A Unique Monocyte Transcriptome Discriminates Sickle Cell Disease From Other Hereditary Hemolytic Anemias and Shows the Particular Importance of Lipid and Interferon Signaling

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Sickle cell disease (SCD) is a relatively common, hereditary hemolytic anemia characterized by complex pathophysiology, including chronic inflammation and oxidative stress. Ischemia-reperfusion injury and hemolysis have been recognized as potent triggers of inflammation, and the inflammatory marker C-reactive protein (CRP) was identified as an independent predictor of early mortality. Emerging studies highlight the importance of pro-inflammatory Toll-like receptor 4 (TLR4) signaling in acute and chronic SCD complications. In general, TLR signaling is dependent on reactive oxygen species (ROS) production by nicotinamide-adenine dinucleotide phosphate oxidase highly expressed on monocyte membranes.

Chronically damaged red blood cells, a hallmark feature of SCD, are known to catalyze redox signaling by participating in the Fenton reaction. ROS production by heme has shown to indirectly activate the nuclear factor kappa B (NF-κB) pathway. Free heme, product of intravascular hemolysis, therefore, serves as a potent modulator of TLR4 signaling in SCD, and TLR4+ cells, monocytes, play an important role in the pathophysiology of SCD. The aim of our study was to identify the main activated pathways in monocytes in response to intravascular hemolysis in SCD patients. In our analyses, we compared SCD to a group of other hemolytic diseases characterized almost exclusively by extravascular hemolysis and thus not subjected to large amounts of intravascular cell-free heme. We investigated gene expression profiles of TLR4+ cells, by positive selection of its co-receptor CD14, from patients with SCD and other hereditary hemolytic anemias to identify differential regulated genes and pathophysiologic pathways. Materials and methods are described in the Section S1, http://links.lww.com/HS/A129. Our results illustrate the importance of the relative contribution of pro- and anti-inflammatory signaling in SCD monocytes, which may contribute to the SCD phenotype.

Data were available from 14 individual SCD patients (ie, 11 HbSS, 3 HbSC), obtained during steady-state disease defined as no acute SCD complications in the preceding month. Table S1 (http://links.lww.com/HS/A129) highlights the main clinical characteristics of the SCD patients without (n = 11) or on deferasirox (DFX) therapy (n = 3), healthy controls (n = 10), and patients with various forms of hereditary hemolytic anemia (n = 46). The latter group consists of patients diagnosed with pyruvate kinase deficiency (n = 14), β-thalassemia (n = 2), hereditary spherocytosis (n = 7), and hereditary xerocytosis (n = 23). None of these patients was treated with DFX at the time of blood sampling. In the SCD patient group, 5 patients required regular exchange red cell transfusions, including 3 patients treated with hydroxyurea.

For visualization of the data, a plot was generated based on a principal component analysis of the 3000 most variable genes in the dataset (Figure 1). The cluster of CD14+ cells of SCD patients not treated with DFX clustered apart from both CD14+ cells derived from healthy controls and cells from patients with various hemolytic anemias. Whereas CD14+ cells of healthy controls and patients with other hemolytic anemias overlapped in the principal component analysis, DFX treatment seemed to correct the transcriptome alterations of SCD patients towards normal.

Next, we analyzed the differentially expressed genes (DEGs) from CD14+ cells of non-DFX SCD patients when compared with either healthy controls or patients with other hemolytic anemias. The analysis rendered 744 genes differentially expressed in the comparison of non-DFX SCD and healthy controls, of which 505 were upregulated in SCD. In the comparison of non-DFX SCD and other hereditary hemolytic anemias, 593 genes were differentially expressed, including 248 genes higher expressed in SCD.
Pathway enrichment analysis showed significant enrichment of genes involved in interferon (IFN) type I and II signaling in the set of individual genes that were differentially expressed in non-DFX SCD when compared with healthy controls (adjusted $P = 4.4 \times 10^{-14}$; Table S2A, http://links.lww.com/HS/A129). We observed a profound upregulation of all components of IFN signaling, including IFN receptors (eg, IFNGR1, IFNGR2), signal transduction molecules (eg, JAK2, STAT1, STAT2, IRF7), and a broad range of IFN-stimulated genes (eg, IFI27, IFTT3, OAS1, ISG15). Furthermore, pathway enrichment analysis showed significant enrichment of pathways involved in chemokine signaling (adjusted $P = 0.06$) and TLR2/4 signaling (adjusted $P = 0.06$) with profound upregulation of the individual genes in CD14$^+$ cells derived from SCD patients.

In addition, pathway enrichment analysis in the set of individual genes differentially expressed between non-DFX SCD and other hereditary hemolytic anemias underlined the importance of chemokine signaling (adjusted $P = 0.01$; Table S2B, http://links.lww.com/HS/A129) with the most profound upregulation of IFN$\gamma$-inducible genes CXCL11 and CXCL9. Our analysis also showed enrichment of genes involved in cholesterol biosynthesis (the mevalonate pathway; adjusted $P = 0.09$), which were all upregulated in SCD, as well as upregulation of genes involved in immune interactions between lymphoid and non-lymphoid cells (adjusted $P = 0.09$).

Next, we aimed to define a core list of protein-encoding genes that represents the specific features of CD14$^+$ cells in SCD. For this purpose, we made a more stringent selection of the individual DEGs (see Statistical analysis in Section S1, http://links.lww.com/HS/A129) and selected those protein-encoding genes that were differentially expressed when comparing CD14$^+$ cells of SCD patients with CD14$^+$ cells of healthy controls and patients with other hereditary hemolytic anemias. This analysis rendered 29 genes, as presented in Figure 2. Several of the encoded proteins in the selection have previously been related to SCD. For the other proteins, an important role in SCD could be presumed based on protein characteristics and actions in other disease models. Again, the list highlights the importance of 2 processes related to immune signaling: CXCR3 (chemokine) signaling by CXCL9 and CXCL11 and lipid metabolism (STARD4, DLC1, SQLE, ME1). Thereby, a role for CD14$^+$ cells in development of vasculopathy in SCD is supported by upregulation of PPAR$G$, GUCY1A1, KL5, CTSL, and CXCR3 signaling (CXCL9 and CXCL11), which all have previously been associated with vascular remodeling and development of pulmonary hypertension.

Interestingly, but not unexpected, heme oxygenase-1 (HMOX1) was one of these genes (versus healthy controls adjusted $P = 5.6 \times 10^{-13}$; versus other hemolytic anemias adjusted $P = 3.3 \times 10^{-13}$). Profound upregulation of HMOX1 in CD14$^+$ cells of SCD patients is in line with the hypothesis that intravascular free heme is an important effector of gene regulation in monocytes. Heme oxygenase-1 (HO-1) mediates heme detoxification. Transcriptional regulation is highly complex and upregulation is mediated by multiple pathways involved in stress and inflammation, as extensively reviewed by others. Monocyte HO-1 has known to be important in prevention of vasculopathic injury in SCD. HO-1 induction required crosstalk with endothelial cells damaged by toxic heme. A large proportion of the immunomodulatory effectivity of HO-1 is contributed to one of the end products of heme degradation, carbon monoxide (CO). So, the strong upregulation of HO-1 in SCD monocytes suggests an essential preventive response to counterbalance continuous pro-inflammatory signaling initiated by a broad range of e-DAMPs.

The regulation of gene expression of many pro-inflammatory genes relies on integration of signals from TLR4 and IFN signaling pathways. Combined action of STAT1-containing transcription factor complexes and NF-kB provides a robust platform for transcriptional activation of a broad range of pro-inflammatory genes. These processes are tightly controlled by HO-1. HO-1 seemed to be required for early activation of the type I IFN-inducing pathway. However, CO suppressed the capacity to
Mevalonate is crucial for induction of trained immunity, which presumably involves the mevalonate pathway (HMG-CoA reductase inhibitors, which prevent conversion of HMG-CoA into mevalonate) could be an effective therapy for treating hyperinflammatory disorders in which trained immunity plays a role. Upregulation of expression of the enzymes of mevalonate synthesis with statins (3-hydroxy-3-methylglutaryl-coenzyme A reductase inhibitors) was previously shown in monocytes from sepsis patients. Moreover, our data hints towards a previously suggested role for lipid rafts or caveolae formation in TLR4 signaling. Remarkably, CO both inhibited translocation of TLR4 and attenuated interaction between Caveolin-1 and TLR4 which dampens TLR4 signaling.

In summary, both the mevalonate pathway and lipid rafts are known enhancers of pro-inflammatory signaling. Our data shows the importance of both processes in pro-inflammatory signaling in SCD monocytes.

In conclusion, our analysis of the CD14+ cell transcriptome in patients with hereditary hemolysis shows that patients with SCD have a characteristic gene expression pattern. This pattern includes upregulation of HOX1, a signature of high intracellular iron and oxidative stress, and thereby underlines previous observations on the importance of e-DAMP molecules (including heme) in initiating pro-inflammatory signaling in SCD. Moreover, it shows that lipid metabolism and IFN signaling are important differentiating pro-immune signaling pathways. The unique SCD monocyte transcriptome also underlines the importance of both pro- and anti-inflammatory pathways. Coexistence of ant- and pro-inflammatory transcriptional activity has previously been shown in monocytes from sepsis patients. And, in line with sepsis and sepsis recovery, the balance might shift in response to, for example, vaso-occlusive crises. We hypothesize that the relative upregulation of both pathways is associated with disease complications, especially vasculopathic complications, in SCD and that HO-1 has an important role in determining this balance. Importantly, all discussed pathways yield potentially druggable targets that possibly could reduce the pro-inflammatory phenotype in SCD and related (vasculopathic) complications.
Disclosures

The authors have no conflicts of interest to disclose.

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