been evaluated. Would this approach be sufficient to overcome the poor prognosis of these patients, or are additional agents needed? Lessons learned from therapies used in relapsed or refractory (R/R) ALL patients have shown that some therapies, such as CD19-directed chimeric antigen receptor-modified (CAR) T-cells, are effective and safe for treatment of CNS leukemia (either with or without concurrent medullary relapse),[9] and these therapies likely will be used as part of the treatment of newly diagnosed patients in the near future, providing an additional strategy of CNS therapy or high-risk prophylaxis. CAR T-cells directed against CD7 or CD5 are actively being investigated in R/R T-ALL and lymphoblastic lymphoma. Data from phase 1 and 2 trials show similar efficacy to that obtained with CD19 CAR T cells for R/R B-ALL, with the same possible implications for CNS prophylaxis or therapy in T-ALL.[10] Apart from cellular therapies, other agents capable of penetrating the CNS (eg, MEK inhibitors or drugs targeting the PI3 kinase pathway, among others) are being actively investigated. Better approaches clearly are needed to treat CNS-3 T-ALL, especially if omission of CRT is a priority. Early intensive therapy to maximize eradication of CNS leukemia should help prevent future CNS disease relapses.

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CLINICAL TRIALS AND OBSERVATIONS

Comment on Prata et al, page 1812

PNH and complement gene variants

Antonio M. Risitano | AORN San Giuseppe Moscati

In this issue of *Blood*, Prata et al report on rare genetic variants of the *complement factor H (CFH)* gene, which are overrepresented in patients diagnosed with paroxysmal nocturnal hemoglobinuria (PNH) and also seem to affect hematologic response to standard anti-C5 treatment with eculizumab.[1]

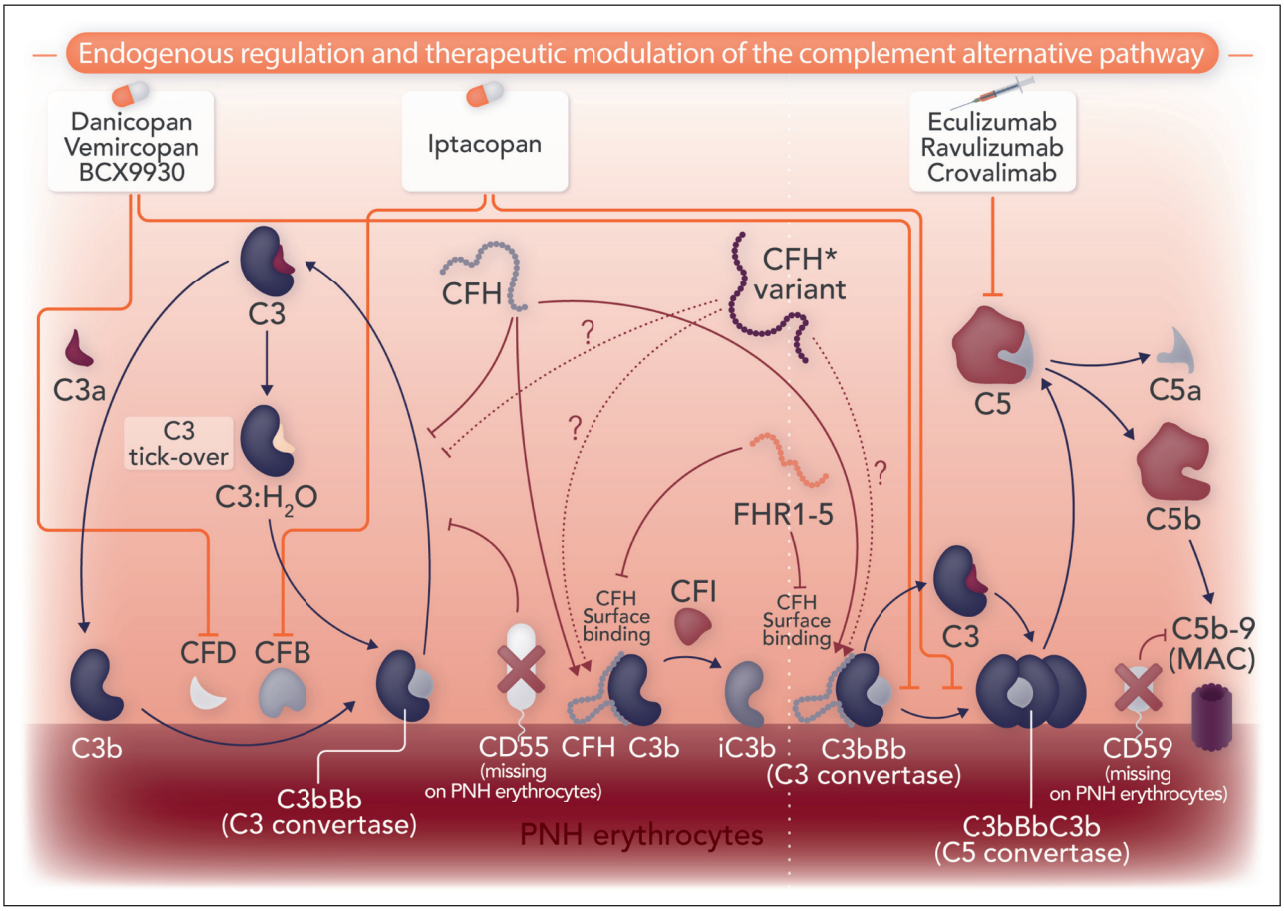
Germline variants in genes coding for different complement components (eg, C3, complement factor B, etc) or complement regulators (eg, CFH, complement factor I [CFI], etc) have been associated with different complement-mediated diseases, such as atypical hemolytic-uremic syndrome, C3 glomerulopathy, age-related macular degeneration, and transplant-associated microangiopathies. In PNH, the impairment of complement regulation is due to the lack of the 2 surface complement regulators CD55 and CD59.[2] Systematic studies on germline variants of complement genes in PNH are rare. The only well-documented variants are the C5 polymorphism p.Arg885His, which prevents eculizumab binding, thereby conferring intrinsic resistance to this anti-C5 agent,[3] and the hypomorphic variant of CR1, which is associated with increased surface C3 opsonization and poor response to eculizumab due to extravascular hemolysis.[4]

In their work, Prata et al systematically screened a sizeable population of 84 PNH patients by next-generation sequencing, looking for germline variants in the complement genes *CFH*, *CFI*, *membrane cofactor protein*, and *C3*. Both common and rare variants were found. Although common variants were found at expected frequencies, rare variants of *CFH* were found at significantly higher frequencies than in healthy individuals, suggesting that they may play a role in PNH pathophysiology. In the second part of their study, Prata et al investigated the possible impact of these germline variants on disease outcomes, including pretreatment presentation and response to eculizumab. Whereas the most common variants *CFH* p.His402Tyr and *CR1* p.His1208Arg were not associated with a different disease presentation or response to eculizumab, rare *CFH* germline variants were associated with statistically significant worse event-free and failure-free survival. Indeed, all patients carrying rare *CFH* variants had red blood cell transfusion, thrombosis, or increase in eculizumab dose within 15 months from treatment initiation, suggesting a functional role for

these gene variants. Development of aplastic anemia or evolution to a myeloid malignancy was not associated with either common or rare complement gene variants.

It is not easy to reconcile the finding of an increased frequency of rare germline *CFH* variants in PNH patients. Indeed, the well-established dual pathophysiology of PNH implies that (1) a *phosphatidylinositol N-acetylglucosaminyltransferase subunit A* (*PIGA*) mutation must occur in somatic hematopoietic stem cells (HSCs) and that (2) these *PIGA*-mutated HSCs expand over normal hematopoiesis owing to an immune privilege.[5] This then suggests that 1 of these 2 independent events may be more frequent in patients carrying these *CFH* variants. It does not seem possible that the inherited *CFH* variant confers a higher somatic mutation rate to affected cells, and, in any case, genetic instability of the *PIGA* gene does not contribute to PNH pathogenesis.[6] Thus, the only alternate possibility, albeit still unlikely, is that these *CFH* variants might increase the likelihood of the T cell–mediated aplastic anemia underlying the expansion of *PIGA*-mutated HSCs. The observation that rare *CFH* variants may impact the response to anticomplement treatment is extremely important, and one may try to interpret their findings based on our knowledge of complement regulation. Endogenous complement regulation is largely individual owing to broad inherited heterogeneity; this interindividual variability may serve as the permissive environment to develop some complement-mediated diseases. But this inherited variability may also shape the clinical phenotype of diseases with an independent pathophysiology, such as PNH, in which complement derangement is the consequence of the disease but not its actual cause.

Endogenous, physiologic regulation of the complement cascade is based on both fluid-phase proteins (CFH and CR1) and membrane-bound proteins (monocyte chemoattractant protein, CD55, and CD59). The pleiotropic role of CFH in complement regulation becomes even more crucial when key players are missing, such as CD55 and CD59 in PNH (see figure). In vitro data suggest that



Pleiotropic role of CFH in complement regulation. Simplified illustration of complement activation/regulation on PNH erythrocytes. Spontaneous, continuous hydrolysis of C3 results in low-grade activation of the alternative pathway through the generation of the fluid phase C3 convertase $C3H_20$:Bb; this activates further C3, with surface-bound C3b leading to surface-bound C3 convertase. This initial step is usually disabled on human cells by CD55, but on PNH erythrocytes, the lack of CD55 results in surface complement activation. PNH erythrocytes have continuous and uncontrolled generation of C3 convertase, which in turn generates surface C5 convertase, which can cleave C5, enabling the terminal pathway of the complement, which is not blocked by CD59 (missing on PNH erythrocytes). As a consequence, PNH erythrocytes undergo continuous complement-mediated lysis owing to the formation of its effector membrane attack complex (MAC). CFH is a fluid-phase complement regulator that prevents the formation and promotes the decay of the C3 convertases. CFH also contributes (as cofactor of CFI) to degrading activated C3 (C3b) into inactivated C3 (iC3b). Inherited variants of the *CFH* gene may account for differences in CFH activity, owing to both variants affecting the CFH complement-regulatory domain or its membrane-binding domain. Furthermore, at least 5 FH-related proteins (FHR-1 to FHR-5) may affect CFH activity by competing with its surface binding; and polymorphisms of FHR genes are quite frequent, with possible functional consequences. In PNH, owing to the lack of the membrane complement regulators, subtle differences in CFH activity may eventually lead to clinical consequences. When the terminal complement is inhibited (eg, by anti-C5 agents such as eculizumab) and MAC-mediated intravascular hemolysis is prevented, these differences may account for different degree of proximal complement activation, leading to different extent of C3-mediated extravascular hemolysis.[4] Professional illustration by Luk Cox, Somersault18:24 (based in part on Ricklin and Cines[11]).

CFH may cooperate with CD55 and CD59 in protecting erythrocytes from complement-mediated lysis, and it may partially protect PNH erythrocytes from immediate lysis.[7] Recently, impaired CFH recruitment on PNH erythrocytes has been implicated in the pathophysiology of PNH.[8] However, the physiologic concentrations of CFH are unable to rescue PNH erythrocytes from lysis,[7] and prevention of MAC-mediated lysis can be observed only with supraphysiologic concentrations (approximately 10-fold higher) or with engineered proteins merging CFH regulatory domains with C3-binding structures, which may increase its affinity for host surface (eg, TT30 and mini-FH).[9] These observations seem in agreement with the finding of this study, given that *CFH* variants with hypothetical impaired function may not necessarily impact the disease phenotype, owing to the dominant role of CD55 and CD59 deficiency. In contrast, functional differences in complement regulation may emerge once the lack of CD59 is overcome by eculizumab. In this scenario, even subtle differences in the regulation of the alternative pathway may lead to different extent of surface C3 activation and C3d deposition,[10] eventually accounting for different degrees of C3-mediated extravascular hemolysis (as demonstrated for CR1[4] but still not proven for these CFH variants). Interestingly, as novel strategies of complement therapeutics are in their advanced development,[9] the next question is how CFH variant may affect the efficacy of proximal complement inhibitors. Because all proximal inhibitors target key components of the alternative pathway, aiming to intercept the same crucial step regulated by CFH (ie, the C3 convertase activity), one would anticipate that functional differences in CFH (and CR1) activity may remain neutral. However, functional differences in CFH (and CR1) activity may result in pharmacodynamic differences leading to breakthrough hemolysis (the most feared complication when proximal inhibitors are used as monotherapy).[9]

In conclusion, it seems conceivable that modest differences in CFH activity may affect the clinical presentation of PNH and eventually shape the hematologic response to anticomplement treatment. Further functional data are needed, especially to anticipate how inherited variants of CFH (and of other complement regulator genes) may impact the response to the novel complement proximal inhibitors.

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IMMUNOBIOLOGY AND IMMUNOTHERAPY

Comment on Penter et al, page 1817

Location, location, location

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In this issue of *Blood*, Penter et al[1] paint a picture of a leukemia bone marrow microenvironment that is immunologically distinct from that of many solid tumors. They present hypothesis-generating results from cutting-edge correlative studies on samples from a phase 1 clinical trial (NCT02890329) combining the CTLA-4 blocking monoclonal antibody ipilimumab with decitabine for patients with relapsed/refractory acute myeloid leukemia or myelodysplastic syndromes. Effective therapies are greatly needed for this patient population, and immune checkpoint blockade has yielded remarkable successes in solid tumors.[2,3] The trial was motivated in part by responses to ipilimumab monotherapy seen in patients with relapsed myeloid neoplasms, primarily extramedullary, after allogeneic hematopoietic cell transplantation (HCT).[4] In the current cohort of patients with predominantly bone marrow disease, the activity of combination therapy was modest in both post-HCT and HCT-naïve individuals and appeared to be primarily driven by decitabine-induced cytoreduction[5]; however, the authors take advantage of the extensive samples collected to explore the immunobiology underlying response and nonresponse to CTLA-4 blockade.

Among their findings were that ipilimumab treatment led to the recruitment of $CD4^+$ regulatory T cells to the bone marrow, in contrast to the recruitment of $CD8^+$ T cells observed previously in patients treated with CTLA-4 blockade