Factor H: a novel modulator in sickle cell disease

Wassim El Nemer[1,2,3] and Bérengère Koehl[1,2,3,4]

[1]Biologie Intégrée du Globule Rouge UMR_S1134, Inserm, Univ. Paris Diderot, Sorbonne Paris Cité, Univ. de la Réunion, Univ. des Antilles; [2]Institut National de la Transfusion Sanguine, F-75015; [3]Laboratoire d'Excellence GR-Ex and [4]Hematology Unit, Sickle Cell Disease Center, Robert Debré Hospital, AP-HP, Paris, France

E-mail: WASSIM EL NEMER - wassim.el-nemer@inserm.fr

doi:10.3324/haematol.2018.214668

Sickle cell disease (SCD) is an autosomal recessive disorder caused by a point mutation in the β globin gene that substitutes glutamic acid (GAG) at position 6 of the protein into valine (GTG).[1] The resulting mutated hemoglobin (HbS) polymerizes under hypoxic conditions driving sickling of red blood cells (RBC). Sickling and dehydration alter the shape of the RBC, decreasing their deformability and increasing their rigidity, which results in significant intravascular hemolysis. These alterations affect blood rheology and microcirculatory flow as well as blood and endothelial cell functions because of the release of hemoglobin and subsequent free heme in the circulation. In addition to chronic hemolysis and related complications, patients with SCD experience frequent vaso-occlusive crises that are painful episodes caused by obstruction of micro-capillaries, believed to be initiated by abnormally adherent RBC or neutrophils reducing the luminal section of the capillaries with subsequent blockage by rigid, deformed RBC.[2,3] Hemolysis and vaso-occlusive crises are critical components of the chronic inflammatory state reported in SCD,[4,5] which in turn is responsible for several cellular dysfunctions including activation of neutrophils that contributes to vaso-occlusive crises in a vicious feedback loop.

SCD is a multisystem disease that has been explored for decades. Despite significant efforts to date and recent advances showing a role for neutrophils in vaso-occlusive crises, the pathogenesis of SCD, in terms of the sequence of molecular events underlying the disease, remains only partially understood. Hemolysis and chronic inflammation are features common to SCD and other pathologies in which complement activation has been reported, such as atypical hemolytic uremic syndromes and paroxysmal nocturnal hemoglobinuria.

The complement system is one of the oldest defense mechanisms against infections during evolution.[6] It is composed of the classical, alternative and lectin pathways that can be activated by specific chemical components. Activation of the classical pathway is initiated by the attachment of the first protein of the complement, C1q, to one of its ligands, the most important being the CH2 domain of the IgG Fc fragment and the CH4 domain of IgM. This activation leads to the cleavage of the C4 component present in plasma into a small C4a fragment and a large C4b fragment that binds covalently to the target surface and subsequently forms the C4bC2a complex, called the "classical C3 convertase" because of its ability to cleave C3.[7] The lectin pathway is activated by the carbohydrate structure of microorganisms. The recognition protein is MBL (mannan-binding lectin) and is associated with serine proteases called MASP-1, -2 or -3. Once activated, MASP acquire the ability to cleave C4 and C2 proteins thus forming the classical C3 convertase C4b2a. The alternative pathway is activated by bacterial products, such as lipopolysaccharides, viruses, and infected, transformed or apoptotic cells; it leads to the formation of the "alternative C3 convertase". It is initiated by the association of soluble C3b with factor B allowing this latter to be cleaved by a serine protease circulating in active form in the plasma, factor D, producing the Ba and Bb fragments. While Ba is excluded from the complex, Bb remains associated with C3b to form the C3bBb complex, named the "alternative C3 convertase", capable of catalyzing the cleavage of C3 to C3b, like the C4b2a complex. Activation of the alternative pathway is capable of self-amplification, which is very important for the recognition and elimination of pathogens in the absence of specific antibodies.[8]

Activation of one of the three pathways leads to succes-

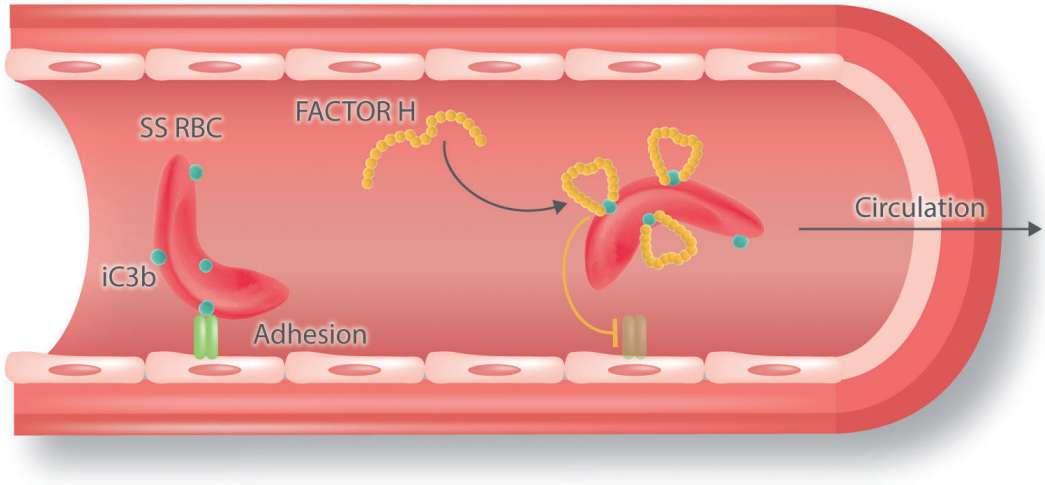

Figure 1. Anti-adhesive role of factor H in sickle cell disease. Activation of the alternative complement pathway in sickle cell disease drives accumulation of C3 cleavage fragments, iC3b in this figure, on the surface of red blood cells triggering their abnormal adhesion to endothelial cells. Factor H binds iC3b and inhibits adhesion of sickle red blood cells to the vascular wall.

sive proteolysis of plasma proteins which converges to the central protein of the complement system called C3. The activation of a C3 convertase (classical or alternative) results in the production of a fragment called C3b which can then initiate different effector pathways: opsonization, recruitment of inflammatory cells, direct destruction of infectious agents by osmotic lysis, elimination of circulating immune complexes and apoptotic cells and modulation of specific immune responses.[9] C3b can be dissociated into inactive fragments (iC3b, C3dg and then C3d) by means of plasma cofactors (factor I and factor H) or membrane co-factors (CMP, CD35 or CR1). The C3 cleavage fragments (C3b, iC3b, C3dg and C3d) can interact with different cellular receptors (CR1 or CD35, CR2 or CD21, CR3 or CD11b/CD18, CR4 or CD11c/CD18), thus modulating the response at the surface of the different immune cells: phagocytosis, presentation of the antigen and modulation of specific immune responses.[10]

As in all activation cascades, a narrow network of circulating or membrane proteins is necessary to closely regulate the different activation pathways. The regulation of the alternative pathway is ensured by factor H which plays a central role in discriminating self from non-self.[11] It controls the initiation of the C3bBb complex (alternative C3 convertase) by competing with factor B for C3b binding and accelerates the dissociation of the alternative C3 convertase.

Evidence for altered alternative complement pathway activity in the sera of SCD patients was reported in 1976 by Koethe and collaborators.[12] In 1985, Chudwin and collaborators showed that 89% of SCD patients' sera had elevated concentrations of C3b derivatives indicative of increased alternative pathway activation.[13] In 1993, Wang and collaborators showed that altered membrane phospholipid exposure of RBC is a critical element of alternative complement pathway activation in SCD patients.[14] Very recently, it was shown that cell-free heme and heme-containing microvesicles resulting from intravascular hemolysis activate complement in SCD.[15] In addition, activation of the complement is suspected to be involved in the delayed hemolytic transfusion reaction in SCD, a suspicion recently supported by good outcomes following injections of an anti-C5 monoclonal antibody (eculizumab).[16]

In this issue of Haematologica, Lombardi and collaborators investigated the activation of the alternative complement pathway as a potential contributor to increased RBC adhesion in SCD patients at steady state and during vaso-occlusive crises.[17] First, they confirmed complement activation *in vivo* by showing increased serum levels of complement activation fragment C5a in SCD patients as well as microvascular deposition of another activation marker, C5b-9, in small vessels of skin biopsies from patients but not from healthy subjects. Investigating blood cells, they found higher numbers of RBC carrying C3d-derived opsonins in SCD patients than in healthy subjects, indicative of alternative complement pathway activation occurring directly on sickle RBC. This was associated with higher proportions of RBC exposing phosphatidylserine at their surface, as previously reported.[14] The authors hypothesized that C3 fragments deposited on the RBC surface may serve as adhesive sites driving abnormal adhesion of sickle RBC to the endothelial wall. They explored this hypothesis by performing *ex vivo* adhesion assays under dynamic conditions, in which they found the expected higher levels of adhesion of sickle RBC on tumor necrosis factor-α-activated endothelial cells as compared to control RBC. Pre-incubating RBC with factor H, a soluble regulator of alternative complement pathway activation that circulates in the plasma and binds to C3b/iC3b on self cells, inhibited sickle RBC adhesion in a dose-dependent manner reaching control levels at high concentrations (Figure 1). This was the first evidence that opsonins of the alternative complement pathway, deposited on the surface of sickle RBC, may play a critical role in mediating these cells' abnormal adhesion to the endothelial wall. The authors tested the inhibitory potential of two factor H fragments and concluded that the factor H 19-20 fragment was sufficient to inhibit sickle RBC adhesion. Finally, using blocking antibodies, the authors showed data suggesting the involvement of P-selectin and Mac-1 in sickle RBC adhesion on the endothelial cell side.

Once activated, the complement pathways play an important role in the induction of tissue lesions, such as recruitment of inflammatory cells (neutrophils, monocytes, macrophages and activated lymphocytes), activation of endothelial cells and platelets, and secretion of pro-inflammatory cytokines. Such dysfunctions are frequent in many pathological situations, including autoimmune diseases,[18] ischemia-reperfusion syndrome and septic shock,[19] making the complement system a potential therapeutic target in these pathologies.[20] The study by Lombardi and collaborators reveals a new role for complement activation in the pathogenesis of SCD, particularly in the adhesive process underlying vaso-occlusive crises.[17] It paves the way for future clinical studies in which modulators of the alternative complement pathway, including factor H-based inhibitors, could be tested as potential new therapeutic options in this pathology.

References

1. Pauling L, Itano HA, et al. Sickle cell anemia, a molecular disease. Science. 1949;109(2835):443.
2. Kaul DK, Fabry ME, Nagel RL. Microvascular sites and characteristics of sickle cell adhesion to vascular endothelium in shear flow conditions: pathophysiological implications. Proc Natl Acad Sci U S A. 1989;86(9):3356-3360.
3. Manwani D, Frenette PS. Vaso-occlusion in sickle cell disease: pathophysiology and novel targeted therapies. Blood. 2013;122(24):3892-3898.
4. Piel FB, Steinberg MH, Rees DC. Sickle cell disease. N Engl J Med. 2017;376(16):1561-1573.
5. Ware RE, de Montalembert M, Tshilolo L, Abboud MR. Sickle cell disease. Lancet. 2017;390(10091):311-323.
6. Nonaka M, Yoshizaki F. Evolution of the complement system. Mol Immunol. 2004;40(12):897-902.
7. Walport MJ. Complement. First of two parts. N Engl J Med. 2001;344(14):1058-1066.
8. Walport MJ. Complement. Second of two parts. N Engl J Med. 2001;344(15):1140-1144.
9. Ricklin D, Hajishengallis G, Yang K, Lambris JD. Complement: a key system for immune surveillance and homeostasis. Nat Immunol. 2010;11(9):785-797.
10. Ricklin D, Reis ES, Mastellos DC, Gros P, Lambris JD. Complement component C3 - the "Swiss Army Knife" of innate immunity and host defense. Immunol Rev. 2016;274(1):33-58.
11. Medjeral-Thomas N, Pickering MC. The complement factor H-related proteins. Immunol Rev. 2016;274(1):191-201.
12. Koethe SM, Casper JT, Rodey GE. Alternative complement pathway activity in sera from patients with sickle cell disease. Clin Exp

Immunol. 1976;23(1):56-60.
13. Chudwin DS, Korenblit AD, Kingzette M, Artrip S, Rao S. Increased activation of the alternative complement pathway in sickle cell disease. Clin Immunol Immunopathol. 1985;37(1):93-97.
14. Wang RH, Phillips G Jr, Medof ME, Mold C. Activation of the alternative complement pathway by exposure of phosphatidylethanolamine and phosphatidylserine on erythrocytes from sickle cell disease patients. J Clin Invest. 1993;92(3):1326-1335.
15. Merle NS, Grunenwald A, Rajaratnam H, et al. Intravascular hemolysis activates complement via cell-free heme and heme-loaded microvesicles. JCI Insight. 2018;3(12).
16. Unnikrishnan A, Pelletier JPR, Bari S, et al. Anti-N and anti-Do(a) immunoglobulin G alloantibody-mediated delayed hemolytic transfusion reaction with hyperhemolysis in sickle cell disease treated with eculizumab and HBOC-201: case report and review of the literature. Transfusion. 2019 Feb 15. [Epub ahead of print]
17. Lombardi, A Matte, A.M. Risitano et al.Factor H interferes with the adhesion of sickle red cells to vascular endothelium: a novel disease-modulating molecule. Haematologica 2019;104(5):919-928.
18. Toubi E, Vadasz Z. Innate immune-responses and their role in driving autoimmunity. Autoimmun Rev. 2019;18(3):306-311.
19. Charchaflieh J, Wei J, Labaze G, et al. The role of complement system in septic shock. Clin Dev Immunol. 2012;2012:407324.
20. Dobo J, Kocsis A, Gal P. Be on target: strategies of targeting alternative and lectin pathway components in complement-mediated diseases. Front Immunol. 2018;9:1851.

A post-transplant optimized transplant-specific risk score in myelodysplastic syndromes

Mahasweta Gooptu and John Koreth

Division of Hematologic Malignancies, Dana-Farber Cancer Institute, Boston, MA, USA

E-mail: MAHASWETA GOOPTU - mahasweta_gooptu@dfci.harvard.edu

doi:10.3324/haematol.2018.214452

Allogeneic hematopoietic stem-cell transplantation (HSCT) remains the only potentially curative therapy for myelodysplastic syndromes (MDS), but treatment risks include relapse and non-relapse mortality (NRM). Whereas relapse following HSCT is typically dictated by disease-related factors, NRM is more influenced by patient- (performance status, co-morbidity, etc.) and transplant-related factors (donor type, conditioning intensity, graft-*versus*-host disease prophylaxis regimen, etc.). In order to improve transplant decision-making for the individual MDS patient, better prediction of HSCT outcomes, by including both relapse and NRM predictors in a comprehensive individualized and dynamic risk model, would be optimal. So where do we stand currently?

The prognosis of MDS has historically been based on the International Prognostic Scoring System (IPSS). For transplant decision-making, Markov models based on the IPSS have documented that MDS patients with low- and intermediate-1-risk MDS have better survival outcomes without transplant, whereas transplantation results in better survival outcomes for patients with intermediate-2- and high-risk MDS.[1,2] The Revised International Prognostic Scoring System (R-IPSS), a refinement of the IPSS, is used to prognosticate MDS at diagnosis, particularly the risk for transformation to acute myeloid leukemia,[3] and is often used as part of the decision to proceed to transplantation or not.[4]

While the IPSS and R-IPSS focus on disease features, they do not consider patient- and transplant related factors relevant to HSCT outcome. Attempts have, therefore, been made to develop MDS transplant-specific risk scores to predict survival better. These scores include the transplantation risk index developed by the *Gruppo Italiano Trapianto di Midollo Osseo* (GITMO)[4] registry using 519 patients as well as a risk score from the Center for International Blood and Marrow Transplant Research (CIBMTR)[5], using 1,519 patients. Both of these indices identified similar prognostic variables (including the R-IPSS), dividing MDS transplant recipients into four risk groups with overall survival rates ranging from 5-76%. However, these indices have not been universally adopted in current practice. While the GITMO index has not been externally validated, the CIBMTR index was validated on a distinct subset of patients from within the CIBMTR database. Gagelmann *et al.* now report on another composite risk score with better predictive ability than the existing indices.[6]

The authors compiled a cohort of 1,059 adult patients (≥18 years) with MDS from the European Society for Blood and Marrow Transplantation (EBMT) registry who underwent HLA-matched HSCT from a related or unrelated donor between 2000 to 2014. Using a Cox proportional hazards model they identified seven variables with significant impact on overall survival: age >50 years, matched unrelated donor, Karnofsky Performance Status <90%, very poor cytogenetics or monosomal karyotype, positive cytomegalovirus status of the recipient, peripheral blood blasts >1% and platelet count ≤50 x 10^9/L. Of these, age and cytogenetic risk were the strongest predictors of survival, based on hazard ratios for death, and given more weight than the other factors in the final score. Four prognostic groups were identified (low, intermediate, high and very-high risk) with overall survival rates of 68.7%, 43.2%, 26.6% and 9.5%, respectively.

How does the EBMT score described in the paper by Gagelmann *et al.* compare to the prior CIBMTR and GITMO scores as well as the R-IPSS itself? One approach would be to compare the concordance or c-statistic (measured as area under the receiver operating curve) of the different indices. The c-statistic is used to compare the goodness of fit of logistic regression models with values that range from 0.5 to 1.0. A c-statistic of 0.5 indicates the predictive ability of the index is no better than chance while a c-statistic in the 0.7-0.8 range has reasonable discriminatory power. Looking at the c-statistic following cross- validation, the EBMT transplant risk index scored 0.609 (95% confidence interval: 0.588 to 0.629), which was better than the CIBMTR (0.555) and GITMO (0.579)