In the study by Risitano et al, the investigators tested the efficacy of several small-molecule complement inhibitors in hemolysis assays using erythrocytes from patients with PNH. The inhibitors tested are all derivatives of compstatin, an agent that prevents cleavage and activation of the complement protein C3. The rationale for these studies is twofold. First, the compstatin derivatives prevent opsonization of PNH erythrocytes with C3b as well as insertion of C5b-9 in the erythrocytes. Second, although other complement inhibitors have been tested in similar systems, most anti-complement therapeutics are large proteins, and their use is limited by immunogenicity of the molecules and high costs of production. The authors suggest that large-scale production costs for the various forms of compstatin could be as low as several dollars per dose. The authors present results demonstrating that these agents prevent C3 deposition and lysis of erythrocytes from PNH patients. They also performed pharmacokinetic experiments in nonhuman primates treated subcutaneously with one of the compstatins and show that this strategy achieved therapeutic levels of the drug.

One of the interesting findings in this study is that the levels of plasma C3 increased in nonhuman primates treated with IV pegylated-Cp40 (a long-acting compstatin derivative). This effect may be due to decreased turnover of C3, and it raises the possibility that the level of compstatin (and other C3 inhibitors) needed to prevent hemolysis could increase over time. Similarly, C3 is part of the acute-phase response and the dose of drug needed to maintain complement inhibition may increase during acute illness. Although the authors carefully measured the levels of the compound in treated animals, they did not test functional inhibition of the complement cascade, so the data presented do not confirm that the achieved levels are sufficient to fully prevent hemolysis. The benefits of fully inhibiting the complement system in patients with PNH must also be weighed against the increased risk of infection that such treatment entails. Blockade of the complement cascade at the level of C5 could theoretically pose a lower risk of infection than does blocking complement at the level of C3. The current study does not address this issue.

Because PNH is a lifelong disease, the ideal drug for this condition would be safe, inexpensive, easily administered, and nonimmunogenic with repeated exposure. Eculizumab has provided a major advance in the treatment of PNH, but it is expensive and does not completely prevent hemolysis. The compstatin derivatives tested in the current study may overcome some of these shortcomings, although these compounds still need to be evaluated in human patients before their benefits can be fully assessed. PNH is a rare disease, so studies in this patient population are not easy to conduct. Yet, the nature of PNH also makes it advantageous for testing the efficacy of new complement inhibitors in vivo: complement activation is central to the disease pathogenesis and there are clear readouts of disease activity. New drugs that prove effective in PNH will be useful for treating other complement-mediated disorders too, so efforts to develop these new therapies will have benefits beyond PNH.

Conflict-of-interest disclosure: J.M.T. is a consultant for Alexion Pharmaceuticals, Inc.

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Comment on Tong et al, page 2026

Shedding light on the asparaginase galaxy

Carmelo Rizzari 1

1 SAN GERARDO HOSPITAL

In this issue of Blood, Tong et al have reported that therapeutic drug monitoring (TDM) of asparaginase (ASP) activity levels in plasma may be an important tool for the optimization of its therapeutic effects in pediatric acute lymphoblastic leukemia (ALL).1

The main and commendable aim of the study conducted by Tong et al was the improvement of ASP therapeutic effects by preventing the onset of allergic reactions and silent inactivation (SI) in children with ALL. These 2 phenomena are in fact among the most

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important factors encountered leading to ASP treatment failure. To achieve this goal (ie, improvement of ASP therapeutic effects), the authors routinely switched their patients to receive pegylated ASP (PEGASP) during consolidation (second exposure), a treatment phase delivered after native Escherichia coli ASP had been given in induction (first exposure). Erwinia chrysanthemi ASP was given instead of PEGASP in patients with allergic reactions or SI occurring during its administration.

To better understand this study, we need to go back in time a little. The antileukemic properties of ASP were discovered in the early 1960s, and since then, ASP has been considered an essential component of modern chemotherapeutic regimens for ALL. Its use has in fact been associated with substantial improvements of cure rates in ALL, especially in children. Currently, 3 different types of ASP are available, 2 derived from the bacteria E coli (the native and the PEGASP forms) and another native form derived from Erwinia C. These 3 products have specific half-lives and distinct immunogenic profiles, and these properties do not make them easily interchangeable. The antileukemic effects of ASP depend on its mechanism of action: ASP in fact hydrolyzes asparagine (ASN) into aspartic acid + ammonia, and this leads to a prolonged and sustained depletion of circulating concentrations of the amino acid. Leukemic blasts, unlike healthy cells, are unable to produce ASN individually because they lack the ASN synthetase enzyme; for this reason, when extracellular ASN disappears, these cells have no chance to survive. ASP treatment effects may be monitored by measuring ASN depletion in plasma or, in an even simpler way, by measuring ASP activity levels in plasma by using relatively simple assays. ASP levels are in fact inversely correlated with ASN depletion. However, resistance to ASP may arise for clinical or biological reasons: among the most frequent clinical reasons are pancreatitis, thrombosis, and allergy. Allergy is by far the most frequent cause of treatment delays, product shift, or treatment discontinuation. Among the biological reasons, one should keep in mind that ASP is a nonhuman protein and its administration in humans may generate an immune response. Antibodies directed against ASP have been associated with the onset of clinical allergy and also with the so-called SI phenomenon (ie, the activity levels in the plasma dramatically decrease and often become undetectable). Because of these events, it can be difficult to predict ASP efficacy, and this can sometimes result in suboptimal treatment. Therefore, a number of important challenges still exist to optimize ASP therapy for individual patients. A basic understanding of the factors influencing the ASP dose-depletion relationship will better equip physicians to identify and adjust therapy in patients that fail to achieve adequate depletion.

From the study by Tong et al, it can be easily inferred that the use of E coli ASP in induction and PEGASP during a later phase in childhood ALL may allow very high PEGASP activity levels in nonhypersensitive patients (70% of the whole cohort), but also that hypersensitivity rates and drug inactivation are found in the remaining 30% of patients (see figure). These findings partially confirm the results of another investigation conducted in the late 1990s, even though it involved a different treatment schedule and setting. On this basis, the authors conclude that in order to prevent this detrimental phenomenon it is advisable to use PEGASP upfront in induction. This treatment approach is already largely applied, as demonstrated by several international protocol studies, such as the ongoing Associazione Italiana Ematologia Oncologia Pediatrica-Berlin-Franklin-Munster ALL 2009 trial, which have already adopted this strategy and have in place from the beginning of the protocol a thorough TDM aimed at the early discovery of patients with SI.

In the study by Tong et al, patients treated with Erwinia C ASP also underwent a strict TDM. Very few of these patients presented with an allergic reaction, and none displayed SI, thus confirming that the Erwinia C product has very low cross-reactivity with the E coli-derived products. Erwinia C ASP was given with a very tight schedule (every 2–3 days) and at high dosage (20 000 IU/m2); as expected, ASP activity levels were much lower than those observed during PEGASP administration, but higher than 100 IU in the first 2 weeks of treatment especially in patients treated with a schedule of every 2 days (see figure). The levels reported by Tong et al are very similar to those recently reported by Vrooman et al; however, they are clearly lower than those reported by Salzer et al with a fixed schedule of every 2 days, a higher dosage (25 000 IU/m2), and the intramuscular route.

Additional findings of the study were that antibodies against PEGASP were found in a number of patients, but their specificity was found to be low. Therefore, the usefulness of antibody (both against ASP and/or PEG) detection remains limited and doubtlessly controversial, with several factors influencing its interpretation.

It is well known that galaxies, elements believed to constitute the universe, are made up of a number of stars, but also of large amounts of gases and dust, elements able to influence each other. There are so many factors influencing ASP activities that this set of elements all together resembles a galaxy. The paper by Tong et al sheds additional light on this “ASP galaxy” and allows scientists to get closer to a better understanding of which elements are the stars, which are the gases, and which are the dust.

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Comment on Price et al, page 1989

Why do T cells cause so much trouble?

Kenneth L. McClain

In this issue of Blood, Price et al document a 20-year experience with autoimmune lymphoproliferative syndrome (ALPS) patients and healthy mutation-positive relatives, showing that defective lymphocyte apoptosis is associated with an increased incidence of lymphomas.1

Nature provides physicians and scientists with opportunities to understand basic biologic mechanisms through the study of rare diseases, and ALPS is no exception. Supplemental Figure 1 of the Price et al article captures the fascinating core issue. Patients and some relatives have heterozygous mutations in the FAS gene which lead to defective apoptosis. Although the relatives do not demonstrate the dramatic lymphadenopathy or splenomegaly found in the patients, relatives do have higher than normal numbers of immature T cells (CD4 neg/CD8 neg, “double negative T cell” [DNTs]), and several markers of ALPS: elevated vitamin B12, interleukin-10, and soluble FAS ligand, although these are lower in the healthy mutation-positive relatives. Clearly, there are other genetic or epigenetic modifiers of this syndrome to account for the differences. One explanation is that patients frequently have multiple mutations, often in the second allele.2 A key message of this work is that the observed over expected ratio of Hodgkin lymphoma in ALPS patients was 149 and for non-Hodgkin lymphoma, 61. It is apparent from this report that the incidence of lymphoma in ALPS patients is similar to that of patients with other congenital immune deficiencies such as severe combined immunodeficiency, X-linked agammaglobulinemia, X-linked lymphoproliferative syndrome, Wiskott-Aldrich syndrome, ataxia telangiectasia, and others.3 The common thread here is that a majority of the malignant lymphoproliferations are in B cells and many were associated with Epstein-Barr virus (EBV) as evidenced by detection of the viral EBV-encoded small RNA by in situ staining of the tumor tissue. Defective T-cell surveillance creates the environment for unregulated B-cell proliferation, often promoted by EBV infection, which leads to malignancy: think EBV and endemic Burkitt lymphoma in patients with high rates of malaria or other environmental causes of T-cell suppression or patients with post-transplant lymphoproliferative disease. In the case of ALPS patients, this connection is only partially defined and is obviously a question ripe for investigation. It is known that the DNTs stimulate B-cell proliferation in ALPS with excessive numbers accumulating because of the apoptosis defect, thus opening the door for malignant changes. Somatic mutations of the FAS genes also occur frequently in lymphomas of nonimmune deficient patients.4

Mutations affecting the intracellular portion of FAS are more frequently in the death domain and allow the defective FAS protein to merge with a normal one, but the defective partner asserts a dominant-negative effect resulting in decreased apoptosis of lymphocytes. Strikingly, all lymphoma patients had the dominant-interfering type of mutation and not the missense mutations which lead to haploinsufficiency as a cause of ALPS.

Price et al have carefully cataloged the clinical features of these patients over the years, and in the current article drive home several fascinating and important points. The National Institutes of Health (NIH) group has treated ALPS patients with mycophenolate and finds that this helps improve the cytopenias that can be life-threatening. The theoretical reason for using mycophenolate (or sirolimus) is increased activity of the serine threonine kinase AKT which leads to stimulation of the mammalian target of rapamycin pathway and constitutive proliferation of the DNTs.5,6 Splenectomy has proven to be a futile and dangerous procedure as the cytopenias return, and overwhelming postsplenectomy is a major cause of mortality.

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