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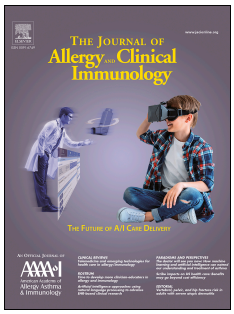

Macrophages and acylcarnitines; New players in aspirin-exacerbated respiratory disease?

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PII: S0091-6749(20)31498-6

DOI: https://doi.org/10.1016/j.jaci.2020.09.040

Reference: YMAI 14811

To appear in: *Journal of Allergy and Clinical Immunology*

Received Date: 6 August 2020

Revised Date: 10 September 2020

Accepted Date: 18 September 2020

Please cite this article as: Laidlaw TM, Balestrieri B, Macrophages and acylcarnitines; New players in aspirin-exacerbated respiratory disease?, *Journal of Allergy and Clinical Immunology* (2020), doi: https://doi.org/10.1016/j.jaci.2020.09.040.

Editorial.

Macrophages and acylcarnitines; New players in aspirin-exacerbated respiratory disease?

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Conflict of Interest: T Laidlaw has served on scientific advisory boards for GlaxoSmithKline and Sanofi-Genzyme, Optinose, and Regeneron. B Balestrieri has no conflicts of interest.

Key words: AERD, macrophages, type 2 inflammation, acylcarnitines, fatty acids, eicosanoids.

In this issue, the authors of a new study report an inflammatory macrophage memory and subsequent overproduction of acylcarnitines that may contribute to the respiratory pathology present in aspirin-exacerbated respiratory disease (AERD) (1). In a field long-dominated by discussions of eosinophils, mast cells, Type 2 cytokines and cysteinyl leukotrienes, this study is strong evidence of the power of enterprise and creativity in science. It presents a number of surprising findings that will, hopefully, serve as a new guide for other researchers to follow novel paths of investigation. The immunological macrophage memory described is immediately evident in the RNAseq analysis comparing alveolar-like monocyte-derived macrophages (aMDM) from patients with AERD to those from healthy donors, which shows that despite a week of in vitro differentiation, the cells from AERD patients have a persistent upregulation of chemotaxis-related genes and downregulation of host defense-related genes. The abnormally activated state of macrophages they identify in AERD is notable for the increased production of cytokines and chemokines and the increased release of proinflammatory lipids. The lipids responsible for the macrophages' activation state include lipids derived from the metabolism of arachidonic acid, particularly leukotrienes produced by 5-lipoxygenase (LO), as previously reported (2), but also acylcarnitine metabolites, which are newer players in this story **(Figure 1)**. The authors also show that compared to healthy individuals, aMDM from AERD patients produce more polyunsaturated fatty acids, and have increased fatty acid oxidation. The consequence of this elevated fatty acid oxidation in AERD is supported by metabolomic data showing reduced methylation of genes involved in fatty acid/acylcarnitine metabolism, and increased levels of acylcarnitine metabolites found in the nasal fluid, sputum, and plasma of AERD patients.

By comparing the transcriptome of macrophages isolated from induced sputum (sMAC) to that of aMDMs differentiated from blood $CD14^+$ monocytes, the authors aimed to determine whether the aberrant macrophage phenotype was confined to the local respiratory tissues or was

reflective of a systemic abnormality. They report a leukotriene-dominant eicosanoid profile in the aMDMs and a 15-LO-dominant profile in the sMACs. Since sMAC are freshly isolated, their proinflammatory memory is dictated by the proinflammatory environment in which they develop (3). However, aMDMs are cultured in granulocyte-macrophage colony-stimulating factor and transforming growth factor-β, which increase the expression of 5-LO and leukotriene C4 synthase (LTC_4-synthase) (4, 5). Therefore, they concluded that the persistent pro-inflammatory macrophage phenotype was present in both the airways and the blood of AERD patients. Upon stimulation with Ca^{++} ionophone, the aMDMs differentiated from AERD patients produced significantly more arachidonic acid- and 5-lipoxygenase-derived metabolites than did aMDMs from healthy controls. Upon stimulation with lipopolysaccharide (LPS), those AERD aMDMs also released more acylcarnitine metabolites than did aMDMs from healthy controls. The overproduction of arachidonic acid-derived eicosanoids is well known in AERD, but the potential for overproduction of acylcarnitines has not been thoroughly studied in this disease. To explore whether cellular changes in metabolite profiles were reflected systemically, the authors performed a metabolomics analysis of plasma from patients with AERD, aspirin-tolerant nasal polyposis, and healthy controls, which revealed that AERD plasma has notably higher levels of sphingomyelins and medium- and long-chain acylcarnitines. This suggests that AERD involves much broader dysregulations in lipid metabolism than has been previously appreciated.

Stimulation of aMDM from AERD patients with LPS and C14-carnitine induced an M2 activation state, a spectrum of activation associated with type 2 inflammation, with upregulation of CCL17 but not of TGM2 or MRC1, two major markers of M2 activation (6). However, both molecules were minimally upregulated by C14-carnitine alone. Since fatty acid oxidation is associated with M2 activation (7), it would be interesting to ascertain with future experiments whether IL-4 +/- C14-carnitine would elicit a novel spectrum of M2 activation in aMDM derived from AERD patients compared to those derived from healthy controls (8). In order to definitively prove that

tissue macrophages in patients with AERD exist in an altered metabolic state of increased activation, further ex vivo studies need to be pursued that include direct isolation of macrophages from the nasal polyps. Ideally, we would want to see comparisons between the macrophages isolated from nasal polyp tissue of patients with AERD and those from patients with aspirin-tolerant chronic rhinosinusitis with nasal polyposis and non-polyp sinus tissue as well. In addition to replicating some of the stimulation assays presented in this study, additional disease-specific experiments would also be of interest, including an exploration of whether aspirin or another cyclooxygenase-1 inhibitor might induce the same transcriptional and metabolic changes that LPS ± C14-carnitine did in the aMDMs examined in this study. The results of that ex vivo cellular aspirin challenge would then inform clinical researchers regarding the potential value in checking polyunsaturated fatty acids and acylcarnitine levels in the nasal fluid and serum of patients with AERD who undergo aspirin challenges and subsequent aspirin-induced reactions.

As with any excellent translational research project, the results presented by the authors here lead us to more questions than answers. First, elevated rates of fatty acid oxidation and acylcarnitine overproduction are often considered in the context of insulin resistance and metabolic disorders, and the authors regarded the potential role of obesity in their study but found no correlation. Acylcarnitines are key regulators of the balance of intracellular sugar and lipid metabolism. Therefore, the finding that glucose metabolism is not abnormal in AERD confirms that the main acylcarnitine pathway altered is dependent on the supply of free fatty acids **(Figure 1)**. Certain chronic inflammatory conditions can also trigger a metabolic reprogramming that upregulates fatty acid oxidation in myeloid cells.(9) The data from this study suggest that the respiratory inflammation in AERD may indeed either lead to or be the result of increased rates of myeloid fatty acid oxidation, and imply that several free fatty acid-induced pathways may be responsible for the inflammatory memory in the macrophages of AERD

patients **(Figure 1)**. Second, one general mechanistic question, which is nearly impossible to answer with human case-control studies, is whether the macrophage immunological memory is causative of the chronic respiratory inflammation in AERD, or a consequence of that chronic inflammation. Attacking this question will likely involve an interventional trial. There may be murine models to guide us, as there have been several studies of pharmacologic inhibition of fatty acid oxidation in murine models of asthma that suggest potential utility. Etomoxir, an inhibitor of the fatty acid oxidation rate-limiting enzyme carnitine palmitoyltransferase-1 (CTP1) that decreases long-chain acylcarnitine production, was used as a treatment thirty minutes after ovalbumin challenge in an ovalbumin-based mouse asthma model. This intervention provided a significant reduction in the recruitment of eosinophils and macrophages into the lungs, and a protection against ovalbumin-induced hyperresponsiveness that was associated with a decrease in Th2 cytokine production (9). Unexpectedly, etomoxir inhibited IL-4-dependent M2 macrophage polarization only at high concentration (200uM), suggesting off-target effects, likely dependent on the reduced availability of free CoA (10). Therefore, although the clinical use of etomoxir itself may be limited due to side effects, there are several other pharmacologic agents in this pathway that are currently under investigation for use in a number of diseases – perhaps AERD should be one of them? Additionally, since fatty acid oxidation is a catabolic pathway secondary to the increase in fatty acid supply, any new therapies for AERD in this area should also take into account limiting various sources of fatty acids.

References

1. Haimerl P, Bernhardt U, Schindela S, Henkel FD, Lechner A, Zissler UM, et al. Inflammatory macrophage memory in NSAID-exacerbated respiratory disease. The Journal of allergy and clinical immunology. 2020.
2. Laidlaw TM, Boyce JA. Aspirin-Exacerbated Respiratory Disease--New Prime Suspects. The New England journal of medicine. 2016;374(5):484-8.
3. Gosselin D, Link VM, Romanoski CE, Fonseca GJ, Eichenfield DZ, Spann NJ, et al. Environment drives selection and function of enhancers controlling tissue-specific macrophage identities. Cell. 2014;159(6):1327-40.
4. Lukic A, Larssen P, Fauland A, Samuelsson B, Wheelock CE, Gabrielsson S, et al. GM-CSF- and M-CSF-primed macrophages present similar resolving but distinct inflammatory lipid mediator signatures. FASEB J. 2017;31(10):4370-81.
5. Steinhilber D, Radmark O, Samuelsson B. Transforming growth factor beta upregulates 5-lipoxygenase activity during myeloid cell maturation. Proc Natl Acad Sci U S A. 1993;90(13):5984-8.
6. Martinez FO, Helming L, Milde R, Varin A, Melgert BN, Draijer C, et al. Genetic programs expressed in resting and IL-4 alternatively activated mouse and human macrophages: similarities and differences. Blood. 2013;121(9):e57-69.
7. Vats D, Mukundan L, Odegaard JI, Zhang L, Smith KL, Morel CR, et al. Oxidative metabolism and PGC-1beta attenuate macrophage-mediated inflammation. Cell metabolism. 2006;4(1):13-24.
8. Xue J, Schmidt SV, Sander J, Draffehn A, Krebs W, Quester I, et al. Transcriptome-based network analysis reveals a spectrum model of human macrophage activation. Immunity. 2014;40(2):274-88.
9. Al-Khami AA, Ghonim MA, Del Valle L, Ibba SV, Zheng L, Pyakurel K, et al. Fuelling the mechanisms of asthma: Increased fatty acid oxidation in inflammatory immune cells may represent a novel therapeutic target. Clin Exp Allergy. 2017;47(9):1170-84.
10. Divakaruni AS, Hsieh WY, Minarrieta L, Duong TN, Kim KKO, Desousa BR, et al. Etomoxir Inhibits Macrophage Polarization by Disrupting CoA Homeostasis. Cell metabolism. 2018;28(3):490-503 e7.

Figure 1. Sources of fatty acids in macrophages and implications in AERD. Fatty acids originate from phospholipase A_2 hydrolysis of membrane glycerophospholipids, which generates lysophospholipids, including lysophosphatidylcholine, and various free fatty acids including arachidonic acid, which is then metabolized to eicosanoids. Lipid droplets (yellow drops) store fatty acids in the cell and fatty acid transporters allow free fatty acids to enter the cell. Acylcarnitines are generated in mitochondria through CPT1 (Carnitine palmitoyl-transferase 1). Underlined are the mediators increased in AERD macrophages at baseline (purple) or after stimulation, with LPS (red), Ca^{++} ionophore (brown-green), C14-carnitine+LPS (blue), and acylcarnitine mix (bright green). In gray font are the mediators of the prostaglandin pathway, of which PGE_2 has been found to be decreased in AERD.

LA (linoleic acid), EPA (eicosapentaenoic acid), DHA (docosahexaenoic acid), 11/13-HDHA (11/13-hydroxy docosahexaenoic acid), HODE (Hydroxyoctadecadienoic acid), HEPE (Hydroxyeicosapentaenoic acid), HETE (Hydroxyeicosatetraenoic acid), HPETE (Hydroperoxyeicosatetraenoic acid), lipoxygenase (LO) , PG (prostaglandin), LT (leukotriene), COX (cyclooxygenase), FFA (free fatty acid), FLAP (5-lipoxygenase-activating protein).