To the editor:

Glucose 6-phosphate dehydrogenase deficiency in an elite long-distance runner

Glucose 6-phosphate dehydrogenase (G6PD) deficiency is the world's most common enzymopathy[1] and caused by mutations in the X-chromosomal *G6PD* gene. The enzyme catalyzes the first reaction of the hexose monophosphate shunt, which results in 2 main products: pentose phosphate sugars and the reduced form of nicotinamide adenine dinucleotide phosphate (NADPH). The latter is involved in reductive processes. G6PD deficiency is most pronounced in erythrocytes, compared with other cells, because red blood cells lack biosynthetic capacity. Therefore, G6PD-deficient erythrocytes are less able to withstand oxidative stress, leading to (acute) hemolytic anemia.[1]

We recently studied a 37-year-old white man who is an elite long distance runner and has attained high class rankings in European, world, and Olympic competitions in the last 15 years while being severely G6PD-deficient. He never showed any clinical signs of hemolysis, and the G6PD deficiency was detected by chance. Routine laboratory investigations showed no abnormalities, except for low haptoglobin and slightly elevated unconjugated bilirubin levels over the years, suggesting the presence of mild chronic hemolysis (Table 1). The athlete's erythrocytes were found to exhibit strongly reduced G6PD activity. G6PD activity in leukocytes was also decreased, though less pronounced. These findings were confirmed by Western blot analysis (Figure 1). DNA analysis revealed a missense mutation in exon 8 (c.844 G>C: Asp282His) of *G6PD*. This mutation has been previously found to underlie several biochemically unique G6PD variants.[2] To further characterize the G6PD variant, kinetic measurements were performed using leukocyte-derived G6PD (Table 1). These results led us to conclude that the kinetic properties of the mutant enzyme were only slightly altered. Hence, G6PD deficiency in this athlete appears to be mainly due to the mutant protein's instability, in particular in erythrocytes (Figure 1).

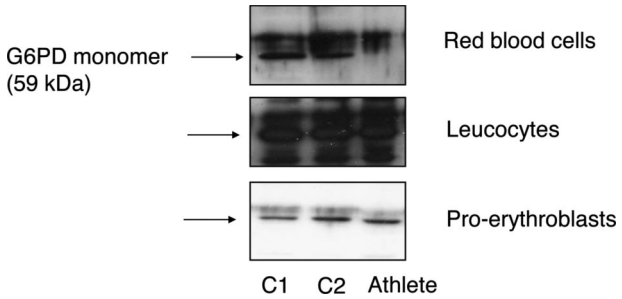

Figure 1. Instability of mutant G6PD. Western blot analysis was performed on red blood cells, leukocytes, and ex vivo–cultured proerythroblasts[10] from 2 controls (C1 and C2) and the athlete, using a polyclonal antibody against G6PD (kindly provided by Robin van Bruggen, Sanquin Research, Amsterdam, The Netherlands). In contrast to leukocytes and ex vivo–cultured proerythroblasts, mutant G6PD was not detectable in the athlete's erythrocytes.

Heavy exercise can accelerate the generation of reactive oxygen species (ROS), exceeding the capacity of antioxidant defenses.[3] Because this may result in hemolysis and muscle

Table 1. Representative results of routine and specialized laboratory investigations

Laboratory investigation	Patient	Reference
Routine		
Hemoglobin, mmol/L	9.4	8.6-10.7
Erythrocytes, $\times 10^{12}$/L	4.8	4.2-5.5
MCV, fL	87	80-97
Reticulocytes, $\times 10^{9}$/L	25	25-120
Creatinine, μmol/L	84	74-120
Creatinine kinase, U/L	114	15-175
γ-glutamyl transferase, U/L	17	15-70
Aspartate aminotransferase, U/L	29	15-45
Alanine aminotransferase, U/L	21	10-50
Lactate dehydrogenase, U/L	494	300-620
Unconjugated bilirubin, μmol/L	24	3-21
Haptoglobin, g/L	< 0.1	0.3-2.0
Specialized laboratory investigation		
G6PD activity (U/g Hb) in erythrocytes	0.8	7.1-11.5
PK activity (U/g Hb) in erythrocytes	7.2	6.9-14.5
HK activity (U/g Hb) in erythrocytes	1.12	1.0-1.6
G6PD activity (U/g protein) in leukocytes	530	815-877
Calculated G6PD activity (U/g protein) in muscle cells*	0.5	3-4.7
K_m for G6P (μmol/L) in leukocytes	40	55-71
K_m for NADP (μmol/L) in leukocytes	45	66-76
K_i value for NADPH (μmol/L) in leukocytes	153	142-159
G6PD activity at pH (range, 6.5-9.0) and elevated temperature (39°C, mimicking increased body temperature)	Normal	Normal
Oxidative hemolysis	1.5%	< 2%

*($y = 0.39x + 0.198$).[9]

degeneration, it has been postulated that G6PD-deficient persons with red blood cell enzyme activities less than 15% of normal should avoid prolonged high-intensity physical exercise.[4] Two recent studies concluded that G6PD-deficient persons are able to perform mild to moderate exercise without higher oxidative stress than G6PD-nondeficients.[5,6] Of particular interest are 2 case reports describing adverse effects of exercise on G6PD-deficient persons. The first describes a 20-year-old G6PD-deficient man who was hospitalized for myalgia and myoglobinuria after intense exercise; he carried the same G6PD variant as our case.[7] The second is a 30-year-old pentathlon-trained athlete with G6PD deficiency who suffered from loss of consciousness and pigmenturia during the last meters of a 12-km competitive run.[8] Contrary to both these reports, our athlete showed no more severe signs of myalgia, myoglobinuria, or hemoglobinuria than other elite long-distance runners, although residual activity in erythrocytes was approximately 9%, and the calculated activity[9] in muscle cells was 13% of normal. We postulate that the athlete described here has developed a unique balance between his G6PD deficiency and exercise-induced disturbances of blood glutathione and lipid peroxidation that allows him to perform strenuous exercise.

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To the editor:

In vivo–activated CD103$^+$ Foxp3$^+$ Tregs: of men and mice

We read with interest the article by Zhao et al[1] on the specific abilities of murine CD103$^+$ T regulatory (Treg) cells to ameliorate ongoing chronic graft-versus-host disease (cGVHD). The authors report here that only in vivo–activated effector/memory-like Tregs that express high amounts of the integrin αE (CD103) are able to efficiently control the disease in a murine model based on allogeneic transfer of DBA/2 donor cells into BALB/c hosts. In conclusion, the authors suggest that the infusion of human CD103$^+$ Tregs might also ameliorate cGVHD in patients, so that the reported findings may offer a new strategy for the treatment of cGVHD patients.

In principle, the use of effector/memory-like Treg cells may be indeed beneficial also in human therapy. We would like to point out, however, that the markers for this Treg subset are not compatible between humans and mice. CD103 is a well-established marker for murine effector/memory-like Treg cells, which have been activated in vivo by antigen in a specific context. In naive mice, up to 30% of CD25$^+$ FOXP3$^+$ lymphocytes in fact express CD103.[2] In contrast to this, cells with this marker are virtually absent among human CD25^{high} Foxp3$^+$ Treg cells. It is expressed by less than 5% of human CD25^{high} Foxp3$^+$ Treg cells in various tissues, including blood.[3-6] This applies also for β7, the integrin β-chain binding to CD103, which is only poorly expressed by human Treg cells.[3,7] Although in CD4$^+$ T cells CD103 expression can be induced by different stimuli in vitro,[5,8] a significant expression of CD103 on human CD4$^+$ cells with regulatory potential ex vivo was reported only in tonsils, here, however, mostly on CD25$^-$ cells.[5]

Given the different expression of CD103 by Foxp3$^+$ Tregs in mice and humans, it is highly questionable whether the results obtained with murine CD103$^+$ Tregs can be translated 1:1 into a clinical setting. There is little doubt that equivalent cell subsets exist in humans and mice, and that effector/memory-like Treg cells fulfill analog functions in both species. Addressing these cells, however, requires considering species-specific differences. In humans, effector/memory-like Treg cells (T_{REM}) are better defined by markers such as CCR6 or CD39.[9,10]

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