The authors also question the “inexplicably high” level of enrichment that we measured in the deoxyribonucleic acid of blood neutrophils. It is important to realize, however, that our method analyzes deuterium enrichment in deoxyribose (dR) derivatives that harbor multiple hydrogen atoms independent of the particular hydrogen location that is enriched. Because the enrichment in body water is low, double-labeling within one dR hardly occurs, and the fraction of single-labeled dR derivatives is thus a nearly linear sum of the enrichment at each of the dR hydrogen locations. This allows the fraction of dR derivatives with one hydrogen atom enriched to exceed the level of enrichment found in the body water, as we and others have previously described.4,5

Taken together, we think the issues raised by Tofts et al do not affect the interpretation of our results.

Li et al, however, raise an alternative possibility that may have large implications for the interpretation of our data. They propose that the slow kinetics of labeled cells observed in the blood may in fact be because of the slow production of neutrophils in the bone marrow, rather than a long half-life in the blood. Indeed, if neutrophil production in the bone marrow were to be the rate limiting kinetic step (ie, if the average time between subsequent divisions of neutrophil precursors in the marrow would be of the order of days), the resulting labeling kinetics of human neutrophils in the blood could be much slower than their actual dynamics in the blood. Unfortunately, in the absence of labeling data from the bone marrow of healthy humans, we cannot exclude this alternative possibility. Our concern remains, however, that previous studies based on ex vivo labeling, on labeling with DFP32 (which might affect neutrophil activation)6 or on non–steady state or toxic conditions, have likely caused aberrant (homing) behavior of neutrophils7 and may thereby have underestimated the circulatory half-life of blood neutrophils. The only way to distinguish between the 2 alternative possibilities proposed by Li et al is to investigate the turnover of bone marrow neutrophils in healthy humans using in vivo labeling techniques such as deuterium labeling, to ensure that neutrophil kinetics are studied under normal physiologic circumstances. Given the importance of the issue, we very much support such future in vivo labeling studies of neutrophils in the bone marrow.

To the editor:

Anemia in congenital nephrotic syndrome: role of urinary copper and ceruloplasmin loss

Mechanisms for anemia in patients with nephrotic syndrome (NS) are complex and incompletely understood. Copper is an essential mineral in red blood cell metabolism. It is absorbed in the intestine, bound to its carrier protein, transported to the liver, and stored. Up to 95% of serum copper is bound to ceruloplasmin. Mouse models have shown that severe copper deficiency results in profound neutropenia1 and anemia.2 In humans, copper deficiency, which has been reported mostly in patients with chronic intestinal dysfunction, leads to anemia, neutropenia,3 and dysmyelopoietic features.4 Copper deficiency is thought to worsen anemia in patients with kidney failure.5 However, copper deficiency anemia has not yet been described in the congenital NS infant.

References

The patient was born at 40 weeks’ gestational age. At 6 months of age, a nephrotic syndrome developed. Clinically, the patient suffered from asthenia, anorexia, and edema. Biologically, proteinuria was 33 g/L, and serum albumin level was as low as 6 g/L before high-dose intravenous albumin infusion. Renal biopsy showed a slightly thickened mesangium, with tubular atrophy and dilated tubuli. Genetic analysis revealed a heterozygote mutation in the \textit{NPHS2} gene (c.714G>T in exon 5, leading to p.Arg238Ser) coding for podocin. No mutations were detected in the \textit{NPHS1} gene.

At 7 months of age, severe aregenerative anemia (hemoglobin level as low as 5.0 g/dL) was detected; neutrophils (1.6 \times 10^9/L) and lymphocytes (2.5 \times 10^9/L) were low. An infectious work-up (including HIV, hepatitis A/B/C, CMV, and EBV) came back negative. Hemoglobin electrophoresis was normal. Hemolysis was ruled out because haptoglobin was not low, and both LDH and unconjugated bilirubin were normal. Antinuclear antibody, SSA, and SSB tests were negative for lupus. Early bone marrow biopsy only showed few hypochromic erythroblasts; no vacuolated erythroids were detected. Total copper serum level was low (0.32 mg/L, normal range 1.08 to 1.44 mg/L), along with ceruloplasmin level (0.2 g/L, normal range 0.22 to 0.61 g/L). Transferrin level was slightly below normal values (1.4 g/L, normal range 2 to 3.5 g/L). Anemia was refractory to erythropoietin (NeoRecormon 6000 UI per week), iron, and vitamin B12 supplementation, which has been described.\(^6\) Blood transfusions had a very transient efficiency. Oral supplementation of copper gluconate was sufficient to increase white blood cell count, high doses were necessary to lead to an increase in hemoglobin level. Therefore, it should be considered to help correct refractory aregenerative anemia in NS patients.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{Figure1.pdf}
\caption{Reticulocyte count and hemoglobin level in infant with NS.}
\end{figure}

\section*{Discussion}

In conclusion, urinary losses because of massive proteinuria may cause copper depletion, involved in the pathogenesis of neutropenia and anemia. Interestingly, whereas normal doses of oral copper gluconate were sufficient to increase white blood cell count, high doses were necessary to lead to an increase in hemoglobin level. Therefore, it should be considered to help correct refractory aregenerative anemia in NS patients.

\section*{Conflict-of-interest disclosure}

The authors declare no competing financial interests.

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