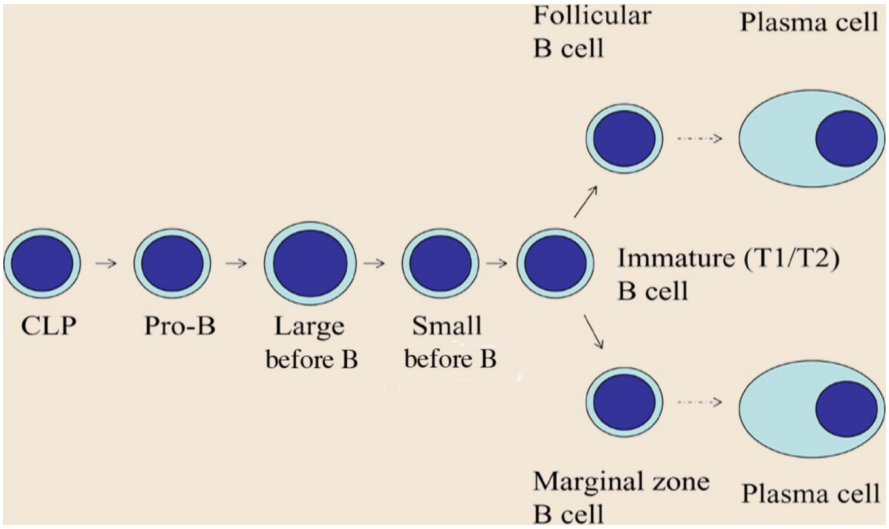

Differentiation of B cells. CLP indicates common lymphoid progenitor. WASp is most important in the more mature B-cell subpopulations.

migration, adhesion, and activation of neutrophils, platelets, macrophages, dendritic cells, natural killer (NK) cells, and T and B cells.[1]

The *WASP* gene is located on the X chromosome. Random X chromosome inactivation in $WASp^{+/-}$ mice would theoretically result in 50% of cells expressing WASp. Westerberg et al show this to be the case in the myeloid compartment, that is, neutrophils, dendritic cells, macrophages, and NK cells. Also, both papers show that in the most immature subsets of T and B cells, such as the double-negative or double-positive thymocytes and the pro-B, pre-B, and immature B cells in the bone marrow, there is no selective advantage for WASp expression. In contrast, in the more mature T and B cells, WASp positive cells had a strong selective advantage (the different stages of B-cell differentiation are shown in the figure). As differentiation proceeds, the advantage of $WASp^{+}$ cells increases. The strongest advantage for WASp expression was found in regulatory T cells and natural killer T cells in spleen and thymus, and in splenic marginal zone (MZ) B cells, in which at least 80% of the cells expressed WASp. In addition, $WASp^{+}$ germinal center B cells had a more pronounced selective advantage than nongerminal center cells. In a particular subpopulation of B cells called B1 cells that reside in the peritoneal cavity, WASp expressing cells were dominant. Finally, in a WAS patient with a revertant mutation, there was evidence for selective advantage of mature peripheral B cells.

Meyer-Bahlburg et al investigated the presence of various subpopulations of B cells in wild-type or WASp-deficient mice. No significant differences were observed in the early populations of pro-B cells to immature B cells, whereas the more mature B-cell populations, that is, IgD^{+} cells in the bone marrow, the follicular and MZ B cells in the spleen were reduced in numbers in mice lacking WASp. In addition, they found that in wild-type mice the mature B-cell subpopulations expressed relatively more WASp as compared with immature B cells.

With reconstitution experiments, the scientists concluded that the relative absence of the WASp negative MZ B cells was due to an intrinsic B-cell deficiency. They went on to analyze the capacity of the cells to divide and found that, surprisingly, the follicular and MZ $WASp^{-}$ B cells had an increased turnover rate as compared with wild-type cells. Thus, the deficiency in the more mature B-cell populations is due to an altered homeostasis and not to a differentiation defect. No evidence was found for an increased rate of apoptosis, but B cells were deficient in the capacity to generate LFA-1-ICAM-1–dependent adhesion complexes. Furthermore, MZ B cells showed impaired migration to sphingosine-1-phosphate. Both LFA-1 - ICAM-1 interactions and sphingosine-1-phosphate have been shown to be important for MZ B-cell positioning.[2,3] This suggests that there is an inefficient localization of mature B cells to specific compartments in the absence of WASp. The cells try to compensate for this by increasing their proliferative rate. It is tempting to speculate that this leads to formation of lymphomas or autoimmune reactions, which are both known to develop in WAS patients.

WAS patients have increased susceptibility to bacterial infections, especially encapsulated pathogens.[1] $WASp^{-/-}$ mice exhibit impaired responses to T-cell independent antigens.[4] The MZ is situated in the outer border of the white pulp of the spleen and consists of B cells and macrophages. It is thought that it provides a first line of defense to blood-borne bacterial antigens. Thus, the papers by both sets of authors give important clues as to how immunodeficiency in WAS develops.

WAS is normally treated with stem cell transplantation. In certain cases, gene therapy might be an alternative. The papers by Meyer-Bahlburg et al and Westerberg et al imply that it is especially important to reconstitute the mature lymphocyte populations.

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

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RED CELLS

Comment on Ganz et al, page 4292

An immunoassay for human serum hepcidin at last: Ganz klar?

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Hepcidin has emerged as the master regulator of iron metabolism. The lack of an assay to measure hepcidin levels in human serum has hampered research in this hot area of iron metabolism biology and pathophysiology. In this issue of *Blood*, Ganz and colleagues provide the initial validation of a novel immunoassay for human serum hepcidin, which promises to be a valuable tool in future studies on the physiology and pathology of iron metabolism.

Hepcidin is the primary regulator of iron homeostasis: hepcidin modulates iron availability by promoting the internalization and degradation of ferroportin, a key iron transporter and so far the only identified mammalian iron exporter, which is essential for both iron absorption in the duodenum and recycling of iron/iron efflux by macrophages. Hepcidin is a negative regulator of iron absorption and mobilization; high hepcidin levels turn off both duodenal iron absorption and release of iron from macrophages while low hepcidin levels promote iron absorption and heme iron recycling/iron mobilization from macrophages. Thus, hepcidin levels are expected to be high in iron overload states and diminished in iron deficient states. Hepcidin production can be induced by inflammation, which explains the reduced availability of iron in the anemia of chronic disease, whereas anemia and hypoxia have been shown to increase iron absorption and mobilization by decreasing hepcidin production.[1]

Previous work on assessing urinary levels of hepcidin was carried out using methods that required mass spectrometry detection and thus are of limited availability.[2,3] Low levels of serum hepcidin have been reported using mass spectrometry detection in blood donors donating at least 13 whole blood units in a 2-year time span.[4] Measurements of prohepcidin, the precursors of the biologically active 25 aa hepcidin, have been generally disappointing because they seem to be poorly correlated with hepcidin and unresponsive to known hepcidin regulators.[5]

The work by Ganz et al validates an immunoassay for human hepcidin levels in serum, which has a lower limit of detection of 5 ng/mL and yields a normal range for serum hepcidin of 29 to 254 ng/mL in men and 16 to 288 ng/mL in women. The assay has enough sensitivity to detect changes in serum hepcidin due to diurnal variation and in response to oral iron.

The next challenge will be to demonstrate what is the additional value of these measurements compared with the traditional diagnostic repertoire for iron metabolism disorders. In particular, what will this assay add to the information presently conveyed by serum ferritin? Since both ferritin and hepcidin are similarly affected by changes in iron availability and inflammation, careful studies will be required to demonstrate the unique additional value of measuring serum hepcidin. The authors correctly point out in their work that hepcidin can change on a time scale much shorter than that of ferritin, and several iron overload conditions, including beta thalassemia, exhibit elevated serum ferritin in conjunction with an abnormally low serum hepcidin. Inappropriately high levels of serum hepcidin are also seen in familial forms of iron-refractory iron deficiency anemia due to mutation in TMPRSS6, a negative regulator of hepcidin transcription.[6,7] Perhaps the greatest promise for the clinical applicability of this new assay for serum hepcidin resides with the diagnosis of iron deficiency at infancy. An abnormally low serum hepcidin could identify infants at the earliest phase of development of iron deficiency before changes in either ferritin or reticulocyte/red cell parameters take place. If such an assay were to be made available and validated for urine samples, it could simplify the screening for iron deficiency of infants. An abnormally low serum or urinary hepcidin could also be of value for identifying adult women who require iron supplementation therapy without using any other laboratory tests. It remains to be seen if the serum hepcidin assay could also help in better identifying patients with anemia of chronic disease and concomitant iron deficiency or patients with anemia of chronic renal failure, both of which are nonresponsive to erythropoietic-stimulating therapies. Finally, in patients with iron deficiency anemia and low ferritin, will an abnormally high hepcidin be of help in identifying patients who are unresponsive to oral iron therapy and require intravenous iron supplements? The availability of this assay opens the way to a variety of exciting studies on iron metabolism in human diseases.

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

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Comment on Atkinson et al, page 4276

SNPs linking *TNF* with anemia

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Anemia is highly prevalent in children in malaria-endemic areas. However, it is difficult to distinguish between IDA and ACD in affected populations. In this issue of *Blood*, Atkinson and colleagues identify SNPs in the *TNFα* gene that are associated with an increased risk of developing IDA during the malaria season.

Single nucleotide polymorphisms (SNPs) in the *TNF* gene locus (lying within the Major Histocompatability Complex class III region on chromosome 6) have been identified as potential risk factors in the etiology of a number of diseases, including malaria. *TNF* promoter polymorphisms are associated with increased *TNF* gene transcription, and previous work has provided strong evidence that plasma tumor necrosis factor alpha (TNFα) levels are significantly elevated following malarial infection.[1] TNFα is known to be a modifier of body iron status and, in their study, Atkinson et al investigated whether functional SNPs and haplotypes across the *TNF* gene locus were associated with anemia during the malaria season. A cohort of 780 children was recruited from rural villages in the malaria-endemic West Kiang region of The Gambia. Blood samples were collected from each child at the start (baseline measurement) and end of the malaria season. The samples were used to assess iron status and inflammation as well as to provide