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Let's Enter the Wonderful World of Immunometabolites

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Over the past decade, cancer metabolism research taught us that metabolites are much more than intermediate or end products of metabolism. As such, the name 'oncometabolite' emerged. Immunometabolism research has developed tremendously over the past few years and, in analogy to the cancer metabolism field, the term 'immunometabolite' has been used for different metabolites and purposes. Here, we propose a definition for the term 'immunometabolite' and provide some historical background and future perspectives on this matter. By doing so, we aim to increase interest in this fast-expanding field and to encourage further research.

The term 'oncometabolite' was first used in the scientific literature by Ward *et al.* in reference to R-2-hydroxyglutarate (R-2HG) in 2010 and now also refers to the metabolically adjacent and structurally similar intermediates succinate, fumarate, and the *S*-2-hydroxyglutarate enantiomer [1] (Figure 1, bottom). The term also refers to intermediates of metabolism that aberrantly accumulate in cancer cells downstream or upstream of mutated genes that encode metabolic enzymes. The increased abundance of oncometabolites can promote tumorigenesis via distinct routes, including immunosuppression [2]. Within cancer cells, these metabolites can drive epigenetic remodeling and trigger oncogenic signaling cascades that contribute to tumorigenesis. Moreover, metabolic rewiring in malignant cells can also affect other cells in the tumor microenvironment (TME) through nutrient competition and the action of oncometabolites as signaling molecules. As such, tumor cell-derived metabolites can negatively influence immune cells to shape a TME that hijacks antitumor immune responses. As an example, R-2-hydroxyglutarate (R-2HG) derived from isocitrate dehydrogenase 1 (*IDH1*)-mutant glioma cells can be actively taken up by T cells, where it results in the suppression of antitumor T cell activities via the perturbation of various signaling cascades [3]. Similarly, lactate produced by tumor cells can induce arginase-1 activity and vascular endothelial growth factor (VEGF) production in tumor-associated macrophages (TAMs), which support tumor growth [4].

Importantly, immune cells also undergo extensive metabolic rewiring upon activation. As in cancer cells, these metabolites serve many roles beyond energy production and directly drive immune cell phenotype and function. Within immune cells, metabolic intermediates can serve as cofactors for (metabolic) enzymes and can mediate post-translational modifications of histones, transcription factors, enzymes, and other key proteins. Moreover, immune cell-derived 'immunometabolites' can leave the cell and signal endogenously to neighboring cells in a cytokine-like manner to affect the local microenvironment and possibly also the systemic environment. In analogy with oncometabolites, immunometabolites can be defined as metabolic intermediates that accumulate in immune cells upon activation. Their overabundance then regulates intracellular pathways that drive the phenotype and specific functions of the cell. The term 'immunometabolite' was first applied to *S*-2-hydroxyglutarate (*S*-2HG) by Tyrakis *et al.* when they observed that it accumulates in $CD8^+$ T cells upon T cell receptor triggering [5]. *S*-2HG production in these cells is regulated by hypoxia-inducible factor (HIF)1α and alters T cell differentiation and proliferation through the modulation of histone and DNA methylation (Figure 1, top left).

Again in the T cell field, Xu *et al.* later discovered that 2HG also regulates the balance between proinflammatory T helper 17 (Th17) cells and anti-inflammatory induced regulatory T (iTreg) cells, in favor of the former [6]. In their search for compounds that reprogram Th17 differentiation towards iTreg cells, they found that 2HG increases in Th17 cells, resulting in the inhibition of demethylases, hypermethylation, and associated inhibition of *Foxp3* gene expression, which is crucial for differentiation towards Th17 cells. Accordingly, inhibition of 2HG production with the small molecule (aminooxy)acetic acid (AOA) blocked Th17 and promoted iTreg differentiation to improve experimental autoimmune encephalomyelitis as a model for multiple sclerosis (MS).

The term 'immunometabolite' was also used by Ma *et al.* for serine when they discovered that this amino acid is an essential metabolite for effector T cell proliferation [7]. However, we would like to encourage the use of the term 'immunometabolite' solely for intermediates that are produced by immune cells upon activation, rather than for exogenous metabolites that affect immune cell function. Otherwise, almost every cellular metabolite could be categorized as an immunometabolite. According to this strict definition, L-2HG, succinate, and itaconate are probably the most recognized and studied immunometabolites currently. While 2HG shapes T cell responses, as discussed earlier, the latter two are crucial controllers of macrophage activation.

Similar to T cells, macrophages come in different 'flavors'. Macrophages stimulated with the bacterial outer membrane molecule lipopolysaccharide (LPS) in the presence and/or absence of the cytokine

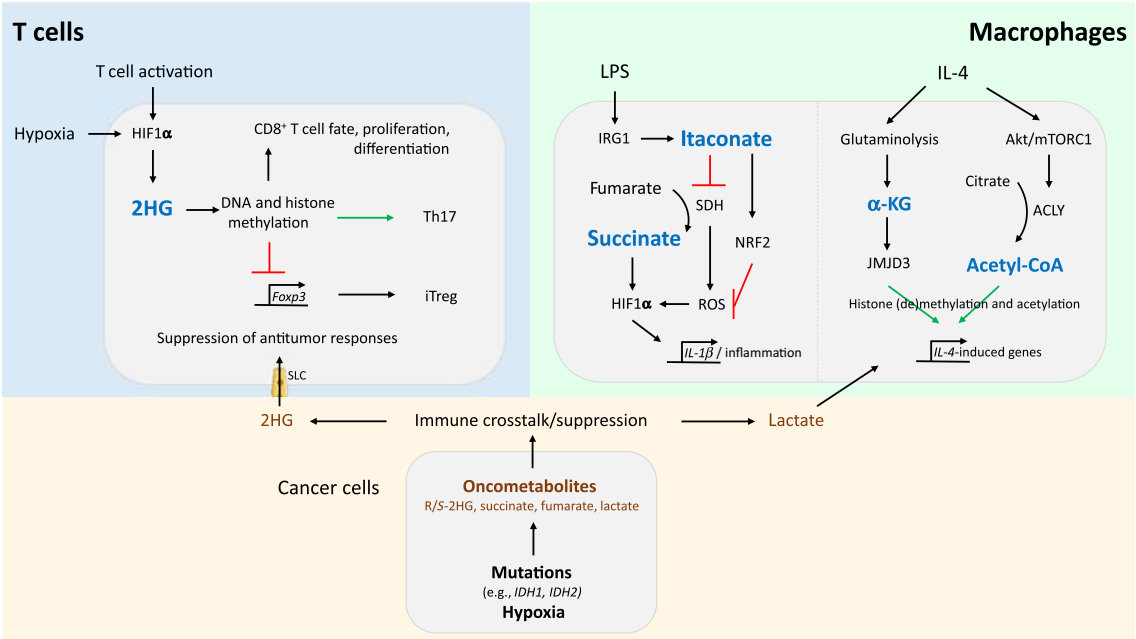

Figure 1. Immunometabolites Accumulate in Immune Cells upon Activation and Regulate Cell Fate and Function. 2-hydroxyglutarate (2HG) emerged as a key immunometabolite that regulates T cell fate. Succinate promotes lipopolysaccharide (LPS)-induced inflammatory responses, whereas itaconate acts as an anti-inflammatory metabolite. α-ketoglutarate and acetyl-CoA were demonstrated to support interleukin (IL)-4-induced macrophage responses via metabolic and/or epigenetic crosstalk. Metabolites (both oncometabolites and immunometabolites) can be released and signal to neighboring cells in the microenvironment. Abbreviations: ACLY, ATP citrate lyase; iTreg, induced regulatory T cell; R-2HG, R-2-hydroxyglutarate; ROS, reactive oxygen species; *S*-2HG, S-2-hydroxyglutarate; SDH, succinate dehydrogenase; SLC, solute carriers; Th17, T helper 17 cell.

interferon (IFN)-γ are prototypical proinflammatory macrophages [$M_{(LPS+/-IFN\gamma)}$], whereas interleukin (IL)-4-treated cells are the most commonly studied model for anti-inflammatory macrophages [$M_{(IL-4)}$]. Akin to cancer cells, inflammatory macrophages show a Warburg-like metabolism characterized by a switch from mitochondrial oxidative phosphorylation towards aerobic glycolysis [8]. Along with this shift, a rewired tricarboxylic acid cycle (TCA) cycle in LPS-treated macrophages causes the accumulation of the Krebs cycle intermediates succinate and itaconate [9]. These metabolites directly control the inflammatory status and function of macrophages, with succinate acting as an inflammatory signal and itaconate having anti-inflammatory effects (Figure 1, top right). Succinate can drive the production of the inflammatory cytokine IL-1β by promoting HIF1α stability and by repurposing the electron transport chain from ATP production to reactive oxygen species (ROS) generation. Moreover, succinate can be released into the extracellular milieu to propagate inflammatory signaling in adjacent cells within the microenvironment via the succinate receptor GPR91/SUCNR1 (for more details on the proinflammatory role, we direct readers to recent reviews on this subject [8,9]).

While 2-HG and succinate are also produced by nonimmune cells, itaconate production appears specific for immune cells, particularly macrophages. As such, it could be considered the prototypical immunometabolite. Inflammatory macrophages make itaconate via the decarboxylation of the TCA cycle metabolite cis-aconitate. In 2013, it was revealed that itaconate production in inflammatory macrophages is mediated by the LPS-induced gene *Irg1*. This 'immune-responsive gene 1' was discovered in 1995, but its function had remained unknown (we refer to a recent extensive review on this interesting immunometabolite for further insight [10]). Irg1-derived itaconate can serve as an endogenous inhibitor of succinate dehydrogenase to counteract one of the proinflammatory effects of succinate [11,12]. In parallel, itaconate can mediate its anti-inflammatory effects via the activation of the antioxidant/anti-inflammatory Nrf2 pathway [13].

Whereas succinate supports inflammatory responses, as discussed earlier, IL-4-induced anti-inflammatory macrophages were shown to require the production of α-ketoglutarate (α-KG) via glutaminolysis [14]. This TCA cycle intermediate serves as a cofactor of the epigenetic enzyme Jmjd3 and promotes IL-4 responses in

macrophages, while repressing proinflammatory cues. As such, the succinate/α-KG ratio within a macrophage appears to be a key controller of its functional fate. In addition to α-KG, ATP citrate lyase (ACLY)-mediated accumulation of the Krebs cycle metabolite acetyl-CoA was shown to support anti-inflammatory macrophage responses by promoting histone acetylation and subsequent transcription of IL-4-induced genes [15]. However, care is needed when interpreting these experiments since they used pharmacological compounds that are now known to show important off-target effects. Therefore, new genetic tools will be key to univocally address the role of the ACLY-derived immunometabolite acetyl-CoA in regulating macrophage responses.

Other future directions for follow-up research concern the ins and outs of immunometabolite transport and localization. How are these distinct immunometabolites transported out of the cell? Furthermore, once outside the cell, what is their actual fate? Which receptors, on which cells, do they target? Although distinct solute carriers (SLCs) were proposed as transporters for succinate and 2-hydroxyglutarate, no such transporter has yet been shown for itaconate. Single-cell metabolomics and other new high-end techniques that enable one to map the metabolic configuration of cells within complex microenvironments will be key to address these questions. Another important point to further investigate is the subcellular location of metabolites and their dynamics. Such spatiotemporal insight should help us to understand how immunometabolites translate metabolic rewiring into functional changes and disease progression. So far, most of our knowledge is derived from mouse models, but how could these data be translated to humans? Moreover, most studies have assessed the immunometabolism of immune cells in bulk; thus, how might this manifest at the single cell level? Finally, the million-dollar question is whether these immunometabolites or their derivatives could provide new therapeutic opportunities. For example, the effects of itaconate and other immunometabolites on macrophage function are mostly assessed by applying derivatives such as dimethyl-itaconate and 4-octyl-itaconate. While these compounds can have strong effects on macrophage function and potential therapeutic value, they do not fully recapitulate the endogenous effects of the 'real' immunometabolites that they mimic. Understanding their specific mode of action will be a key area of research and should clarify their applicability for future therapy. While waiting elucidation of these questions, we have also emphasized the importance of immunometabolites in regulating immune cell fate and disease outcome. Moreover, we stress the necessity of carefully defining them as metabolites that are generated within immune cells after activation, rather than as external metabolites that affect immune cell function.

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Forum

The First Hormone: Adrenaline

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It is not often that three misconceptions are associated with one molecule for more than a century. This is the case with adrenaline. The aim here is to clarify that adrenaline was the first hormone, with the discovery of its activity and chemical purification being prior to secretin. Adrenaline is the correct name given by Jōkichi Takamine, epinephrine being its inactive benzoyl derivative.

While adrenaline is a well-known molecule, there have long been three misconceptions. It has not been recognized as the first hormone, its discovery is obscure,