Published in final edited form as:
Cell Metab. 2015 May 5; 21(5): 650–651. doi:10.1016/j.cmet.2015.04.014.

Ether Lipid Deficiency Does Not Cause Neutropenia or Leukopenia in Mice and Men

Fabian Dorninger[1], **Christoph Wiesinger**[1], **Nancy E. Braverman**[2], **Sonja Forss-Petter**[1], and **Johannes Berger**[1,*]

[1]Department of Pathobiology of the Nervous System, Center for Brain Research, Medical University of Vienna, 1090 Vienna, Austria

[2]Department of Human Genetics and Pediatrics, McGill University-Montreal Children's Hospital, Montreal, QC H3Z 2Z3, Canada

In a recent report in *Cell Metabolism*, Lodhi and colleagues present intriguing findings on the importance of fatty acid metabolism for the hematopoietic system (Lodhi et al., 2015). It is convincingly shown that mice with a conditional deficiency either in FAS (fatty acid synthase) or in PexRAP (peroxisomal reductase activating PPARγ) have drastically reduced levels of white blood cells, in particular concerning neutrophil granulocytes, but also lymphocytes. From the facts that PexRAP is involved in ether phospholipid biosynthesis and that *Fas* knockout (KO) mice show a decrease in several choline-containing ether lipid species, the authors conclude that the observed loss of neutrophils is caused by the deficit in ether lipids. This came as a surprise to us considering that neutropenia or leukopenia has not been described in any of the previously established mouse models of ether lipid deficiency or in case reports of human patients suffering from rhizomelic chondrodysplasia punctata (RCDP), resulting from inborn errors in peroxisomal ether lipid biosynthesis.

Prompted by this apparent discrepancy, we set out to clarify whether the absence of ether lipids is indeed sufficient to cause severe neutropenia. To this end, we employed a mouse model of complete ether lipid deficiency (Rodemer et al., 2003). These animals lack glyceronephosphate O-acyltransferase (GNPAT; alias: DAPAT, DHAPAT), the first enzyme in the ether lipid biosynthesis pathway, resulting in a complete inability to synthesize ether lipids. Strikingly, we found no abnormalities in the counts of neutrophils, lymphocytes, or total white blood cells in peripheral blood from the tail of male *Gnpat* KO mice (Figure S1A) or in samples collected by cardiac puncture from female *Gnpat* KO and wild-type littermates (Figure S1B).

Our results indicate that the mechanism leading to neutropenia and leukopenia, as observed by Lodhi and collaborators, presumably cannot be explained by ether lipid deficiency alone. Instead, it seems likely that other factors, apart from a reduction in ether lipids, contribute to the observed phenotype. PexRAP has been described as an enzyme that catalyzes the reduction of 1-O-alkyl-dihydroxy-acetonephosphate (1-O-alkyl-DHAP) (Lodhi et al., 2012). Therefore, it is conceivable that the observed apoptosis of neutrophils could be caused by a

*Correspondence: johannes.berger@meduniwien.ac.at.

Europe PMC Funders Author Manuscripts

toxic accumulation of the intermediate 1-O-alkyl-DHAP due to the disruption of PexRAP (official gene symbol: *Dhrs7b*). So far, no other substrates for PexRAP have been identified; however, these may exist and, thus, upon PexRAP deficiency, pathways other than ether lipid biosynthesis could be affected. For example, in the original description of PexRAP function, the same authors also reported that the levels of several nonether phospholipids are diminished after knockdown of PexRAP and, more importantly, that the lack of PexRAP function has major consequences for PPARγ signaling (Lodhi et al., 2012).

A similar phenotype concerning white blood cell counts as in the conditional PexRAP KO mice was also found in conditional *Fas* KO mice. However, in these animals, a wide variety of metabolic pathways and functions must be expected to be disturbed, thereby potentially influencing blood cell counts. For example, based on the data presented by Lodhi and collaborators, there appears to be a general depletion of choline phospholipids, which might also contribute to membrane alterations and cause the severe neutro- and leukopenia observed in these mice.

Gnpat KO mice differ from the inducible KO models described by Lodhi and collaborators in that they represent a model of complete ether lipid deficiency that is present from birth on and affects all tissues. First, it cannot be excluded that toxicity caused by the induction of Cre recombinase contributes to the described hematological abnormalities. It has been shown previously that the activation of Cre by tamoxifen treatment produces a variety of detrimental changes in the hematopoietic system (Higashi et al., 2009), in particular when analyzed within a short time frame after the treatment. Although lower tamoxifen concentrations, as used by Lodhi and colleagues, may attenuate these problems (Higashi et al., 2009; Uhmann et al., 2009), a similar effect could account for some of the alterations that were observed in both inducible KO models. Indeed, the authors consider this possibility in their paper; however, their conclusions may be challenged, as the presented experiments allow alternative interpretations. Lodhi and collaborators show results derived from heterozygous mice ($PexRAP^{flox/WT}$; see Table S3 in Lodhi et al., 2015), demonstrating remarkable, even if not statistically significant, decreases in white blood cells upon Cre induction. They attribute these alterations to a gene dosage effect, but in light of the observations reported by Higashi and collaborators, toxicity resulting from Cre activation may also contribute to the observed phenotype. This issue could only be solved by a comparison of $PexRAP^{WT/WT}$ (−CreER) and $PexRAP^{WT/WT}$ (+CreER) mice.

Furthermore, the blood cell investigations in conditional PexRAP and *Fas* KO mice were conducted a few days after the initiation of tamoxifen treatment (induction of the knockout), a time frame that may not allow for blood cells and their progenitors to adapt to the changed condition, leading to neutrophil loss. We cannot exclude that in *Gnpat* KO mice, such adaptation processes to the condition of ether lipid deficiency occur and counteract the loss of neutrophils and leukocytes observed in the inducible models. Especially in light of possible therapeutic implications of the findings of Lodhi and collaborators, these considerations could be of high relevance.

In order to confirm the results derived from the *Gnpat* mouse model, we also obtained data from patients with RCDP that had been deposited in an RCDP database at the RhizoKids

registry. Also upon inherited ether lipid deficiency in humans, no signs of neutropenia or leukopenia could be observed (Figure S1C). A trend toward increased leukocyte and neutrophil levels can be explained by the fact that the presented data were recorded in a clinical setting and that these patients frequently suffer from respiratory tract infections.

Due to the potential importance of the findings of Lodhi and colleagues for a variety of abundant disease conditions, caution is warranted for the interpretation of these data. Therefore, pathways other than the disruption of ether lipid biosynthesis, which—as we have demonstrated here—alone is not sufficient to induce severe neutropenia, may need to be explored in further studies to understand in greater detail the mechanisms underlying the observed phenotype.

Supplemental Information

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors thank Gerhard Zeitler for technical assistance. Funding was provided by the Austrian Science Fund (FWF; P 24843-B24). The RCDP patient registry and natural history study is funded by RhizoKids International, a non-profit organization. The registry is maintained at the Skeletal Dysplasia Center, Nemours Children's Hospital, Wilmington, Delaware, USA.

References

Higashi AY, Ikawa T, Muramatsu M, Economides AN, Niwa A, Okuda T, Murphy AJ, Rojas J, Heike T, Nakahata T, et al. J Immunol. 2009; 182:5633–5640. [PubMed: 19380810]

Lodhi IJ, Yin L, Jensen-Urstad AP, Funai K, Coleman T, Baird JH, El Ramahi MK, Razani B, Song H, Fu-Hsu F, et al. Cell Metab. 2012; 16:189–201. [PubMed: 22863804]

Lodhi IJ, Wei X, Yin L, Feng C, Adak S, Abou-Ezzi G, Hsu FF, Link DC, Semenkovich CF. Cell Metab. 2015; 21:51–64. [PubMed: 25565205]

Rodemer C, Thai TP, Brugger B, Kaercher T, Werner H, Nave KA, Wieland F, Gorgas K, Just WW. Hum Mol Genet. 2003; 12:1881–1895. [PubMed: 12874108]

Uhmann A, Dittmann K, Wienands J, Hahn H. J Immunol. 2009; 183:2891. author reply 2891–2892. [PubMed: 19696426]

Europe PMC Funders Author Manuscripts