that clonally expand in an “adaptive” manner as well as innate-like cells that home to tissues such as epidermis, dermis, intestine, lungs, and uterus. They are not restricted to recognize peptides bound to major histocompatibility complex (MHC) molecules, and the Vγ9 Vδ2+ subset can recognize metabolites of the mevalonate pathway on the surface of tumor cells and kill acute myeloid leukemia blasts (see figure). Finally, reactivation of human cytomegalovirus (CMV) after allogeneic stem cell transplantation is associated with the in vivo expansion of Vδ2+ γδ T cells that react against CMV-infected cells, and such expansion is correlated with clearance of the virus.9

In this issue, Airoldi and colleagues characterize functional and phenotypic reconstitution of γδ T cells in 27 children with malignant or nonmalignant disorders treated with haplo-HSCT using grafts depleted of αβ T cells and CD19+ B cells.1 Compared with recipients of grafts positively selected for CD34+ cells, recipients of TCRαβ- /CD19- grafts had higher percentages of γδ T cells among total T cells and Vδ2+ cells among γδ T cells at 3 months after transplantation, demonstrating that selective retention of the γδ subset in the graft affects the kinetics and pattern of immune reconstitution following haplo-HSCT. Perhaps most relevant to clinicians, the reconstituting γδ T cells were found to have lytic activity against human cytomegalovirus and against acute myeloid leukemia blasts. The Vδ1+ subset expanded significantly in the 15 patients who experienced CMV reactivation and contained more terminally differentiated cells, suggesting that this subset was actually fighting the virus in vivo. Furthermore, ex vivo–expanded Vγ9- Vδ2+ T cells efficiently killed acute myeloid leukemia or acute lymphoid leukemia blasts that had been exposed to zoledronic acid (ZOL), an aminobisphosphonate that induces the upregulation of isopentenyl pyrophosphate (IPP), a ligand of Vγ9- Vδ2+ T cells, by inhibiting a key enzyme of the mevalonate pathway.9 Taken together, the results suggest that selective depletion of TCR-αβ+ T cells from HLA-haploidentical peripheral blood grafts enhances the functional and phenotypic reconstitution of TCR-γδ+ T cells and offers the possibility of reducing the risk of leukemia relapse by treating recipients with aminobisphosphonate drugs. Additionally, the ability of γδ T cells to kill both CMV-infected and leukemic targets may account for the possible correlation between CMV reactivation and a decreased risk of leukemia relapse10 (see figure).

How will the results of this study impact allogeneic stem cell transplantation? It is perhaps too early to tell. Follow-up studies of haplo-HSCT with TCR-αβ/CD19 depletion will need to focus on the correlation of immune reconstitution with the incidence of CMV reactivation and leukemia relapse. The possibility of reducing relapse by treating recipients with ZOL is intriguing but remains speculative. What the study does accomplish is to demonstrate the potential to impact immune reconstitution and graft-versus-leukemia effects through intelligent graft engineering. As haplo-HSCT gains in acceptance, more studies like this one will be needed to define exactly which cells are needed in the graft and why, and which ones can be thrown away.

Conflict-of-interest disclosure: The author declares no competing financial interests.

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Comment on Schramm et al, page 2359

Mutations in complement C3 from aHUS patients

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In this issue of Blood, Schramm et al demonstrate that the majority of mutations in complement C3 identified in atypical hemolytic uremic syndrome (aHUS) patients cause dysregulation in the alternative pathway of complement.1

The underlying molecular mechanism is shown to be decreased cofactor activity of complement regulators. The resulting increased formation and stability of the C3 degradation product C3b are translated into increased C3 deposition onto endothelial cells, and the majority of aHUS patients carrying mutations in C3 were observed to exhibit low levels of plasma C3. The mechanistic outcome of the study is the very similar effects observed for the majority of the C3 mutations on the cofactor activity of factor H (FH) and membrane cofactor protein (MCP), suggesting that their binding surfaces on C3b are strongly overlapping. C3b is generated by proteolysis of C3 on complement activation. It recruits factor B (FB), which is subsequently cleaved into Bb, and thus the alternative pathway (AP) C3

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The impaired F1 degradation of mutant C3 molecules identified in aHUS patients can be rationalized by crystal structures of C3b-FH complexes. (A) The structure of C3b in complex with FH CCP domains 1 to 46 combined with that of C3d in complex with FH CCP19-20 and a model GAG. C3d is a final degradation product of C3b, comprising basically only the thioester-containing domain (TED). Notice that the 2 extremes of FH may bind to the same C3b molecule with the intervening CCP domains looping out, but simultaneous interaction of FH with 2 activator-bound C3b molecules cannot be excluded either. FH CCP1-4 is recognized by 4 MG domains, the CUB domain, and the TED in C3b, whereas FH CCP19-20 is only contacting the C3b TED. An additional complication is that FH CCP19 interacts with the C3b TED, whereas FH CCP20 can interact with host cell membrane-linked GAG as indicated here or a nearby molecule of C3d. (B) A large number of mutant C3 residues identified in aHUS patients by Schramm et al give rise to lower cofactor activity of FH and MCP (colored circles). They are all located directly in or near to the binding interface for FH on C3b (blue residues). The figure was prepared with PyMOL by the combination of Protein Data Bank ID codes 4ONT7 and 2WII.

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convertase C3bBb is formed, and a positive feedback amplification loop leads to further convertase assembly. This amplification is suppressed on healthy host cells by complement regulators. One of the best studied complement regulators is FH, which binds to host surfaces through sialic acid, heparin, and sulfated glycosaminoglycans (GAGs) and in this manner attenuates the AP on host cells. FH accelerates convertase decay by irreversibly dissociating Bb from the C3 convertase and may subsequently serve as cofactor for the serine protease factor I (FI), which cleaves C3b into iC3b, which is unable to rebind FB. Two other cofactors are MCP and complement receptor 1 (CR1), which in contrast to the soluble FH are anchored in host cell membranes.

The pathogenesis of aHUS leading to endothelial damage and microvascular thrombosis has long been linked to impaired complement regulation. Genetic defects leading to mutations in the components of the alternative pathway or FH autoantibodies have been identified in roughly 60% of aHUS patients. Mutations are commonly found in FH and MCP, whereas mutations within C3 and FI occur at lower frequencies. Patients with C3 mutations develop severe disease, leading to end-stage renal failure in 55% to 65% of cases. The terminal complement pathway is required for the induction of endothelial lesions in aHUS. Schramm et al constructed their database of mutations by sequencing the C3 gene in almost 1300 aHUS patients from France, Italy, the United Kingdom, and the United States. In combination with already published mutations, they could list 48 different genetic changes in C3 associated with aHUS. Using the C3b-containing crystal structure, the authors could hypothesize why the mutations conferred AP dysregulation. A striking finding was that 27 of the mutated residues map within or close to the binding sites of FH CCP domains 1 to 4 (CCP1-4) or CCP19-20 on C3b (see figure panel A), suggesting that these mutations affect the cofactor activity of FH, leading to decreased conversion of C3b to iC3b. A similar explanation could be offered for a single mutant residue close to a FI cleavage site in the complement C1r/C1s, Uegf, Bmp1 (CUB) domain of C3b. To experimentally investigate how the C3 mutants induce dysregulation of the AP, the authors prepared an impressive collection of 23 mutant recombinant C3 molecules. One caveat concerning this collection is that, due to the procedure for recombinant expression, these C3 variants do not contain the internal thioester found in circulating C3. Instead the recombinant proteins were in the form of the naturally occurring thioester tick-over product C3(H2O). This adopts a C3b-like conformation and is capable of forming a fluid phase AP C3 convertase with FB, and C3(H2O) is also subject to FI degradation. Hence, although the recombinant C3 variants still contain the anaphylatoxin domain released as C3a from C3 by C3 convertases, they represent a valuable model system for examining how mutations in C3 affect binding of cofactors and the subsequent conversion of C3b to iC3b by FI. However, it should be kept in mind that this model system only approximates the degradation of activator surface bound C3b to iC3b.

The recombinant C3 variants were evaluated for their binding to the immobilized cofactors FH, MCP, and CR1 through surface plasmon resonance. Of the 23 mutants evaluated, 17 displayed decreased binding to ≥1 of the 3 regulators compared with wild-type (WT) C3. Intriguingly, the authors observed a very strong correlation between the binding of the C3 variants to FH and MCP, suggesting that their C3 binding modes are very similar. In contrast, binding to CR1 was much less affected. The impact of the mutations was further investigated by examining cofactor activity when mutant C3 acted as a substrate for FI. For 14 C3 variants examined, 12 showed diminished cofactor activity for MCP or FH compared with degradation of WT C3, whereas both regulators displayed a lowered cofactor activity against 8 C3 variants (see figure panel B). The C3 mutations associated with lower cofactor activity were located in or around the binding sites for FH CCP2-4 and CCP19-20. In contrast, CR1 cofactor activity was much less affected by mutations in C3.

An impaired degradation of C3b to iC3b predicts an increased C3 deposition on self-tissue from patients. To validate this, Schramm et al measured the deposition of C3 onto human umbilical vein endothelial cells (HUVECs) by FH-depleted serum. One patient serum contained C3 with the A1072D mutation, which in the FI degradation assay gave rise to a lower cofactor activity of both FH and MCP, whereas another patient serum had C3 with the mutation R139W, which only gave lower cofactor activity for MCP. As predicted, with serum containing C3 A1072D, much more exogenous FH was required to decrease C3 deposition on HUVECs compared...
with serum containing either C3 R139W or WT C3.

The work of Schramm et al further strengthens the idea that the mechanisms keeping the complement AP in check on host cells are compromised in aHUS patients. The authors also demonstrate in an elegant manner how the combination of genetic data from a large number of patients, functional assays, and structural biology paves the way for personalized medicine.

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Comment on Paiva et al, page 2370

Macro-Quest

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In this issue of Blood, Paiva et al provide important information on the cell of origin in Waldenstrom macroglobulinemia (WM), a longstanding puzzle due to conflicting and incomplete data.1 W is not Hodgkin lymphoma. Both are eponymous diseases that carry the name of the physician who initially described them. In both, there has been longstanding confusion about the cell of origin, but the similarity mostly stops there. In Hodgkin lymphoma, the finding that malignant Reed-Sternberg cells originate from B cells was not established until the 1990s, with the use of single cell analysis.2 Until then, we were not fully sure if the malignancy arose from B cells, T cells, or the monocyte lineage. In WM, we have had no such problem: the fact that the malignant cells were lymphoid in origin and secreted immunoglobulin has been known almost from the outset. However, the problem in WM has been more subtle and revolves around the precise nature of the B-cell lineage differentiation stage (from a pregerminal center B cell up to fully differentiated plasma cells) that is responsible for the malignancy. Paiva et al clearly show that WM, and its precursors immunoglobulin-M (IgM) monoclonal gammapathy of undetermined significance (MGUS), and smoldering WM, arise from CD25+ CD22+low activated B lymphocytes based on sensitive multiparametric flow cytometry and gene expression profiling (GEP) studies.3 They also confirm that the clonal cells in WM are almost uniformly negative for CD5, CD10, CD11c, and CD101. These results are not just of historic and biologic relevance, but also provide a unique immunophenotype that can be used to discriminate patients with WM from other related B-cell lymphoproliferative disorders. This would be of particular value in reaching the correct diagnosis in WM patients with nonmutated MYD88, as well as those with other related (non-WM) B-cell disorders that harbor the MYD88 mutation.

Most patients with WM have a recurrent somatic mutation, L265P, involving the MYD88 gene.4,5 Subsequent studies have found that this mutation is also present in the precursor stages of the disease, including IgM MGUS, suggesting that MYD88 L265P is an early event and that additional cytogenetic changes are necessary for the evolution of the malignancy.5 In line with this, Paiva et al demonstrate that certain cytogenetic copy number abnormalities including +4, +12, del(6q23.3-6q25.3), and +18q21.1 are more prevalent in patients with malignant transformation to WM and need further study. It is, however, interesting that as in myeloma, none of these changes resulted in a major shift in GEP. Although larger series of patients are needed to confirm these findings, they found no significant differences on GEP between the clonal cells of WM and IgM MGUS. In contrast, clonal cells of WM and IgM MGUS displayed several deregulated genes in comparison with normal resting B cells, and these changes involved many signaling pathways. Some of these genes and signaling pathways should be explored to determine whether it is possible to therapeutically revert the WM clone into a resting phenotype.

Most importantly, the paper also clarifies semantics related to WM that have been the source of much confusion and controversy for a long time. Some guidelines have suggested that any level of infiltration of the bone marrow with clonal WM cells with any level of IgM monoclonal protein be termed WM.6 Such a definition would make WM the most common malignancy in the world because an IgM MGUS is present in ~1% of the general population over the age of 50, and as the authors show, infiltration of the bone marrow by clonal cells can be demonstrated in most cases of IgM MGUS using sensitive cytometry. Clonal cells detected in IgM MGUS are indistinguishable from the clonal cells in WM using current techniques in a reliable manner. We know that patients with IgM MGUS defined as <10% clonal bone marrow involvement and no end-organ damage have a low risk of progression to symptomatic WM (1.5%/year)7-8 and have an overall survival similar to or even better than an age-matched general population.9 Thus, I agree with the
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