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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References

1. Cappellini MD, Fiorelli G. Glucose-6-phosphate dehydrogenase deficiency. Lancet. 2008;371:64-74.
2. Vulliamy TJ, Luzzatto L. Glucose-6-phosphate dehydrogenase deficiency and related disorders. In: Handin RI, Lux SE IV, eds. Blood Principles and Practice of Hematology, 2nd ed. Philadelphia, PA: Lippincott Williams & Wilkins, 2003;1921-1950.
3. Finaud, J, Lac G, Filaire E. Oxidative stress: relationship with exercise and training. Sports Med. 2006;36:327-358.
4. Ninfali P, Bresolin N. Muscle glucose 6-phosphate dehydrogenase (G6PD) deficiency and oxidant stress during physical exercise. Cell Biochem Funct. 1995; 13:297-298.
5. Jamurtas AZ, Fatouros IG, Koukosias N, et al. Effect of exercise on oxidative stress in individuals with glucose-6-phosphate dehydrogenase deficiency. In Vivo. 2006;20:875-880.
6. Nikolaidis MG, Jamurtas AZ, Paschalis V, et al. Exercise-induced oxidative stress in G6PD-deficient individuals. Med Sci Sports Exerc. 2006;38:1443-1450.
7. Ninfali P, Bresolin N, Baronciani L, et al. Glucose-6-phosphate dehydrogenase Lodi^{844C}: a study on its expression in blood cells and muscle. Enzyme. 1991; 45:180-187.
8. Bresolin N, Bet L, Moggio M, et al. Muscle glucose-6-phosphate dehydrogenase deficiency. J Neurol. 1989;236:193-198.
9. Ninfali P, Baronciani L, Bardoni A, Bresolin N. Muscle expression of glucose-6-phosphate dehydrogenase deficiency in different variants. Clin Genet. 1991;48:232-237.
10. van Wijk R, van Wesel AC, Thomas AA, Rijksen G, van Solinge WW. Ex vivo analysis of aberrant splicing induced by two donor site mutations in PKLR of a patient with severe pyruvate kinase deficiency. Br J Haematol. 2004;125:253-263.

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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References

1. Zhao D, Zhang C, Yi T, et al. In vivo-activated CD103+CD4+ regulatory T cells ameliorate ongoing chronic graft-versus-host disease. Blood. 2008;112:2129-2138.
2. Huehn J, Siegmund K, Lehmann JC, et al. Developmental stage, phenotype, and migration distinguish naive- and effector/memory-like CD4+ regulatory T cells. J Exp Med. 2004;199:303-313.

3. Iellem A, Colantonio L, D'Ambrosio D. Skin-versus gut-skewed homing receptor expression and intrinsic CCR4 expression on human peripheral blood CD4+CD25+ suppressor T cells. Eur J Immunol. 2003;33:1488-1496.
4. Lim HW, Broxmeyer HE, Kim CH. Regulation of trafficking receptor expression in human forkhead box P3+ regulatory T cells. J Immunol. 2006;177:840-851.
5. Allakhverdi Z, Fitzpatrick D, Boisvert A, et al. Expression of CD103 identifies human regulatory T-cell subsets. J Allergy Clin Immunol. 2006;118:1342-1349.
6. Venken K, Hellings N, Thewissen M, et al. Compromised CD4+ CD25(high) regulatory T-cell function in patients with relapsing-remitting multiple sclerosis is correlated with a reduced frequency of FOXP3-positive cells and reduced FOXP3 expression at the single-cell level. Immunology. 2008;123:79-89.
7. Hirahara K, Liu L, Clark RA, Yamanaka K, Fuhlbrigge RC, Kupper TS. The majority of human peripheral blood CD4+CD25highFoxp3+ regulatory T cells bear functional skin-homing receptors. J Immunol. 2006;177:4488-4494.
8. Rao PE, Petrone AL, Ponath PD. Differentiation and expansion of T cells with regulatory function from human peripheral lymphocytes by stimulation in the presence of TGF-beta. J Immunol. 2005;174:1446-1455.
9. Kleinewietfeld M, Puentes F, Borsellino G, Battistini L, Rotzschke O, Falk K. CCR6 expression defines regulatory effector/memory-like cells within the CD25(+)CD4+ T-cell subset. Blood. 2005;105:2877-2886.
10. Borsellino G, Kleinewietfeld M, Di Mitri D, et al. Expression of ectonucleotidase CD39 by Foxp3+ Treg cells: hydrolysis of extracellular ATP and immune suppression. Blood. 2007;110:1225-1232.

Response

Is CD103 a good surface marker for in vivo–activated effector/memory Treg cells in patients with chronic inflammation? Unknown yet

We observed that the percentage of $CD103^+$ cells among $CD4^+FoxP3^+$ regulatory T (Treg) cells of donor DBA/2 mice was less than 10% before transplantation, and the percentage of $CD103^+$ cells among donor-type $CD4^+Foxp3^+$ Treg cells increased to more than 55% in BALB/c recipients with chronic graft-versus-host disease (GVHD) and 70% in recipients without chronic GVHD after hematopoietic cell transplantation (HCT).[1] We also observed that the percentage of $CD103^+$ cells among $Foxp3^+CD4^+$ Treg cells in donor C57BL/6 mice was approximately 20% (mean ± SE: 20.7 ± 1.7%, N = 4) before HCT, and the percentage of $CD103^+$ Treg cells increased to around 40% (mean ± SE: 44.3 ± 2.0%, N = 4) in BALB/c recipients with acute GVHD. These results indicate that inflammation, especially chronic inflammation, can augment the expression of CD103 on mouse $Foxp3^+$ Treg cells, although the percentage of $CD103^+$ cells among $Foxp3^+$ Treg cells can differ up to certain levels in different mouse strains or different housing environments, because the percentage of $CD103^+$ Treg cells in our DBA/2 mice is around 10%, but the percentage of our C57BL/6 mice is around 20%; the percentage of $CD103^+$ Treg cells in our C57BL/6 mice is approximately 20%, but the percentage in C57BL/6 mice in Hühn's reports is approximately 30%.[2]

It has been well documented that the percentage of $CD103^+$ cells among $Foxp3^+CD4^+$ Treg cells in healthy humans is low, around 5%.[3-5] However, it is still unknown whether $Fopx3^+$ Treg cells in human HCT recipients express CD103. It has been shown that the percentage of $CD103^+$ cells among $CD25^{hi}CD4^+$ T cells was significantly higher than that among $CD25^-CD4^+$ T cells in patients with multiple sclerosis.[6] In addition, in vitro TGF-β–induced Treg cells were all $CD103^+$.[7] TGF-β secretion is usually up-regulated in patients with chronic inflammation.[8] Therefore, although most of the $Foxp3^+$ Treg cells in normal humans do not express CD103, we cannot exclude the possibility that $Foxp3^+$ Treg cells in patients with chronic inflammation (such as chronic GVHD) will up-regulate CD103 expression. Because it has been reported that effector/memory $Foxp3^+$ Treg cells express CCR6 in mice[9] and they express CD39 in multiple sclerosis patients,[10] comparison of expression of CD103, CCR6, and CD39 by $Foxp3^+$ Treg cells in patients with chronic inflammation is required to find out which one is best for identifying the Foxp3 Treg cells.

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References

1. Zhao D, Zhang C, Yi T, et al. In vivo-activated $CD103^+CD4^+$ regulatory T cells ameliorate ongoing chronic graft-versus-host disease. Blood. 2008;112:2129-2138.
2. Siewert C, Lauer U, Cording S, et al. Experience-driven development: effector/memory-like alphaE^+Foxp3^+ regulatory T cells originate from both naive T cells and naturally occurring naive-like regulatory T cells. J Immunol. 2008;180:146-155.
3. Iellem A, Colantonio L, D'Ambrosio D. Skin-versus gut-skewed homing receptor expression and intrinsic CCR4 expression on human peripheral blood $CD4^+CD25^+$ suppressor T cells. Eur J Immunol. 2003;33:1488-1496.
4. Lim HW, Broxmeyer HE, Kim CH. Regulation of trafficking receptor expression in human forkhead box $P3^+$ regulatory T cells. J Immunol. 2006;177:840-851.
5. Allakhverdi Z, Fitzpatrick D, Boisvert A, et al. Expression of CD103 identifies human regulatory T-cell subsets. J Allergy Clin Immunol. 2006;118:1342-1349.
6. Venken K, Hellings N, Thewissen M, et al. Compromised $CD4^+$ CD25(high) regulatory T-cell function in patients with relapsing-remitting multiple sclerosis is correlated with a reduced frequency of FOXP3-positive cells and reduced FOXP3 expression at the single-cell level. Immunology. 2008;123:79-89.
7. Rao PE, Petrone AL, Ponath PD. Differentiation and expansion of T cells with regulatory function from human peripheral lymphocytes by stimulation in the presence of TGF-{beta}. J Immunol. 2005;174:1446-1455.
8. Toda M, Leung DY, Molet S, et al. Polarized in vivo expression of IL-11 and IL-17 between acute and chronic skin lesions. J Allergy Clin Immunol. 2003; 111:875-881.
9. Lee JH, Kang SG, Kim CH. $FoxP3^+$ T cells undergo conventional first switch to lymphoid tissue homing receptors in thymus but accelerated second switch to nonlymphoid tissue homing receptors in secondary lymphoid tissues. J Immunol. 2007;178:301-311.
10. Borsellino G, Kleinewietfeld M, Di Mitri D, et al. Expression of ectonucleotidase CD39 by $Foxp3^+$ Treg cells: hydrolysis of extracellular ATP and immune suppression. Blood. 2007;110:1225-1232.