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Immune Regulation of sickle Cell Alloimmunization

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Abstract

Red blood cell (RBC) transfusion remains an important treatment for patients with sickle cell disease (SCD) and the majority of patients receive transfusions by adulthood. However, SCD patients are at a high risk of alloimmunization, which can cause life-threatening complications. The high rate of alloimmunization can in part be explained by chronic inflammatory condition in SCD characterized by significant immune and inflammatory activation. Heightened immune effector cell responses and/or impaired regulatory networks are likely to drive alloantibody production in alloimmunized SCD patients. In support of this, altered T cell immunoregulation, known to control antibody responses, have been reported in alloimmunized SCD patients. In addition, stronger follicular help T cell responses that help antibody production by B cells were described in alloimmunized as compared to non-alloimmunized SCD patients. Furthermore, several innate immune abnormalities have been identified in alloimmunized SCD patients, including a compromised anti-inflammatory response against extracellular cell free heme. The data support a model in which alloimmunized SCD patients are unable to switch off their proinflammatory state in response to the ongoing hemolytic state characteristic of SCD, placing this patient subset at a higher risk to develop a strong immune response against allogeneic determinants on transfused RBCs, thus increasing the risk of further alloimmunization. A detailed mechanistic understanding of innate immune abnormalities that can contribute to pathogenic T cell responses in alloimmunized SCD patients will lay the foundation for identification of biomarkers of alloimmunization with the goal that this information will ultimately help guide therapy in these patients.

Keywords

sickle cell; alloimmunization; hemolysis; heme oxygenase; Tregs; dendritic cells; CD83; NFκB; Follicular helper T cells; TIGIT

Introduction

Sickle cell disease (SCD) results from a mutation in the β -globin gene which causes hemoglobin S containing red blood cells (RBCs) to become rigid and sticky in sickle-prone conditions. As a consequence, patients suffer from a range of clinical complications

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Disclosure of interest

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including periodic painful crises, end organ damage, stroke, acute chest syndrome and chronic hemolytic anemia. RBC transfusions remain an important modality of treatment for SCD complications, including primary and secondary stroke prophylaxis and treatment, acute chest syndrome treatment and preoperative prophylaxis. However, transfusions carry the risk of alloimmunization, the development of alloantibodies against transfused cells, causing potential life-threatening delayed hemolytic transfusion reactions in SCD patients in which the transfused *and* patient's own RBCs are destroyed.¹ In addition, once the patients make alloantibodies, finding compatible units can be difficult and time-consuming, resulting in transfusion delays. SCD alloimmunization rates are higher than any other transfused population such as patients with thalassemia major or general hospital population.¹ The higher alloimmunization rate has been ascribed to antigenic differences between mostly Caucasian donors and recipients who are of African descent.²⁻⁴ Since most of the alloantibodies are against C, E and K1 antigens, many centers in the US have adopted the use of C, E and K phenotypically matched units from primarily African American donors for SCD patients. However, even with the provision of extended antigen-matched donor RBCs from the same racial background, SCD patients continue to develop antibodies due to high degree of polymorphisms in the immunogenic RBC antigens, encoded in the *RH* locus, in individuals of African ancestry.⁵ This highlights the need for better characterization of triggers of alloimmunization and identification of risk factors in this highly vulnerable population.

Immune regulation in SCD alloimmunization

In addition to antigenic differences between donor and recipient,²⁻⁴ and numbers of cumulative transfusions,^{3,6-9} less well understood genetic predisposing factors and acquired patient-related factors are likely to influence the process of alloimmunization.^{1,7;10-12} Immune profiling of transfused SCD patients have identified altered phenotypes and/or activity in alloimmunized CD4⁺ helper T cells (T_H) whose role in generation of antigen-specific B cell memory and plasma cells have been known for decades.¹³⁻¹⁷ In a small study of chronically transfused patients with SCD, we have reported reduced peripheral T cell regulatory (T_{REG}) suppressive function and altered T_H responses with higher circulating IFN- γ , but lower IL-10 levels in allosensitized as compared to non-sensitized group.¹³ In a separate study, differences in T_{REG} activity between alloimmunized and non-alloimmunized SCD patients were not detected, although their assay conditions included accessory cells,¹⁷ known to alter T_{REG} functional activities.¹⁸ Our group has also shown that B regulatory (B_{REG}) cells from alloimmunized SCD group have altered functional activity: alloimmunized B_{REG} cells were impaired in their ability to inhibit monocyte proinflammatory cytokine production.¹⁹ We speculate that alloimmunized SCD patients have an imbalance between regulatory (T_{REG}) and effector (T_H) cells, resulting in weakened immunoregulatory state and/or heightened T cell responses which can promote pathogenic immune response against transfused cells and increase the risk of alloimmunization. The altered immunoregulatory state in alloimmunized SCD patients may be genetically inherited as has been predicted by mathematical modeling¹⁰ or may only be established after the patient becomes alloimmunized. Interestingly, once a patient, regardless whether they have

SCD or not, becomes alloimmunized, it is more likely that they make additional alloantibodies.^{3;20;21}

Follicular helper T cells and SCD alloimmunization

A first step toward understanding the adaptive immune response in SCD alloimmunization is to characterize CD4⁺ T helper cell responses that control IgG production. Since T helper 2 (T_H2) cells produce IL-4 which induces isotype switching and antibody secretion, they were classically regarded as the key T cell subset in driving antibody responses.^{22–24} However, mice deficient in key T_H2 developmental pathway components are capable of developing T-dependent antibody responses, arguing against T_H2 as the sole drivers of humoral response.^{25–27} More recently, T follicular helper (T_{FH}) cells have emerged as the main effector CD4⁺ T cells specialized in providing help B cells to generate the initial wave of antibody response as well as in supporting B cell differentiation into high affinity antibody producing B cells and long-lasting IgG antibodies.²⁷ Studies on human T_{FH} cells were made possible by the identification of blood T_{FH} cells sharing phenotypic and functional similarities with secondary lymphoid T_{FH} cells, including the expression of CXCR5 chemokine receptor (which allows their migration into B cell follicles in response to the specific ligand CXCL13^{28–30}). In addition, a positive correlation was also shown between circulating CXCR5⁺CD4⁺ T cells and either protective antibodies or autoantibodies.^{31–35} As with lymphoid T_{FH} cells, circulating T_{FH} cells upregulate ICOS (inducible T cell costimulator^{27;34} required for T cell proliferation and T-B cell interactions³⁶) and CD40L,^{27;37} (which is a potent activator of B cells, inducing their activation and differentiation,^{38;39}) as well as produce IL-21 (a highly potent cytokine for B cell growth, differentiation, and class switching^{32;40;41}) following stimulation. Additional features include the expression of high levels of inhibitory costimulatory molecule PD-1 (program cell death 1) that is associated with heightened humoral responses,^{42–45} although some studies have also proposed a inhibitory role for PD-1 in T_{FH} function.^{46;47} Given their key role in providing help to B cells to support their activation, expansion and differentiation, T_{FH} cells are likely to be critical in the induction as well as persistence of the alloantibody response. In support of a potential role of T_{FH} cells in SCD alloimmunization, Vingert et al¹⁶ found antigen-specific T_{FH} cells responses in peripheral blood from 3/9 patients with SCD who had developed antibodies to Kidd system antigen, Jk^b.

TIGIT (T cell immunoreceptor with Ig and immunoreceptor tyrosine-based inhibitory domains) is also a costimulatory molecule, highly expressed on T_{FH} cells and was reported to be involved in interactions between T cells and follicular dendritic cells to regulate B cell responses.^{37;48;49} On human blood T_{FH} cells, we found that TIGIT mediates T_{FH} activity. Indeed, TIGIT⁺ T_{FH} cells provide the most efficient B cell help for IgG production (Fig. 1).¹⁴ In the SCD setting, we found that despite comparable levels of TIGIT⁺ T_{FH} cells, the functional activity of TIGIT⁺ T_{FH} cells differs between alloimmunized and non-alloimmunized SCD patients: only TIGIT⁺ T_{FH} cells from alloimmunized SCD patients were able to induce RBC-specific IgG production by B cells.¹⁴ Furthermore, TIGIT⁺ T_{FH} cells from alloimmunized SCD patients expressed significantly more IL-21, and higher levels of costimulation marker CD40L as compared to non-alloimmunized SCD patients. Although we hypothesize that heightened TIGIT⁺ T_{FH} responses in the alloimmunized

group is due to increased TIGIT signaling, it may also be that non-alloimmunized SCD patients have impaired TIGIT-mediated responses and TIGIT signaling pathway(s). Current studies are focused on characterizing the differences in TIGIT-mediated responses between alloimmunized and non-alloimmunized SCD patients to help toward future identification of TIGIT⁺ T_{FH} associated biomarkers of alloimmunization and TIGIT⁺ T_{FH}-based therapeutic strategies such as TIGIT blockade to prevent alloimmunization. Interestingly, in SCD patients, the titers of many alloantibodies fall below detection levels after several months,⁵ an incompletely understood phenomenon termed evanescence that occurs at an estimated 50% occurrence even in general transfused population.⁵⁰ We hypothesize that the quality/quantity of T_{FH} cells/subsets by affecting the generation and maintenance of alloantibodies in SCD patients, are critical for persistence/disappearance of alloantibodies over time.

Hemolysis and SCD alloimmunization

Patients with SCD suffer from chronic hemolysis which results in substantial release of free hemoglobin and its oxidized form heme in the circulation.^{51;52} Heme triggers TLR4 signaling in endothelial cells, resulting in expression of adhesion molecules and vaso-occlusive crisis (VOC) in mouse models of SCD.⁵³ Our group recently showed that hemolysis impairs the ability of monocytes from alloimmunized patients to dampen proinflammatory T cell responses.⁵⁴ As compared to non-alloimmunized SCD patients, alloimmunized patients expressed lower levels of heme oxygenase 1 (HO-1), the cyto-protective enzyme that breaks down heme and is associated with anti-inflammatory responses,^{55;56} in their monocytes and specifically in their CD16⁺ monocyte subset.⁵⁴ We hypothesize that HO-1 levels/activity in response to cell free heme may be a critical parameter to switch the activity of monocytes from proinflammatory to immunoregulatory. In response to heme, human monocyte derived dendritic cells (moDCs) from healthy volunteers downregulate their maturation marker (CD83) and prime and induce fewer proinflammatory (T_H1) effector T cells.⁵⁷ These data suggest that if exposed to free heme, DCs from an otherwise healthy individual become less likely in mounting an immune response. Taking this one step further, we hypothesize that in patients with SCD who experience ongoing intravascular hemolysis, alloimmunization may be avoided if the heme response can render DCs less able to provoke an immune response. Consistent with this working model, we demonstrated that DCs from non-alloimmunized SCD patients downregulate their CD83 maturation marker in response to free heme and effectively inhibit pro-inflammatory (T_H1) priming similar to healthy donor DCs. In contrast, DCs from alloimmunized patients are refractory to the effects of heme: DC maturation and induction of pro-inflammatory CD4⁺ cells were not inhibited in response to heme in this patient group.⁵⁷ We found that the underlying mechanism for CD83 regulation in DCs depended on TLR4, but not on HO-1.⁵⁷ Exposure to cell free heme resulted in the downregulation of prototypical pro-inflammatory signaling pathway NF- κ B transcription factor, specifically p50 and RelB subunits, in DCs from non-alloimmunized SCD patients.⁵⁷ In contrast, alloimmunized SCD DCs had an impaired response to heme as evidenced by inability to inhibit NF- κ B proinflammatory transcription factors.⁵⁷ These data suggest that cell free heme dampens DCs to elicit an immune response through downregulation of CD83/p50/RelB and that this pathway is defective in alloimmunized SCD patients (Fig. 2).

Based on these data, we speculate that the ongoing hemolysis in SCD can dampen DC maturation and T cell priming in non-alloimmunized patients through downregulation of CD83/p50/RelB, thereby lowering the immune response and subsequent risk of alloimmunization. However, when this CD83/NF- κ B heme/hemolysis response pathway is defective, inflammation is induced and/or maintained, which in turn heightens the risk of alloimmunization. Deciphering the components of this defective pathway can possibly lead to identification of targets to restore the anti-inflammatory capacity of DCs when exposed to free heme, thereby potentially reducing the risk of alloimmunization.

It is interesting to note that whereas heme/heme analogs drive anti-inflammatory responses in DCs, most of the published literature implicates a pro-inflammatory effect of heme/TLR4 signaling in other cell types and preclinical models.^{53;58–61} Differences in cell types at the level of co-receptors and/or signaling molecules, involving other NF- κ B subunits besides p50 and RelB^{53;62} may explain this difference. Furthermore, LPS signaling, which also occurs through TLR4 signaling in DCs is proinflammatory, and involves upregulation of NF- κ B.^{53;63} In contrast, heme signaling blocks the TLR4/proinflammatory signal in DCs through inhibition of the NF- κ B activation pathway involving p50 and RelB, at least in healthy donors and in non-alloimmunized SCD patients. We speculate that LPS and heme have different binding sites or binding affinities for TLR4, or require additional molecules that can induce a pro-vs anti-inflammatory downstream signal in DCs. Studies to identify the differential TLR4 signaling as well as the components of heme defective pathway in alloimmunized may help identification of targets to restore the anti-inflammatory capacity of DCs when exposed to free heme as a strategy to avert alloimmunization.

Conclusions

Alloimmunization remains a barrier for safe and effective transfusion for patients with SCD. Altered T cell subsets including stronger follicular help T cell responses that help antibody production by B cells as well as defects in the innate immune response to hemolysis have been identified in alloimmunized SCD patients. A detailed mechanistic understanding of the ways in which innate immune abnormalities can contribute to pathogenic T cell responses in alloimmunized SCD patients will provide a foundation for the development of not only diagnostic tools to identify in advance patients at risk of alloimmunization, but also therapeutic approaches to cure selectively the hyperactive immune cells responsible for antibody production against transfused blood.

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Figure 1.

$TIGIT^{+} CD4^{+} T_{FH}$ cells are more potent in driving B cell responses, including higher expression levels of costimulatory markers CD40L and ICOS as well increased IL-21 expression levels than $CD4^{+} T_{FH}$ lacking TIGIT.

Figure 2.

In response to heme, TLR-4 signaling inhibits NF- κ B and downstream CD83 gene expression in DCs from non-alloimmunized patients, resulting in lower expression of maturation marker ($CD83^{low}$). As such, DCs prime naïve T cell to produce lower levels of IFN γ . In contrast, heme driven signaling pathway is defective in DCs from alloimmunized patients, thereby preventing the inhibition of NF- κ B and CD83. As a result, primed naïve T cells produce higher levels of IFN γ ($IFN\gamma^{high}$). We speculate that this pathway represents a mechanism by which enhanced inflammation is induced and/or maintained in alloimmunized patients.