deletions and result in a frameshift into the alternative reading frame 1. Familial predisposition to MPNs also frequently leads to somatic JAK2-V617F mutations that are found in the majority of the affected family members.5 Because polycythemia vera (PV), ET, and PMF phenotypes can be observed in these pedigrees, we tested whether mutations in CALR also occur in familial MPNs.

We studied 12 pedigrees with familial MPNs in which at least 1 affected family member carried a somatic JAK2-V617F mutation, and we also examined 11 pedigrees with hereditary thrombocytosis (HT), in which we excluded mutations in the THPO, MPL, and JAK2 genes (data not shown). We found a CALR mutation in 3 of the 12 familial MPN pedigrees (Figure 1A). In each of these 3 pedigrees, 1 affected family member carried a 52-base deletion in exon 9 of the CALR gene (not shown), which represents the most frequent form of the CALR mutations (type 1). One of these patients was initially diagnosed with ET7 and later progressed to PMF (MPN family 1), and the two remaining patients had ET (MPN families 2 and 3). All CALR mutations occurred in patients with an MPN diagnosis, whereas a somatic JAK2-V617F mutation was found in 1 healthy family member with platelets in the upper normal range but otherwise normal blood counts (MPN family 5; data not shown). A similar finding was reported previously.8 We did not detect CALR mutations in any of the 11 families with HT and a total of 44 affected and family members (Figure 1B).

In the 12 pedigrees with familial MPNs, all affected family members carried either a somatic JAK2-V617F mutation or a mutation in CALR. In MPN family 1, the underlying predisposition is inherited as an autosomal dominant trait with low penetrance (Figure 1A), whereas in other pedigrees, the mode of transmission is more difficult to determine because too few family members in too few generations were available (eg, MPN families 2 and 3). Two mechanistic models have been proposed regarding how such a germline predisposition may result in MPNs with clonal hematopoiesis.9,10 First, the germline mutation could increase the mutation rate in JAK2 and CALR genes (hypermutability hypothesis), or second, it could synergize with JAK2-V617F or CALR in MPN disease initiation (fertile ground hypothesis). Because the G>T transition in JAK2-V617F and the 52-base deletion in CALR are mechanistically very different, it seems unlikely that they could be promoted by the same germline mutation through a hypermutability mechanism. Therefore, our findings favor the fertile ground hypothesis of germline predisposition to MPN.

To the editor:

Physical activity limits pulmonary endothelial activation in sickle cell SAD mice

Vaso-occlusion (VOC) in sickle cell disease (SCD) results from many pathophysiological mechanisms including sickling of red blood cells, hemolysis, inflammation, vascular adhesion, and reduced nitric oxide (NO) bioavailability.1 All these mechanisms interact together to trap blood cells and to accentuate the local blood flow decrease and subsequent local hypoxia.

In healthy and pathological conditions, physical training results in physiological and molecular adaptations including anti-inflammatory

References


Acknowledgments: This work was supported by grants 310000-120724/1 and 32003BB_135712/1 from the Swiss National Science Foundation and KLS-02398-02-2009 from the Swiss Cancer League (R.C.S.).

Contribution: P.L. designed and performed research, analyzed data, and wrote the paper; R.N. performed research and analyzed data; A.A., F.C., and M.M.P.-E. provided clinical data and patient samples and analyzed results; and R.C.S. designed research, analyzed data, and wrote the paper.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

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Figure 1. Effect of HR stress in PA or CON C57Bl/6 (C57; n = 7 per group) and SAD mice (n = 6 per group). Eight weeks of a voluntary wheel-running protocol were conducted in C57 and SAD mice. Before euthanizing the animals, HR stress (4 hours hypoxic stress at 6.5% oxygen followed by 2 hours of reoxygenation in ambient air) was used to stimulate the pathophysiological parameters known to trigger the VOC in this mouse model. (A) P-selectin and (B) VCAM-1 immunostaining scores in the lung. Immunohistologic slides were blindly semiquantified by 3 experienced anatomists for VCAM-1 and P-selectin with the staining intensity. For each vessel (15 to 20 vessels per mouse), a score from 0 to 3 was attributed (0, no immunostaining; 1, <25% vessel staining; 2, <50% vessel staining; 3, >50% vessel staining). For each mouse, the immunostaining score was the mean of each vessel score from a section. (C) Representative staining for VCAM-1 and P-selectin in the blood vessel of lungs. Magnification ×40. (D) Nitrite/nitrate (NOx) concentrations in the lungs. The Griess method was used: the sum of nitrite and nitrate in the plasma is considered an index of NO production. (E) Plasma concentrations of lactate dehydrogenase (LDH). *P < .05; #, P < .05 vs C57CONHR; §, P < .05 vs C57PAHR; a, P = .07. Values are means ± standard deviation. All variables were tested for normality and variance homogeneity. P-selectin immunostaining and NOx concentrations were tested with nonparametric Kruskall-Wallis test followed by Mann-Whitney U test. The other variables were compared using factorial analysis of variance followed by planned comparisons. Nhx, normoxic conditions.
effects and endothelial activation limitations. In SCD, these adaptations could be beneficial by limiting physiological factors involved in the VOC. In previous studies, we demonstrated that physical training blunted plasma soluble vascular cell adhesion-1 (VCAM-1) and improved NO bioavailability in sickle cell trait carriers. The present study investigated the effects of chronic physical activity at baseline and in response to a VOC stress in the lungs of sickle SAD mice.

Healthy (C57Bl/6J) and SAD male mice were used in this study. The SAD transgene reproduces the hypoxia-induced vaso-occlusive events of human SCD. Control mice (CON) lived in standard mouse cages, whereas physical activity mice (PA) were housed in cages equipped with running wheels. After 8 weeks, mice were euthanized either after normoxic conditions or after exposure to an acute 4 hours of vaso-occlusive hypoxic stress followed by 2 hours of reoxygenation in ambient air (HR).

Interestingly, baseline P-selectin expression in the lungs (Figure 1A,C) was more evident in SADCON than in SADPA mice. Because P-selectin is involved in leukocytes and sickle cells trapping initiation, this decreased expression with physical activity may lead to a beneficial reduction in cell aggregate formation and consequently the risk of VOC. Contrary to SADCON mice, the expression of the adhesion molecule VCAM-1 in the PA group was not increased by HR stress (Figure 1B), suggesting that physical activity could limit pulmonary endothelial activation.

Endothelial activation has been shown to be notably mediated by decreased NO bioavailability. After HR stress, higher concentrations of NOx were measured in the lungs of SADPA compared with SADCON (+63%, Figure 1D). These results strongly suggest that physical activity could improve pulmonary endothelial function, as already observed in healthy subjects. This improvement of NO metabolism may also participate in the decreased VCAM-1 expression observed after HR, via nuclear factor kB inhibition. Hemolysis is also known to trigger endothelial activation and vasculopathy. After HR, plasma LDH concentrations increased only in SADCON mice, whereas no significant variation was observed in the SADPA group, suggesting that PA could limit HR-induced hemolysis in SAD mice.

In conclusion, this study is the first to demonstrate the beneficial effects of physical activity in response to an HR stress in SCD mice. Similar exercise programs could be a relevant option to prevent pulmonary complications in SCD patients because it has been observed in SCD subjects that moderate acute exercise does not alter their hemorheologic, oxidative stress, or hematologic parameters. From these results, further studies performed on more severe SCD mice models and in SCD patients will have to confirm the efficiency and/or the benefits of PA in SCD, and to describe the underlying mechanisms of the therapeutic effect of exercise training in SCD.

**References**


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Physical activity limits pulmonary endothelial activation in sickle cell SAD mice

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