only slightly smaller in size (original magnification in sectors is X36,000) as compared with those detected on ISCs (Fig 1A and B, original magnification X18,000). The ratio of IgG-binding loci detected is approximately 250:1 for ISCs: low-density HbS erythrocytes.

Our studies also characterized RBC membrane proteins that could comprise the IgG-binding loci on sickle RBCs. Specifically, we eluted surface-bound IgG from dense ISC-enriched sickle RBCs and then determined which autologous RBC membrane proteins bind
Fig 1. Visualization of autologous IgG-binding loci on the surface of dense irreversibly sickled erythrocytes. The SEM micrographs in (A) and (B) show the surface ultrastructure of ISC obtained from two different HbS subjects (A and B, respectively). The cell surface is photographed in sequential segments along the vertical axis of the cell body. (A) a, dense sickle erythrocyte subpopulation from subject A, in which the black arrowhead designates the ISC illustrated in b through g; bar = 4 μm. (b, c, and d) Sequential secondary images of the immunogold-labeled ISC designated in (a). (e, f, and g) Sequential backscattered electron images for b, c, and d, respectively, show the gold-labeled human-IgG binding loci on the cell surface (indicated by black arrowheads); bar = 0.5 μm. (B) a, secondary image of ISC from HbS subject B; bar = 0.8 μm. (b, c, and d) Sequential sectors along the vertical axis of the cell in (a) show that backscattered electron images of gold-labeled antihuman-IgG binding loci are located on the cell surface (IgG-positive signal indicated by black arrowhead in [c]). (B) d, the product of membrane vesiculation; bar = 0.5 μm.
Previous studies analyzed hemichrome-stabilized protein aggregates derived from sickle RBCs and showed that greater than 75% of the sickle RBC-bound IgG was attached to these aggregates. The approximately 200 molecules of IgG/RBC were concentrated among 2 to 4 of the Heinz body sites per cell. We interpret our observations on low-density discoid HbS RBCs to be consistent with these calculations. In contrast, IgG-binding loci on dense ISC (Fig IA and B) were significantly greater in number (250:1), suggesting that the loci could arise from changes in RBC membrane structure produced in vivo. Mechanisms for these membrane alterations remain to be defined; however, our study supports the concept that IgG-binding loci are comprised primarily by transmembrane protein band 3 clusters.

Our study presents the first visualization of IgG-binding loci on the surface of intact irreversibly sickled erythrocytes. These definitive findings confirm previous reports that (1) sickle RBCs bind IgG in vivo; and (2) dense ISC bind comparatively larger quantities of autologous IgG antibody in vivo than low-density sickle RBCs and provide evidence supporting the possibility that the recognition of discrete sickle RBC subsets by autologous IgG antibody in vivo represents a significant component of SCD pathophysiology.

**REFERENCES**


Fig 2. Autologous IgG binding to low-density sickle erythrocytes. (a) SEM-micrographs show that most low-density sickle cells exhibit a discoid morphology; bar = 4 μm. SEM-micrographs (b) and (d) illustrate the sectors of immunogold-labeled low-density sickle RBCs that have been magnified and then assayed by backscattered electron imaging (c) and (e), respectively, to detect surface-bound autologous IgG. IgG-positive signal is indicated by the black arrowhead; bar = 1 μm.

Visualization of IgG-binding loci on the surface of sickle erythrocytes [letter]

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