inside blood commentary

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RED CELLS, IRON, AND ERYTHROPOIESIS

Comment on Stefanova et al, page 245

Ironing out the role of hepcidin in infection

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In this issue of *Blood*, Stefanova et al provide direct proof (which was previously lacking) that hepcidin is a major component of innate immunity.[1]

Shortly after hepcidin was identified and isolated[2,3] but before it was elucidated as a master regulator of iron metabolism,[4] it became clear that inflammation was one of the principal regulators of hepcidin. Since then, it has been thought that hepcidin may protect against some infections. Proliferation in some so-called siderophilic bacteria is limited by access to iron, whereas other bacteria are unaffected by iron status. The article by Stefanova took advantage of that fact and established the basis for differential parameters of bacterial virulence directly regulated by hepcidin. It was essential for these investigators to establish crucial experimental conditions. First, they needed to show that only the unbound iron from transferrin was essential for the virulence of those bacterial strains that required iron. The first requirement was to show that iron loading leads to free or unbound iron (not transferrin-bound) and that this free, unbound iron is essential for the virulence of those bacterial strains. Thus, they developed an assay for free plasma iron (ie, the redox-active, chelatable component of non–transferrin-bound iron [NTBI]). The second requirement was to use the same conditions in situations in which hepcidin was absent, and they had a suitable animal model: a mouse with a knockout hepcidin gene (HKO mouse).[5] It was also helpful that 2 strains of *Yersinia enterocolitica* were available to them, one siderophilic and the other nonsiderophilic. Third, if they could show that hepcidin plays a major role in the virulence of siderophilic bacteria, then the hepcidin analog they previously made and verified, which they termed minihepcidin PR73,[6] might revert bacterial virulence in animals with iron overload. They could then proceed with their well-designed experiments.

In the initial experiments, Stefanova et al indeed showed that iron makes siderophilic *Y enterocolitica* more virulent than the nonsiderophilic strain. They demonstrated that the iron-overloaded HKO mouse had 100% mortality upon infection with the siderophilic bacteria, which was reversed by iron depletion and, importantly, by minihepcidin. Minihepcidin was also protective in wild-type mice that were iron overloaded. In additional experiments, they demonstrated that the virulence of these siderophilic bacteria was entirely the result of the presence of NTBI rather than neutrophil recruitment and bacterial killing. These manipulations were relevant only to the siderophilic bacteria, because hepcidin and iron manipulation had no effect on the virulence of *Staphylococcus aureus* or *Mycobacterium tuberculosis*.

Along with this important improvement in our understanding of the role of hepcidin in protection from certain types of infections, more work in divergent hepcidin roles in other pathophysiological areas is needed. Clearly, inflammation is not the only regulator of hepcidin expression, and hepcidin is regulated by other pathophysiological situations that contribute to numerous hematologic diseases. Although the relationship of iron status and regulation of hepcidin is clear, another important question was only recently answered by Park et al[3]: Why is hyperactive erythropoiesis in chronic hemolysis resulting from deficiencies in red cell enzymes (such as pyruvate kinase deficiency) or inefficient erythropoiesis in dyserythropoietic anemias and thalassemia frequently accompanied by, and at times diagnosed by, clinically significant iron overload? The answer was that the erythropoietin-regulated hormone erythroferrone downregulates hepcidin transcripts in the liver.[7] Hepcidin is also regulated independently by hypoxia, which mediates the expression of many genes by modulating the levels of hypoxia-induced master transcription factors (HIFs). HIFs are dimers of α subunits and the common β subunit; there are 3 α homologs, HIF-1α, HIF-2α, and HIF-3α, that constitute HIF-1, HIF-2, and HIF-3. Of these, HIF-1 controls hepcidin expression. In the HIF-1α knockout mouse, which is embryonically lethal, hepcidin transcription was increased in these embryos, proving that hypoxia via HIF-1 downregulates hepcidin.[8] However, the molecular mechanism of HIF-1 hepcidin downregulation still remains to be deciphered.

There may be other pleotropic roles of hepcidin. For example, there are reports of the presence and production of hepcidin in astrocytes and glial cells in the brain.[9,10] Much more work remains to be done to understand its function, regulation, and significance in neurocognition and brain pathophysiology. Thus, more investigative work on the role of hepcidin in health and disease is awaited. Nevertheless, the article by Stefanova et al represents a great step forward in our understanding of the role of hepcidin in

protecting against certain infections and provides tempting potential therapeutic applications for hepcidin analogs.

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● ● ● CLINICAL TRIALS AND OBSERVATIONS

Comment on Zinzani et al, page 267

Check this checkpoint inhibitor in lymphoma

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In this issue of *Blood*, Zinzani et al[1] report that the immune check point inhibitor pembrolizumab is active against relapsed/refractory primary mediastinal large B-cell lymphoma (PMLBCL). This unique clinicopathologic entity accounts for up to 10% of all diffuse large B-cell lymphomas (DLBCLs) and is most commonly seen in females between 20 and 40 years of age. Gene expression profiling has shown that the genes expressed in PMLBCL are essentially those of Hodgkin lymphoma (HL).[2] Some of these genes are related to the signaling pathways of NF-κB and JAK-STAT (see figure). Of interest is that chromosome 9p24.1 alterations (see figure), which have been associated with programmed death ligand (*PD-L1*)/L2 upregulation in classical HL, have also been observed in PMLBCL.[3] In view of these similarities in gene expression between PMLBCL and HL, it should not come as a surprise that overexpression of the *PD-L1* gene, typically seen in HL, is also observed in PMLBCL.

In the KEYNOTE-013 trial reported by Zinzani et al, pembrolizumab was administered to a subset of 17 patients with PMLBCL, of which 7 (41%) responded and an additional 6 (35%) of patients had some tumor shrinkage.

The study was a phase 1 trial. It is well-known that patients entered into that type of study usually have far advanced disease and are very refractory to treatment. Response rates are usually poor. As expected, the population entered consisted of multiply relapsed patients with 3 prior lines of therapy, which translates into fourth-line therapy. As compared with previous salvage studies in aggressive lymphomas, in which the average number of prior lines of therapy is 1 or 2, clearly this population was more heavily pretreated. In that context, the response rate of 41%, which at first glance might not appear extremely encouraging, must be considered in a different light. Despite the advanced nature of the patient population, it was encouraging that at a median follow-up of 11.3 months, median duration of response has not been reached. Only 5 of 17 patients have progressed and only 4 have died. Two patients reached the maximum 2-year treatment duration and remain in remission; furthermore, only 1 of the responders has relapsed.

Another crucial point to consider is that the duration of response in all cases was longer than their response to first-line therapy, a highly unusual finding. In addition, there were 3 cases whose response to first-line therapy was only stable disease, yet their response to pembrolizumab was complete response in 1 case and partial response in the other 2.

Currently, the preferred front-line treatment of PMLBCL is an intensive approach reported by Dunleavy et al using chemotherapy with cyclophosphamide, doxorubicin, etoposide, prednisone, rituximab, and vincristine sulfate (DA-R-EPOCH) without radiation therapy.[4] With that approach, the failure-free survival is 93%, leaving little room for salvage therapy with pembrolizumab. Although real-world results perhaps might not be as impressive as those reported by Dunleavy et al, the future use of pembrolizumab, instead of rescue therapy, might consist of its use as front-line therapy combined with the classical R-CHOP regimen (rituximab, cyclophosphamide, doxorubicin, prednisone, vincristine sulfate), which is much less toxic than DA-R-EPOCH. By doing so, the toxicity of DA-R-EPOCH might be obviated. Most patients with PMLBCL are females of child-bearing potential, which makes the goal of preserving fertility an important objective. Pembrolizumab might be the first step in developing a chemotherapy-free combination with other biological agents that have little or no gonadal toxicity, certainly a desirable goal.

We are being faced with increasingly new knowledge about the biology of lymphomatous disorders, which is already having an impact on our treatment choices. We must stand ready to integrate this new knowledge and apply it to our therapeutic strategy for these tumors.

As clinicians, we have grown accustomed to perceiving lymphoid disorders in a histological context, but it is time we start developing novel approaches to categorizing them that will be more clinically meaningful and relevant. The