Time and demand are two critical dimensions of immunometabolism: the process of macrophage activation and the pentose phosphate pathway

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A process is a function of time; in immunometabolism, this is reflected by the stepwise adaptation of metabolism to sustain the bio-energetic demand of an immune-response in its various states and shades. This perspective article starts by presenting an early attempt to investigate the physiology of inflammation, in order to illustrate one of the basic concepts of immunometabolism, wherein an adapted metabolism of infiltrating immune cells affects tissue function and inflammation. We then focus on the process of macrophage activation and aim to delineate the factor time within the current molecular context of metabolic-rewiring important for adapting primary carbohydrate metabolism. In the last section, we will provide information on how the pentose phosphate pathway might be of importance to provide both nucleotide precursors and redox-equivalents, and speculate how carbon-scrambling events in the non-oxidative pentose phosphate pathway might be regulated within cells by demand. We conclude that the adapted metabolism of inflammation is specific in respect to the effector-function and appears as a well-orchestrated event, dynamic by nature, and based on a functional interplay of signaling- and metabolic-pathways.

Keywords: immunometabolism, inflammation, macrophage activation, metabolic reprograming, primary carbohydrate metabolism, pentose phosphate pathway, sedoheptulose kinase, time and demand

CONCEPTS OF IMMUNOMETABOLISM

The first concepts of immunometabolism date back to the pre-genomic age of biomedical research. As early as 1912, Levene and Meyer used dog blood-derived leukocytes to directly demonstrate that hexoses are converted into two molecules, each containing a chain of three carbons (1). They also provided further evidence that hexoses are the source of lactate and assumed that this process accounts for “synthetic purposes by the leukocytes.” This period is widely recognized as the onset of modern biochemistry and furthermore of immunometabolism.

Immunometabolism is also tightly linked to research on cancer metabolism, especially with regard to the pioneering work of Otto Warburg, wherein he further developed the concept of cellular physiology (2). It was revealed that exudate leukocytes have high aerobic glycolysis, while respiration was very low and it was concluded then that white blood cells must have a cancer metabolism (3, 4). However, they differentiated immune cell and cancer metabolism in that cancer cells use aerobic glycolysis to live, while aerobic glycolysis in white blood cells is a sign of aging or dying off. With this background, Walter Kempner and Ernst Peschel, both from the Bergmann’sche Institut at the Charité in Berlin, published their work with the German title: “Stoffwechsel der Entzündung” (Metabolism of Inflammation) (5). In 1930, they formulated two fundamental questions: what are the specific reactions of inflammation? Which processes lead to cell migration and subsequently to tissue swelling or necrosis? They presumed that an adapted cellular metabolism of white blood cells may play a major role in these processes. They tested their hypothesis in a human in vivo model of sterile-inflammation and provided fundamental new insights, which are still of relevance for today’s concepts of immunometabolism. Kempner and Peschel used the beetle-juice (cantharidin)-induced skin blister model and metabolically defined the inflamed human tissue in order to examine the physiology of inflammation. They observed a disrupted equilibrium of oxygen, CO₂, sugar, lactate, and bicarbonate as a result of inflammation and concluded that this was induced by the metabolism found in infiltrating immune cells. They expected this to happen as a function of time. They demonstrated a drop in glucose over a period of 6–90 h and pulsed oral glucose administrations indicating that glucose replenishment from healthy tissue was also gradually declining. Within the inflamed area (the blister) also oxygen concentration declined. This was again attributed to high cellular respiration of infiltrated cells and a reduced gas-exchange with the healthy tissue. In addition to that, they measured a time-dependent increase in lactate and a decrease in the bicarbonate levels, which together could explain the decrease in pH of inflamed tissue, previously observed by Schade (6). Kempner and Peschel identified metabolic changes in inflamed tissue as a function of time, which is actively established by infiltrating “injured” immune cells with an adapted cellular metabolism (5). Thereby, they delineated a complex interplay between cellular metabolism and the physiology of inflammation. In 2011, the cantharidin-induced skin blister was re-evaluated and recommended as an excellent human in vivo model to study inflammation (7). This report also reveals that the infiltrating cells in this model are mainly neutrophils and monocytes/
macrophages; these cells were probably also the cause for the observation by Kempner and Peschel.

Since then, a new school of immunobiology has started to reveal the molecular mechanism behind the observed metabolic-adaptation in various immune cells and models of immunology. As an example, the action of the pentose phosphate pathway (PPP) and the power of redox-biology, including superoxide production, were identified as essential in forming the respiratory-burst of phagocytes (8, 9). Also amino acid and lipid metabolism, as well as their adaptations, were characterized as fundamental to properly fuel the function of an immune response (10, 11). In recent years, however, new concepts in immunometabolism have evolved and further mechanistic-details have surfaced that enable us to better understand how these metabolic-adaptions are reached and regulated.

TIME RESOLVED METABOLIC-ADAPTATIONS DURING MACROPHAGE ACTIVATION

Macrophages are important immune cells, which regulate tissue homeostasis by sensing and interpreting cell injury and infection, the classic triggers of an inflammatory response (12). Today, macrophages are classified according to the activation stimuli into at least two polarization states, the classic M1 (representing a pro-inflammatory phenotype) and the alternative M2 macrophage (representing an anti-inflammatory or homeostasis inducing phenotype), in order to discriminate between the effector phenotypes resulting from the distinct activation signals (13). However, in vivo macrophages rather appear to blend into various "shades of activation," while retaining some of their plasticity (14–18). Furthermore, macrophage populations and phenotypes can dramatically change over time, as exemplified by the finding that the inflammatory response is a spatially and temporally coordinated process. Recently, the polarization process of macrophages has been further associated with the reprogramming of cellular metabolism (19–25). Information processing by signal-transduction pathways starts shortly after activation and is temporally coordinated, reflected by the phosphorylation and de-phosphorylation of signal transducers and effector molecules. The question arises how the reprogramming of primary carbohydrate metabolism is timed in the process of macrophage activation. We would like to present more detailed and more importantly time-resolved information on key events, which appear to establish a pro-inflammatory M1-like metabolic-phenotype induced by lipopolysaccharide (LPS, Figure 1).

After only 20 min of in vitro LPS-stimulation, simultaneously with prime signaling events, the glucose uptake of cells approximately doubles (26). At the same time, the extracellular acidification rate (ECAR), an indirect measure of aerobic glycolysis, also increases until reaching a certain plateau-state, to then adapt, and further increase (21). This response indicates that LPS leads to a rapid induction of glycolytic flux, which is modulated and amplified in multiple steps (Figure 1). The extension phase of ECAR is accompanied by a slow and marginal decrease in the oxygen consumption rate (OCR). The molecular mechanisms leading to these immediate early metabolic events, however, are not known and acidification may also result from sources other than the formation of lactic acid.

However, 1 h after LPS stimulation, the mRNA of the glucose transporter (GLUT1) is induced and the uptake of glucose further increases (26). After uptake of glucose, it becomes phosphorylated by hexokinases (HK) to glucose 6-phosphate (G6P), which can then be diverted into various catabolic and anabolic pathways. Non-stationary metabolic flux analysis, tracking the fate of intracellular glucose during macrophage activation, reveals that already 1 h after LPS-exposure a considerable amount of glucose is used by both, glycolysis and the PPP (21). In rat-Kupffer cells, which are specialized liver macrophages, as well as murine dendritic cells, HK-II was shown to associate with mitochondria within an hour after LPS stimulation (27, 28). A similar mechanism is observed in cancer cells, where mitochondrial matrix derived ATP is channeled to HK-II and thereby augmenting the glycolytic flux (29). Recently, the sedoheptulose kinase (Shpk, formerly known as CARKL) was characterized as a unique heptose kinase, phosphorylating sedoheptulose (a ketohexose) to sedoheptulose 7-phosphate (S7P), which can then act as a reaction partner of glyceraldehyde 3-phosphate (G3P) in the non-oxidative PPP (21, 30–32). In macrophages, the mRNA of Shpk is rapidly down-regulated by LPS but not by interleukin (IL)-4 stimulation (21). Regulation of Shpk will be further discussed in the next section. Also, approximately after 1 h, LPS specifically induces pyruvate kinase M2 (PKM2) protein expression and phosphorylation, which becomes further augmented in the late phase of macrophage activation (23). Phosphorylation of PKM2 favors dimeric configuration and PKM2 translocation into the nucleus, where it acts together with hypoxia-inducible factor 1-alpha (HIF1α) as a transcriptional inducer of interleukin 1-beta (IL-1β) and more importantly of glycolytic genes like PKF, constituting an amplification loop in the intermediate and late phase of macrophage activation (23, 33). Within 2–4 h after activation by LPS, an isoform switch from the liver-type 6-phosphofructo-2-kinase (PFKFB1 aka PKF2) to the ubiquitous and more active PFKFB3 occurs (34). This is also observed when LPS is used in combination with interferon gamma (IFNγ) to induce a pro-inflammatory macrophage activation (20). PFKFB3 produces augmented levels of fructose 2, 6-bisphosphate (F2,6bP), which then functions as an allosteric activator of 6-phosphofructo-1-kinase (PFK1) to further sustain the pro-glycolytic program (Figure 1). Interestingly in yeast, PFK1 derived F1,6bP allosterically activates PKM2, indicating the presence of metabolic feedback loops (35).

Approximately 4–6 h after macrophage activation, the export of glycolytic lactate appears to become mandatory for the activation process as indicated by the increased expression of monocarboxylate transporter 4 (MCT4) (36). Knockdown of MCT4 results in enhanced intracellular lactate accumulation, a decreased expression of LPS-induced glycolytic enzymes and an attenuated secretion of tumor necrosis factor-alpha (TNFα) and IL-6. Accumulating intracellular lactate might decrease glycolytic activity by inhibiting PFK1, an enzyme which may reach maximal activity in the later phase, as indicated by peaking F2,6bP concentrations and PFKFB3 mRNA levels at 6–12 h (34, 37). Also, approximately 4 h after initiation of macrophage polarization by LPS, the tricarboxylic acid (TCA) cycle changes its operational mode from a catabolic pathway to a partly anabolic system (21, 22). The TCA-cycle metabolite succinate accumulates in a
FIGURE 1 | Time-resolved metabolic reprogramming during pro-inflammatory macrophage polarization. This model illustrates the activation of a macrophage as a function of time and is based on the literature discussed in the main text. LPS-induced activation can be grouped into an initiation-, an early metabolic-reprogramming-, and an amplification-phase. The initiation phase of the metabolic response is characterized by an increase in glucose consumption and in the extracellular acidification rate (ECAR). The early metabolic reprogramming phase depicts the increase and rerouting of carbon flux through glycolysis and the PPP, events which also regulate the cellular redox-state. In this setting, the mitochondrial association of hexokinase-II (HKII) appears to provide sufficient levels of glucose 6-phosphate (G6P), while the downregulation of sedoheptulose kinase (Shpk, previously known as CARKL) appears to be necessary to maintain appropriate carbon flux at the interface of glycolysis and the PPP. During the amplification phase, this pro-glycolytic metabolic-phenotype is further strengthened. A switch toward the more active 6-phosphofructo-2-kinase (PFK2) enzyme PFKFB3 produces higher levels of fructose 2-bisphosphate [F2,6bP], thus allosterically activating PFK1 and enhancing glycolytic flux. Dimers of the pyruvate kinase M2 (PKM2), as well as accumulating succinate further augment metabolic reprogramming by supporting HIF-1α dependent transcriptional induction of glycolytic genes. In the amplification phase, also the export of intracellular glycolysis-derived lactate through monocarboxylate transporter 4 (MCT4) becomes obligatory, which may otherwise inhibit PFK1. These initial events lead to more prominent metabolic changes observed 24 h after macrophages have encountered the pro-inflammatory stimuli. However, further time-resolved data is required to refine these processes and our current perspective, how cellular metabolism of macrophages adapts during activation.

macrophage cell line and bone marrow derived macrophages (BMDMs) (21, 22). Succinate, derived by glutamine-dependent anerplerosis and gamma-aminobutyric acid (GABA)-shunt, was shown to inhibit the prolyl hydroxylase-dependent degradation of HIF1α and to enhance IL-1β production (22). Increased succinate levels may also increase succinylation of metabolic enzymes such as glyceraldehyde 3-phosphate dehydrogenase (GAPDH), transaldolase (TALDO), and lactate dehydrogenase (LDH) A-chain, possibly further shaping late phase metabolic adaptations (22, 38). Succinate dependent HIF1α stabilization as well as increased succinylation are both suppressed by the inhibition of glycolysis, indicating that these processes are dependent on increased glycolytic
The PPP represents a prime example on how increased carbon-flux can contribute to mount the specific effector functions of LPS-activated macrophages by complementing their appropriate demands through supplying both redox-power and ribose moieties either at the same time or independently from each other. The PPP is divided into the oxidative (oxPPP) and non-oxidative branch (non-oxPPP). Briefly, the oxPPP, with glucose 6-phosphate dehydrogenase (G6PD) as its rate-limiting enzyme, is highly active in macrophages (21, 47), decarboxylates G6P, and forms ribose 5-phosphate (R5P) through three irreversible reactions, while simultaneously reducing two molecules of NADP+ to NADPH and liberating one molecule of CO2. The non-ox PPP consists of reversible reactions, which can either recycle R5P to glycolytic intermediates or use the latter to generate pentose phosphates (C5P) through reverse flux. The general aspects of PPP architecture and function have been reviewed elsewhere in great detail (48–50).

Oxidative PPP derived NADPH serves as a cofactor for NADPH-oxidase dependent reactive oxygen species (ROS) production, while also reducing oxidized redox-couples to simultaneously sustain an anti-oxidant response (i.e., glutathione and thioredoxin systems), thereby partly controlling the redox balance during macrophage activation (51, 52). Also, the function of many redox-sensitive signaling proteins, which are associated with the process of activation, are potentially dependent on increased flux through the oxPPP (53–59). Furthermore, NADPH is also critical for reductive biosynthesis serving activation associated membrane expansion and the production of lipid mediators such as prostaglandins. Remarkably, it was reported that NADPH levels undergo periodic oscillations in macrophages and neutrophils, which are tightly linked to superoxide oscillations and adapt, upon LPS stimulations to a higher frequency (60–63). These oscillations may depend on periodic glucose influx and PPP activity, and appear to encode information in their amplitude and frequency (64, 65). Severe G6PD deficient leukocytes have been associated with impairments in their oxidative burst, their bactericidal activity (66–68), their resistance to oxidative stress (69), as well as modified cytokine responses (70–72). Overexpression of G6PD in a macrophage cell line enhanced the activation of NfκB and p38-MAPK signaling pathways and potentiated the expression of pro-inflammatory cytokines as well as ROS production (73). In contrast to IL-1β, the production of TNFα and IL-6 does not appear to be directly dependent on aerobic glycolysis, as recently suggested by the activation of PKM2 and 2-deoxyglucose (2-DG) treatment, respectively (22, 23). Notably, 2-DG is a glycolytic inhibitor downstream from hexokinase and can therefore become phosphorylated to 2-deoxyglucose 6-phosphate, which is partly metabolized by the oxPPP in red blood cells (74). Whether this also occurs in macrophages or not remains to be investigated. Inhibition of G6PD or LDH, however, was shown to decrease TNFα and IL-6 levels, implicating that these cytokines are rather regulated by redox-state than simply by the increased glycolytic-flux (21). Apart from redox-power, the macrophage activation process also demands a large amount of pentose phosphates probably to sustain de novo nucleotide synthesis for their characteristic transcriptional response. In contrast to M2, M1 macrophages drastically change their transcriptional profile (40). Isotope distribution analysis of a non-stationary metabolic flux experiment with asymmetrically labeled glucose after 1 h of LPS-induced macrophage activation indicated that both ox- and non-oxPPP flux rates increase, while most of the pentose phosphates (C5) are derived from the non-ox branch (21).

The non-oxPPP relies on transketolase (TK) and TALDO catalyzed reversible transfer of keto-groups to various aldose acceptors. TK uses thiamine pyrophosphatase as cofactor to transfer two carbon (C2) -units, while TALDO can transfer C3-units by forming Schiff base intermediates (75, 76). Thereby, this pathway interconverts carbohydrate-phosphates of different chain length (C3P to C7P), without the need of energy in form of ATP (carbon scrambling, Figure 2A). The regulation of non-oxPPP is complex due to its reversible nature and still not fully understood. The flux-rate and its direction are generally thought to depend on thermodynamics, which impose a major constraint on the structure of metabolic pathways (77). However, the recent identification of Shpk indicates additional regulatory mechanism, which was previously not considered (Figure 2B) (78). In contrast to TK or TALDO, Shpk is reported to be regulated differently during LPS- and IL-4 induced polarization (21). LPS stimulation leads to a rapid down-regulation of Shpk mRNA in the early phase of macrophage activation in mice and humans likewise and in vitro as well as in vivo. In contrast to LPS, IL-4 stimulation...
FIGURE 2 | The function and regulation of the non-oxidative PPP.

(A) represents a simplified model, which illustrates how transketolase (TK) and transaldolase (TALDO) may interchange carbohydrate-phosphates of three to seven carbon-atoms in length (C3P to C7P) without the need of energy (carbon-scrambling) to account for the cellular demand, which in part defines cell function (indicated by the symbol \( f(x) \)). In (B), we theoretically evaluate the regulatory effect of Shpk-derived sedoheptulose 7-phosphate ([C7P]Shpk) on non-oxPPP flux in the presence of TK and TALDO. Flux through the non-oxPPP, by its reversible reactions, is dependent on the stoichiometry of the reactants (indicated by green arrowheads). In contrast to TK- and TALDO-derived S7P, the phosphorylation of free sedoheptulose to S7P by Shpk requires energy in form of ATP. Assuming a constant contribution by Shpk, this additional source of S7P may therefore act as a thermodynamic buffer, which can be actively regulated to induce a non-equilibrium. In theory, perturbation of Shpk can either increase the resistance (increased [C7P]Shpk) or lower it (decreased [C7P]Shpk) to support shunting through the non-oxPPP. However, in the presence of TK or TALDO, an increased [C7P]Shpk will promote the incorporation of glycolytic-G3P into the PPP. This model further illustrates that non-oxPPP flux-direction is also dependent on the demand of respective molecules (indicated by red arrowheads).

Maintains or even slightly increases Shpk levels (21). Counterbalancing LPS-induced down-regulation of Shpk by overexpression in a macrophage cell line resulted in an accumulation of pentose phosphates and an imbalance of the cellular redox system, as indicated by the accumulation of oxidized redox couples as well as blunted LPS-induced intracellular superoxide production (21). In theory, Shpk, by the formation of rate-limiting S7P, should increase the shunting of glycolysis-derived G3P into the non-oxPPP (78) and regulate oxPPP activity through the formation or recycling of pentose phosphates (79). So far, we have no confirmed mode-of-action, how Shpk activity actually regulates carbon-flux through the non-oxPPP, and no information on its activity and local distribution during macrophage activation. Therefore, we can only speculate on the consequences of Shpk regulation for the process of metabolic-adaptation (Figure 2B). Shpk-derived S7P may act as a thermodynamic buffer to support a stable non-equilibrium, which drives (low S7P) or inhibits (high S7P) carbon-flux through the non-oxPPP. However, flux-direction seems to be determined by demand and by the presence of TK and TALDO (Figure 2B). In addition to that, high S7P levels can directly modulate glycolytic flux through the inhibition of hexose phosphate isomerase, as well as by competitively inhibiting fructose 6-phosphate (F6P) phosphorylation by PFK (80, 81). Therefore, the consequences of Shpk regulation appear as strictly context dependent, which is defined by the demand of metabolites (i.e., C5P) and the presence or absence of other enzymes. We know that Shpk only partially colocalizes with G6PD in the cytoplasm of cells, which points out that there are instances where the ox- and the non-oxPPP are coupled to and uncoupled from each other (21). Information on the function of TK and TALDO in the process of macrophage activation is rare; however, both enzymes were tightly linked to oxidative stress-defense in other cell types (82–84). Notably, yeast seems to lack a Shpk homolog but utilizes a specific sedoheptulose–bisphosphatase [dephosphorylates sedoheptulose 1,7-bisphosphate (S1,7bP) to S7P] for ribonogenesis when the demand for nucleotide precursors is high (85). S1,7bP was previously reported to also exist in rat liver tissue (86, 87); however, there appear to be some major differences in the architecture of heptose metabolism (heptolysis) between fungi and vertebrates (78, 85).

In summary, these findings indicate that during macrophage activation the cellular demands are covered by a precisely coordinated interplay of many pathways to sustain such profound polarization events. The PPP appears as a versatile hub to reroute carbon moieties within the network of primary carbohydrate metabolism while independently controlling cellular redox-states.

CONCLUSION

This collection of findings may support our perspective that time and demands are critical to understand the molecular events important to mount an immune response. Immunometabolism demonstrates its consequences for physiology at various levels including cells, tissues, organisms, and entire populations, as we currently experience with diseases like cancer, cardiovascular disease, obesity, and diabetes to name but a few. Already, Kempner and Peschel considered diabetic patients in their investigations...
and noted a sustained glucose supply together with a prolonged inflammatory response compared to non-diabetics. Since then, many excellent studies further delineated the complex interplay of metabolism, the immune system and tissue function, and malfunction. At the molecular level, macrophages adapt their metabolism very early in the polarization process, which then become amplified over time. This highlights that we need to strongly consider the process leading to activation and not only the phenotypic “endpoints.” A macrophage located within a complex tissue microenvironment may go through multiple, subsequently occurring, activation events, which then may amplify or antagonize each other. It will be interesting to test in vivo if, and importantly how, subsequent or parallel cross-presentation of multiple activation-stimuli (i.e., pro- and anti-inflammatory signals such as LPS, IFNγ, TNFα, IL-6, IL-4, or IL-10) may skew and define the process of metabolic reprogramming in macrophages.

**REFERENCES**

1. Levene PA, Meyer GM. The action of leucocytes on glucose. *J Biol Chem* (1912) 12:361–70.
2. Warburg O. Stoffwechsel der Tumoren. Berlin: Springer (1926).
3. Bakker A. Einige ebeneinmussungen im stoffwechsel der carcinozellen und exsudateleukocyten. *Klin Wochenschr* (1927) 6:252–4. doi:10.1007/BF01710710
4. Fleischmann W, Kubowitz F. Ueber den stoffwechsel der leukocyten. *Biochim Ztschr* (1927) 181:395.
5. Kempsner W, Plesch E. Stoffwechsel der enzündung. *Ztschr f klin Med* (1930) 114:43–59.
6. Schade H, Clauden F, Habler C, Höffl F, Mochizucki N, Birner M. Weitere unter-suchungen der molekularpathologie der entzündung: die exsudate. *Ztschr f d ges Physiol* (1930) 114:334–40. doi:10.1007/BF02622491
7. Dinh PH, Corraza F, Mestdagh K, Kassengera Z, Doyen V, Michel O. Validation changes during the ingestion of particles by polymorphonuclear leukocytes. *J Biol Chem* (1959) 234(6):1355–62.
8. Newsholme P, Curi R, Gordon S, Newsholme EA. Metabolism of glucose, glutamine, long-chain fatty acids and ketone bodies by murine macrophages. *Biochem J* (1986) 239(1):121–5.
9. Newsholme P, Newsholme EA. Rates of utilization of glucose, glutamine and oleate and formation of end-products by mouse peritoneal macrophages in culture. *Biochem J* (1989) 261(1):211–8.
10. Murphy C, Newsholme P. Importance of glutamine metabolism in murine macrophages and human monocytes to strongly consider the process leading to activation and not only the phenotypic “endpoints.” A macrophage located within a complex tissue microenvironment may go through multiple, subsequently occurring, activation events, which then may amplify or antagonize each other. It will be interesting to test in vivo if, and importantly how, subsequent or parallel cross-presentation of multiple activation-stimuli (i.e., pro- and anti-inflammatory signals such as LPS, IFNγ, TNFα, IL-6, IL-4, or IL-10) may skew and define the process of metabolic reprogramming in macrophages.

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19. Galvan-Pena S, O’Neill LA. Metabolic reprogramming in macrophage polarization. *Front Immunol* (2014) 5:420. doi:10.3389/fimmu.2014.00420
20. Rodriguez-Prados JC, Traves PG, Guerra C, Rico D, Aragones J, Martin-San P, et al. Substrate fate in activated macrophages: a comparison between innate, classic, and alternative activation. *J Immunol* (2010) 185(1):605–14. doi:10.4049/jimmunol.0901698
21. Haschemi A, Kosma P, Gille L, Evans CR, Burzant CF, Starkl P, et al. The sedoheptulose kinase CARKL directs macrophage polarization through control of glucose metabolism. *Cell Metab* (2012) 15(6):813–26. doi:10.1016/j.cmet.2012.04.023
22. Tannahl GM, Curtis AM, Adamik J, Palsson-McDermott EM, McGettrick AF, Goel G, et al. Succinate is an inflammatory signal that induces IL-1beta through HIF-1alpha. *Nature* (2013) 496(7444):238–42. doi:10.1038/nature12086
23. Palsson-McDermott EM, Curtis AM, Goel G, Lauterbach MA, Sheedy FJ, Gleeson LE, et al. Pyruvate kinase M2 regulates Hif-1alpha activity and IL-1beta induction and is a critical determinant of the Warburg effect in LPS-activated macrophages. *Cell Metab* (2015) 21(1):65–80. doi:10.1016/j.cmet.2014.12.005
24. Vats D, Mukundan L, Odegard I, Zhang L, Smith KL, Morel CR, et al. Oxidative metabolism and PGC-1beta mediate activated macrophage-mediated inflammation. *Cell Metab* (2008) 4(1):13–24. doi:10.1016/j.cmet.2008.08.006
25. Graham T, Yamanishi Y, Cauden BE, Forster I, Pawlinski R, Mackman N, et al. HIF-1alpha is essential for myeloid cell-mediated inflammation. *Cell* (2003) 112(5):654–57. doi:10.1016/S0092-8674(03)00154-5
26. Fukushima M, Shinomiya H, Shimiuzi Y, Oshiki K, Utsunomi S. Endotoxin-induced enhancement of glucose influx into murine peritoneal macrophages via GLUT1. *Infect Immun* (1996) 64(1):108–12.
27. Shulga N, Pastorino JG. Hexokinase II binding to mitochondria is necessary for Kupffer cell activation and is potentiated by ethanol exposure. *J Biol Chem* (2014) 289(24):26213–25. doi:10.1074/jbc.M114.580175
28. Everts B, Amiel E, Huang SC, Smith AM, Chang CH, Lam WY, et al. TLR-driven early glycolytic reprogramming via the kinases TBK1-IKKeaperson supports the anabolic demands of dendritic cell activation. *Nat Immunol* (2014) 15(4):323–32. doi:10.1038/ni.2833
29. Mathupala SP, Ke YH, Pedersen PL. Hexokinase II: cancer’s double-edged sword acting as both facilitator and gatekeeper of malignancy when bound to mitochon-dria. *Oncogene* (2006) 25(34):4777–86. doi:10.1038/sj.onc.1209603
30. Wamelink MM, Struys EA, Jansen EE, Levtchenko EN, Zijlstra FS, Engelke U, et al. Substrate fate in activated macrophages: a comparison between innate, classic, and alternative activation. *Front Immunol* (2014) 5:420. doi:10.3389/fimmu.2014.00420
31. Mounier R, Theret M, Arnold L, Cuvellier S, Bultot L, Goransson O, et al. AMP-Kalpah regulates macrophage skewing at the time of resolution of inflamation during skeletal muscle regeneration. *Cell Metab* (2013) 18(2):251–64. doi:10.1016/j.cmet.2013.06.017
32. Mouser DM, Edwards JP. Exploring the full spectrum of macrophage activation. *Nat Rev Immunol* (2008) 8(12):958–69. doi:10.1038/nri2448
33. Foster SL, Hargreasve DC, Medzhitov R. Gene-specific control of inflammation by TLR-activated chromatin modifications. *Nature* (2007) 447(7147):972–8. doi:10.1038/nature05836
44. O'Neill LA, Hardie DG. Metabolism of inflammation limited by
45. Pearce EL, Pearce EJ. Metabolic pathways in immune cell activation and quies-
43. Huang SC, Everts B, Ivanova Y, O'Sullivan D, Nascimento M, Smith AM,
42. Imtiyaz HZ, Simon MC. Hypoxia-inducible factors as essential regulators of
41. T ornatore L, Thotakura AK, Bennett J, Moretti M, Franzoso G. The nuclear

38. Du J, Zhou Y, Xu X, Yu JI, Khan S, Jiang H, et al. Sirt5 is a NAD-dependent pro-
37. Kumar S, Rabson AB, Gelinas C. The RxxRxRxxC motif conserved in all
36. Pollak N, Dolle C, Ziegler M. The power to reduce: pyridine nucleotides –
35. Nagy and Haschemi Time and demand in immunometabolism
34. Martinez FO, Gordon S, Locati M, Mantovani A. Transcriptional profiling of the
33. Huang SC, Everts B, Ivanova Y, O'Sullivan D, Nascimento M, Smith AM, et al. Cell-intrinsic lysosomal lipolysis is essential for alternative activation of macrophages. Nat Immunol (2014) 15(9):846–55. doi:10.1038/ni.2956
32. Imtiyaz HZ, Simon MC. Hypoxia-inducible factors as essential regulators of
31. Pearce EL, Pearce EJ. Metabolic pathways in immune cell activation and quies-
30. O'Neill LA, Hardie DG. Metabolism of inflammation limited by

29. Ray PD, Huang BW, Tsuji Y. Reactive oxygen species (ROS) homeostasis
28. Pollak N, Dolle C, Ziegler M. The power to reduce: pyridine nucleotides –
27. Board M, Humm S, Newsholme EA. Maximum activities of key enzymes of gly-
26. Odegard H, Chawla A. Alternative macrophage activation and metabolism.
25. Board M, Humm S, Newsholme EA. Maximum activities of key enzymes of gly-
24. O'Neill LA, Hardie DG. Metabolism of inflammation limited by

23. Du J, Zhou Y, Su X, Yu JJ, Khan S, Jiang H, et al. Sirt5 is a NAD-dependent pro-
22. Martinez FO, Gordon S, Locati M, Mantovani A. Transcriptional profiling of the
21. Pearce EL, Pearce EJ. Metabolic pathways in immune cell activation and quies-
20. T ornatore L, Thotakura AK, Bennett J, Moretti M, Franzoso G. The nuclear
19. Du J, Zhou Y, Su X, Yu JJ, Khan S, Jiang H, et al. Sirt5 is a NAD-dependent pro-
18. Martinez FO, Gordon S, Locati M, Mantovani A. Transcriptional profiling of the
17. Du J, Zhou Y, Su X, Yu JJ, Khan S, Jiang H, et al. Sirt5 is a NAD-dependent pro-
16. Odegard H, Chawla A. Alternative macrophage activation and metabolism.
15. Imtiyaz HZ, Simon MC. Hypoxia-inducible factors as essential regulators of

14. O'Neill LA, Hardie DG. Metabolism of inflammation limited by
13. Huang SC, Everts B, Ivanova Y, O'Sullivan D, Nascimento M, Smith AM, et al. Cell-intrinsic lysosomal lipolysis is essential for alternative activation of macrophages. Nat Immunol (2014) 15(9):846–55. doi:10.1038/ni.2956
12. Imtiyaz HZ, Simon MC. Hypoxia-inducible factors as essential regulators of
11. T ornatore L, Thotakura AK, Bennett J, Moretti M, Franzoso G. The nuclear

10. Du J, Zhou Y, Su X, Yu JJ, Khan S, Jiang H, et al. Sirt5 is a NAD-dependent pro-
9. Odegard H, Chawla A. Alternative macrophage activation and metabolism.
8. Martinez FO, Gordon S, Locati M, Mantovani A. Transcriptional profiling of the
7. Du J, Zhou Y, Su X, Yu JJ, Khan S, Jiang H, et al. Sirt5 is a NAD-dependent pro-
6. Odegard H, Chawla A. Alternative macrophage activation and metabolism.
5. Nagy and Haschemi Time and demand in immunometabolism
4. O'Neill LA, Hardie DG. Metabolism of inflammation limited by
3. Huang SC, Everts B, Ivanova Y, O'Sullivan D, Nascimento M, Smith AM, et al. Cell-intrinsic lysosomal lipolysis is essential for alternative activation of macrophages. Nat Immunol (2014) 15(9):846–55. doi:10.1038/ni.2956
2. Imtiyaz HZ, Simon MC. Hypoxia-inducible factors as essential regulators of

1. Du J, Zhou Y, Su X, Yu JI, Khan S, Jiang H, et al. Sirt5 is a NAD-dependent pro-

45. Pearce EL, Pearce EJ. Metabolic pathways in immune cell activation and quies-
44. O'Neill LA, Hardie DG. Metabolism of inflammation limited by
43. Huang SC, Everts B, Ivanova Y, O'Sullivan D, Nascimento M, Smith AM, et al. Cell-intrinsic lysosomal lipolysis is essential for alternative activation of macrophages. Nat Immunol (2014) 15(9):846–55. doi:10.1038/ni.2956
42. Imtiyaz HZ, Simon MC. Hypoxia-inducible factors as essential regulators of
41. T ornatore L, Thotakura AK, Bennett J, Moretti M, Franzoso G. The nuclear
38. Du J, Zhou Y, Su X, Yu JJ, Khan S, Jiang H, et al. Sirt5 is a NAD-dependent pro-
37. Kumar S, Rabson AB, Gelinas C. The RxxRxRxxC motif conserved in all
36. Pollak N, Dolle C, Ziegler M. The power to reduce: pyridine nucleotides –
35. Nagy and Haschemi Time and demand in immunometabolism
34. Martinez FO, Gordon S, Locati M, Mantovani A. Transcriptional profiling of the
33. Huang SC, Everts B, Ivanova Y, O'Sullivan D, Nascimento M, Smith AM, et al. Cell-intrinsic lysosomal lipolysis is essential for alternative activation of macrophages. Nat Immunol (2014) 15(9):846–55. doi:10.1038/ni.2956
32. Imtiyaz HZ, Simon MC. Hypoxia-inducible factors as essential regulators of
31. Pearce EL, Pearce EJ. Metabolic pathways in immune cell activation and quies-
30. O'Neill LA, Hardie DG. Metabolism of inflammation limited by

29. Ray PD, Huang BW, Tsuji Y. Reactive oxygen species (ROS) homeostasis and redox regulation in cellular signaling. Cell Signal (2012) 24(5):981–90. doi:10.1016/j.cellsig.2012.01.008
28. Pollak N, Dolle C, Ziegler M. The power to reduce: pyridine nucleotides – small molecules with a multitude of functions. Biochem J (2007) 402(2):205–18. doi:10.1042/BJ20061638
27. Board M, Humm S, Newsholme EA. Maximum activities of key enzymes of gly-
26. Odegard H, Chawla A. Alternative macrophage activation and metabolism.
25. Board M, Humm S, Newsholme EA. Maximum activities of key enzymes of gly-
24. O'Neill LA, Hardie DG. Metabolism of inflammation limited by

23. Du J, Zhou Y, Su X, Yu JJ, Khan S, Jiang H, et al. Sirt5 is a NAD-dependent pro-
22. Martinez FO, Gordon S, Locati M, Mantovani A. Transcriptional profiling of the
21. Pearce EL, Pearce EJ. Metabolic pathways in immune cell activation and quies-
20. T ornatore L, Thotakura AK, Bennett J, Moretti M, Franzoso G. The nuclear
19. Du J, Zhou Y, Su X, Yu JJ, Khan S, Jiang H, et al. Sirt5 is a NAD-dependent pro-
18. Martinez FO, Gordon S, Locati M, Mantovani A. Transcriptional profiling of the
17. Du J, Zhou Y, Su X, Yu JJ, Khan S, Jiang H, et al. Sirt5 is a NAD-dependent pro-
16. Odegard H, Chawla A. Alternative macrophage activation and metabolism.
15. Imtiyaz HZ, Simon MC. Hypoxia-inducible factors as essential regulators of
14. O'Neill LA, Hardie DG. Metabolism of inflammation limited by
13. Huang SC, Everts B, Ivanova Y, O'Sullivan D, Nascimento M, Smith AM, et al. Cell-intrinsic lysosomal lipolysis is essential for alternative activation of macrophages. Nat Immunol (2014) 15(9):846–55. doi:10.1038/ni.2956
12. Imtiyaz HZ, Simon MC. Hypoxia-inducible factors as essential regulators of
11. T ornatore L, Thotakura AK, Bennett J, Moretti M, Franzoso G. The nuclear

10. Du J, Zhou Y, Su X, Yu JI, Khan S, Jiang H, et al. Sirt5 is a NAD-dependent pro-
9. Odegard H, Chawla A. Alternative macrophage activation and metabolism.
8. Martinez FO, Gordon S, Locati M, Mantovani A. Transcriptional profiling of the
7. Du J, Zhou Y, Su X, Yu JJ, Khan S, Jiang H, et al. Sirt5 is a NAD-dependent pro-
6. Odegard H, Chawla A. Alternative macrophage activation and metabolism.
5. Nagy and Haschemi Time and demand in immunometabolism
4. O'Neill LA, Hardie DG. Metabolism of inflammation limited by
3. Huang SC, Everts B, Ivanova Y, O'Sullivan D, Nascimento M, Smith AM, et al. Cell-intrinsic lysosomal lipolysis is essential for alternative activation of macrophages. Nat Immunol (2014) 15(9):846–55. doi:10.1038/ni.2956
2. Imtiyaz HZ, Simon MC. Hypoxia-inducible factors as essential regulators of

1. Du J, Zhou Y, Su X, Yu JI, Khan S, Jiang H, et al. Sirt5 is a NAD-dependent pro-

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Nagy and Haschemi Time and demand in immunometabolism
80. Karadsheh NS, Tejwani GA, Ramaiyah A. Sedoheptulose-7-phosphate kinase activity of phosphofructokinase from the different tissues of rabbit. *Biochim Biophys Acta* (1973) 327(1):66–81. doi:10.1016/0005-2744(73)90104-6
81. Venkataraman R, Racker E. Mechanism of action of transaldolase. I. Crystalization and properties of yeast enzyme. *J Biol Chem* (1961) 236:1876–82.
82. Lassen N, Black WJ, Estey T, Vasiliev V. The role of corneal crystallins in the cellular defense mechanisms against oxidative stress. *Semin Cell Dev Biol* (2008) 19(2):100–12. doi:10.1016/j.semcdb.2007.10.004
83. Xue M, Qian Q, Adaikalakoteswari A, Rabbani N, Bahabei Jadidi R, Thornalley PJ. Activation of NF-E2-related factor-2 reverses biochemical dysfunction of endothelial cells induced by hyperglycemia linked to vascular disease. *Diabetes* (2008) 57(10):2809–17. doi:10.2337/db06-1003
84. Hancerko R, Fernandez DR, Doherty E, Qian Y, Vas G, Niland B, et al. Prevention of hepatocarcinogenesis and increased susceptibility to acetaminophen-induced liver failure in transaldolase-deficient mice by N-acetylcysteine. *J Clin Invest* (2009) 119(6):1546–57. doi:10.1172/JCI35722
85. Clasquin MF, Melamud E, Singer A, Gooding JR, Xu X, Dong A, et al. Ribonoeogenesis in yeast. *Cell* (2011) 145(6):969–80. doi:10.1016/j.cell.2011.05.022
86. Blackmore PF, Shuman EA. Regulation of hepatic alto heptulose 1,7-bisphosphate levels and control of flux through the pentose pathway by fructose 2,6-bisphosphate. *FEBS Lett* (1982) 142(2):255–9. doi:10.1016/0014-5793(82)80147-6
87. Belyaeva NF, Golubev MA, Grigorovich JA, Dubinsky ZV, Semenova NA, Pitkanen E, et al. The involvement of fructose 2,6-bisphosphate in substrate cycle control in the nonoxidative stage of the pentose phosphate pathway. *A phosphorus magnetic resonance spectroscopy study. Experientia* (1994) 50(8):780–4. doi:10.1007/BF01919382

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