continued up to 24 hours. Accumulation of neutrophils was many times higher in animals with CGD than in wild-type animals. In a similar manner, the concentration of LTB4 increased significantly in CGD animals. By using neutrophil-depleted mice, they verified that neutrophils are responsible for the majority of LTB4 production in the lungs, although other leukocytes are also capable of synthesizing LTB4. To prove the concept, Song et al show that the zymosan-induced hyperinflammation in CGD mice can be almost completely prevented by inhibiting LTB4 synthesis or by blocking LTB4 receptors. With these findings, the important role of neutrophil-derived LTB4 in induction of sterile lung inflammation in CGD mice is clearly supported.

Interestingly, neutrophil recruitment to the lungs was also significantly reduced at 24 hours if the inhibitor of LTB4 synthesis was administered 8 hours after the zymosan treatment. However, if the inhibitor was administered 24 hours after the animal had been exposed to zymosan, it no longer had a protective effect. These findings reveal that LTB4 has a crucial role in development of the aberrant inflammation only in the early period, and they provide another example of the sequential participation of different mediators in neutrophil recruitment.[9]

The new data communicated by the authors clearly indicate the pathogenetic role of excess LTB4 produced as a result of the enhanced calcium signaling in CGD cells, a process independent of, but adding to, the problem of deficient ROS-related elimination of infectious microbes. However, LTB4 synthesis may not be the only calcium-dependent process that is enhanced because of the missing electrogenic function of Nox2, and may potentially contribute to the complex pathology in CGD.

As with all animal experiments, and specifically in the field of immunology, the relevance to the human disease is a final major question. *Aspergillus* is a typical microbe that causes serious infections, most often pneumonia with pyogranulomatous infection and abscess formation in patients with CGD[2]; thus, investigation of the effect of yeast cell wall extract is highly relevant. Production of LTB4 is about 10 times higher in murine neutrophils than in human neutrophils, but the supplemental data that accompany the article by Song et al confirm that direction and proportion of changes are the same. LTB4 was found to play an important role in directing recruitment of human neutrophils in general and in pulmonary infiltration in particular. The final proof, however, will come from clinical experience when drugs that attack the LTB4-LTB4 receptor axis or the neutrophil calcium channels are tested in the management of CGD patients.

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CLINICAL TRIALS AND OBSERVATIONS

Comment on Röth et al, page 912

A complementary new drug for PNH

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In this issue of *Blood*, Röth et al show in a phase 1/2 trial that crovalimab, a subcutaneously administered monoclonal antibody that targets C5, is safe and effective in treating paroxysmal nocturnal hemoglobinuria (PNH).[1] Crovalimab targets a C5 epitope that is different from the IV C5 inhibitors, eculizumab and ravulizumab. Crovalimab is administered subcutaneously every 4 weeks.

PNH is a clonal hematopoietic stem cell disorder caused by somatic mutation of the X-linked gene, *PIGA*.[2] The *PIGA* gene product is needed for biosynthesis of glycosylphosphatidylinositol (GPI)-anchored proteins; hence, the PNH stem cell and all of its progeny are missing GPI-anchored proteins. Two GPI-anchored proteins, CD55 and CD59, are complement regulatory proteins. CD59 works downstream of C5 to regulate terminal complement, and CD55 works upstream of C5 and regulates the C3 and C5 convertases. Failure to regulate complement on the surface of PNH blood cells leads to intravascular hemolysis, fatigue, and thrombosis. Until recently, thrombosis was the leading cause of death from PNH, and the median survival was 15 to 20 years.[3]

In 2007, eculizumab became the first US Food and Drug Administration (FDA)-approved drug for the treatment of PNH.[4] Eculizumab is a humanized, monoclonal antibody that binds C5 and prevents cleavage of C5 to C5a and C5b by the C5 convertases. Without C5b, the membrane attack complex does not form, and PNH red cells are protected from intravascular hemolysis. For the first 5 weeks, eculizumab

is administered IV every 7 days and then every 2 weeks thereafter. The drug stops intravascular hemolysis, stabilizes hemoglobin levels without the need for blood transfusions in 80% of patients, improves quality of life, and mitigates the risk for thrombosis. Life expectancy for PNH patients on eculizumab is comparable to age-matched controls.[5] Drawbacks of the drug are cost, frequent IV infusions, and extravascular hemolysis from failure of eculizumab to control C3b deposition on PNH red cells due to the absence of CD55. This failure to limit C3 fragments from depositing on PNH red cells leads to opsonization in the liver and spleen.[6] PNH patients on eculizumab therefore have a chronic, variably compensated, extravascular hemolytic anemia. In addition, PNH patients on eculizumab may experience "breakthrough" intravascular hemolysis due to failure of the drug to sufficiently lower free C5 levels for the full 2 weeks (pharmacokinetic breakthrough) and/or from complement amplifying conditions, such as infection, pregnancy, and surgery (pharmacodynamic breakthrough). Last, there is a rare C5 polymorphism (R885H) found in 3% of the Japanese population that prevents eculizumab from binding to C5.[7] Another C5 inhibitor, ravulizumab, has recently been approved for treatment of adults with PNH by the FDA and the European Medicines Agency.[8] Ravulizumab is an Fc modification of eculizumab, with a longer half-life allowing maintenance dosing every 8 weeks. Ravulizumab is noninferior to eculizumab. It targets the same C5 epitope as eculizumab; thus, it does not prevent extravascular hemolysis in PNH. Ravulizumab effectively eliminates pharmacokinetic breakthrough but not pharmacodynamic breakthrough.[9]

Crovalimab employs sequential monoclonal antibody recycling technology to inhibit terminal complement at C5. Simply, this is a long-acting anti-C5 monoclonal that uses pH-dependent binding to target C5. This innovative approach promotes degradation of C5 in lysosomes and recycling of the monoclonal antibody to the plasma. This leads to sustained C5 blockade with low-volume subcutaneous injections. The study by Röth and colleagues, sponsored by Hoffmann-La Roche and Chugai Pharmaceutical, is a 3-part open-label adaptive phase 1/2 study of crovalomib. Part 1 explored pharmacokinetics and pharmacodynamics in healthy controls. Part 2 administered crovalomib to 10 treatment-naive PNH patients, and part 3 gave the drug to 19 eculizumab-treated patients. In part 2, patients were loaded with 2 doses of IV crovalimib followed by weekly subcutaneous crovalimab, and in part 3, patients received an IV loading dose of crovalimab 2 weeks after the last dose of eculizumab and were then randomized 1:1:1 to 1 of 3 subcutaneous doses (680 mg every 4 weeks; 340 mg every 2 weeks, or 170 mg weekly) for 20 weeks. The authors demonstrate that subcutaneous dosing of crovalimab at 680 mg every 4 weeks is safe and effective for treating PNH, even for patients with the C5 (R885H) polymorphism that prevents binding of eculizumab. The drug appears to prevent pharmacokinetic breakthrough but does not prevent pharmacodynamic breakthrough or stop extravascular hemolysis associated with complement amplifying conditions.

In summary, crovalimab represents a modest advance in treating PNH. For sure, some PNH patients will prefer subcutaneous dosing every 4 weeks (cravolimab) over IV infusions every 8 weeks (ravulizumab); however, neither of these drugs prevent extravascular hemolysis or pharmacodynamic breakthrough. Proximal complement inhibitors (upstream of C5) are in clinical development and may ultimately prove to be superior to C5 inhibitors in controlling intravascular and extravascular hemolysis in PNH.

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