To the editor:

Are Mpl glycosylation defects in polycythemia vera secondary to artifactual hypoglycemia?

In their report on altered processing of the thrombopoietin receptor (Mpl) in patients with polycythemia vera, Moliterno and Spivak 1 (Oct 15 issue) show that Mpl glycosylation is associated with disease progression. The authors demonstrate that the nonglycosylated Mpl isoform has reduced expression and is impaired in its ability to transit to the cell membrane, thus rendering the cells resistant to thrombopoietin. 1, 2 While the authors suggest that these findings indicate that Mpl glycosylation defects may serve as a marker for disease progression, an alternate hypothesis should be entertained.

Artifactual hypoglycemia has been reported to occur in polycythemia vera and is caused by in vitro autoglycolysis due to an exaggerated consumption of glucose by blood cells. 3 In polycythemia vera, this phenomenon can occur even with only modest leukocytosis and may be due to both red and white cell-induced enhanced glycolysis. 4- 5 Serum glucose concentrations can drop significantly within 2 hours after sample collection, and longer intervals of time are associated with greater reductions in glucose levels. Additionally, recent work from other laboratories has indicated that glucose deprivation causes defects in receptor processing and glycosylation. 6- 8 By depriving cells of glucose, both the epidermal growth factor and insulin receptors were impaired in their glycosylation and exhibited reduced surface expression, similar to the findings of Moliterno et al with regard to Mpl. Therefore, it is possible that there is an artifactual association between Mpl glycosylation and disease progression due to factitious hypoglycemia. The authors further state that this defect is a “fossil record” of a progenitor cell because “there is no evidence that platelets can synthesize or metabolize Mpl”, however, others have shown that platelets can efficiently synthesize protein. 9

To their credit, the authors use controls with other myeloproliferative disorders, some of which exhibit the potential for in vitro artifactual hypoglycemia; however, no blood cell count data is provided in this report. Interestingly, in their previous paper, 2 the authors show that the mean white cell count in the polycythemia vera patients is much greater than in any of their controls. Furthermore, no other hematopoietic cytokine receptors were studied to prove specificity of the effect. It is unclear whether artifactual hypoglycemia has influenced the validity of the Moliterno study; however, if this methodology is to be used to stage disease progression then the possible effects of artifactual hypoglycemia should be considered.

Kevin G. Otto
Division of Hematology
University of Washington
Seattle, WA

Response:

Mpl glycosylation defects in polycythemia vera are not secondary to artifactual hypoglycemia

We thank Dr Otto for his hypothesis, which is directly refuted by our published observation 1 that the Mpl abnormality observed in polycythemia vera (PV) platelets was also present in PV megakaryocytes as demonstrated by in situ immunohistochemical staining of fixed bone marrow biopsy sections, an approach not subject to confounding by artifactual leukocyte-induced in vitro hypoglycemia. Nevertheless, given the biologic significance of our observations with respect to the pathophysiology of PV, we think that it is important to individually address the specific contentions upon which Dr Otto’s hypothesis is based.

As Dr Otto indicates, leukocyte-induced in vitro hypoglycemia in blood samples kept at room temperature in the absence of metabolic inhibitors can be seen in myeloproliferative disorders such as PV 2, 3 as well as with normal leukocytes 4 Dr Otto, however, cites a publication demonstrating in vitro hypoglycemia induced by PV leukocytes when the leukocyte count was less than 20 000/µL, although not when it was normal 5 This observation is not only unprecedented, 3 but the authors also observed artifactual hypoglycemia equivalent to that observed with PV leukocytes in a secondary erythrocytosis patient with a normal leukocyte count. However, 4 hours of in vitro incubation were required to obtain these results. 3 Dr Otto does note that we included the appropriate leukocytosis controls in our initial studies 1 and thus he cannot have it both ways. We have never observed impaired platelet Mpl

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glycosylation in patients who did not have a myeloproliferative disorder, regardless of the height of the leukocyte count. Conversely, we have documented impaired platelet Mpl glycosylation in patients with PV with normal leukocyte counts (35% of our patients) as well as in IMF patients with leukopenia. At the same time, we have never seen impaired platelet Mpl glycosylation in patients with secondary erythrocytosis.

Dr Otto also notes that in our second publication, unlike the first, we failed to provide patient leukocyte counts. That was because, as we stated in the paper, they were irrelevant to the results. For example, in the patient illustrated in Figure 6 of the paper showing progression of the platelet Mpl glycosylation defect, the leukocyte count was 13 100/µL in S/19 and 14 700/µL in 1/99, hardly a change to which even Dr. Otto would ascribe biochemical significance. However, since the issue was raised, we wish to state for the record that the mean leukocyte count for the “A” group in Table 1 was 13 200/µL (range 6200-22 400), for the “A=B” group, 9500/µL (range 6600-28 100), for the “B=A” group, 31 660/µL (range 7000-50 000), and for the “B” group 45 300/µL (range 9800-145 437). While there is the expected correlation between disease duration and mean leukocyte count, it is equally clear from both the means and the ranges that there is no correlation between the leukocyte counts and the extent of the Mpl glycosylation defect. We should also add that while we routinely process our clinical blood samples within 2 hours of collection, we have found that the integrity of Mpl remains intact in normal platelets in whole blood shipped unrefrigerated overnight. Thus, Dr Otto’s contention that the leukocyte count is a confounder with platelets is undefendable.

The second component of Dr Otto’s hypothesis with respect to blood platelets concerns the effect of hypoglycemia on receptor processing. However, when one examines Dr Otto’s chosen examples, they fail to recapitulate the type of defect that we observed. In one study, glucose deprivation caused a reduction in insulin receptor gene transcription. However, the mRNA content of Mpl in PV platelets was not different than normal. In a second study, while glucose deprivation was associated with impaired insulin receptor glycosylation, the improperly processed receptor was endoglycosidase H-resistant. This is contrary to what we observed, namely, Mpl in PV platelets was endoglycosidase H-sensitive. Furthermore, where the conditions of glucose deprivation were defined, the glucose concentrations ranged from 0 to 5.5 mM. However, the range of normal for blood glucose is 3.3 to 5.5 mM, implying that Mpl processing should be impaired in normal circulating platelets and of course it is not. Additionally, we demonstrated both A and B forms of Mpl in Dami cells in the absence of hypoglycemia, which strongly supports our contention that glucose deprivation is irrelevant to the PV Mpl defect.

Finally, Dr Otto’s hypothesis is also based on the ability of platelets to synthesize protein. However, in the publication he cites, protein synthesis was observed only in thrombin-activated platelets and even then required a time lag. We only studied unstimulated platelets and have never been able to obtain incorporation of 35S-methionine into Mpl in resting normal platelets. Also, we did not study other hematopoietic growth factor receptors simply because none of the relevant ones (IL-3 beta and gp130) were immunologically detectable in platelets as clearly stated in our paper. We did, however, examine 2 important integral membrane platelet glycoproteins, gpIIb and multimerin. Neither were abnormally glycosylated in a PV patient with markedly impaired Mpl processing (see reference 6, figure 5), a specificity, given the abundance of these former proteins, that would be unusual with a nonspecific metabolic insult such as hypoglycemia. Unfortunately, Dr Otto fails to mention these important observations that also refute his hypothesis.

Taking everything together, it is very clear that artifactual, leukocyte-induced, in vitro hypoglycemia cannot explain either the specific impairment of Mpl processing in PV megakaryocytes and platelets or its progression with disease extent and duration.

Alison R. Moliterno and Jerry L. Spivak
Division of Hematology
Johns Hopkins University
School of Medicine
Baltimore, Maryland

References

To the editor:

HFE polymorphism and accurate diagnosis of C282Y hereditary hemochromatosis carriers

Mutations in the HFE gene are responsible for more than 90% of the cases of hereditary hemochromatosis in the Caucasian population. Three mutations have been reported to contribute to disease, including C282Y, H63D, and S65C.

We read with interest and concern 2 articles published recently with respect to the accuracy of hereditary hemochromatosis DNA testing. Each of these articles describes the presence of a G→A polymorphism at nucleotide 6117 within the primer sequence for the amplification of exon 4, which contains the C282Y mutation. (Genbank #Z92910 has been updated since the publication of Jeffery et al [1999] and Somerville et al [1999]. These nucleotide numbers represent the current nucleotide numbers in the HFE gene.)
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