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Interferon Gamma Induces Reversible Metabolic Reprogramming of M1 Macrophages to Sustain Cell Viability and Pro-Inflammatory Activity

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ABSTRACT

Classical activation of M1 macrophages with lipopolysaccharide (LPS) is associated with a metabolic switch from oxidative phosphorylation to glycolysis. However, the generalizability of such metabolic remodeling to other modes of M1 macrophage stimulation, e.g. type II interferons (IFNs) such as IFNγ, has remained unknown as has the functional significance of aerobic glycolysis during macrophage activation. Here we demonstrate that IFNγ induces a rapid activation of aerobic glycolysis followed by a reduction in oxidative phosphorylation in M1 macrophages. Elevated glycolytic flux sustains cell viability and inflammatory activity, while limiting reliance on mitochondrial oxidative metabolism. Adenosine triphosphate (ATP) distributed by aerobic glycolysis is critical for sustaining IFN-γ triggered JAK (Janus tyrosine kinase)-STAT-1 (Signal Transducer and Activator of Transcription 1) signaling with phosphorylation of the transcription factor STAT-1 as its signature trait. Inhibition of aerobic glycolysis not only blocks the M1 phenotype and pro-inflammatory cytokine/chemokine production in murine macrophages and also human monocytes/macrophages. These findings extend on the potential functional role of immuno-metabolism from LPS- to IFNγ-linked diseases such as atherosclerosis and autoimmune disease.

1. Introduction

The observation that cancer cells utilize aerobic glycolysis despite sufficient oxygen availability was first reported by Otto Warburg in 1923 (Warburg, 1923). Since then this phenotype has been observed in a number of highly proliferative cell types (Hsu and Sabatini, 2008; Vander Heiden et al., 2009). Moreover, it has recently been described that classically (lipopolysaccharide (LPS)) stimulated macrophages also display a Warburg metabolic phenotype (Tannahill et al., 2013; Palsson-McDermott et al., 2015; Yang et al., 2014; Mills et al., 2016). Macrophages are critical for both innate nonspecific host defense and the adaptive specific immune response (Akira et al., 2006), which broadly impacts diverse disease entities. Accordingly, glucose metabolism and aerobic glycolysis specifically may be of significance not only for LPS-linked infectious disease processes, but also interferon (IFN) γ-linked disease processes such as atherosclerosis and autoimmune diseases (McLaren and Ramji, 2009; Galkina and Ley, 2009, Shirai et al., 2016). This is of relevance as a "switch" from oxidative phosphorylation (OXPHOS) to aerobic glycolysis is not only a hallmark of T cell activation but required for T cell effector functions such as IFN-γ production (Chang et al., 2013; Peng et al., 2016). Of interest, while type II IFNs are known to lead to classical M1 macrophages, it is less well known if they induce a metabolic switch similar to LPS. Importantly, type I IFNs such as IFN-α have been shown induce alternative changes in the cellular metabolism of plasmacytoid dendritic cells consisting of increased fatty acid oxidation and OXPHOS (Wu et al., 2016).

Macrophages are not one homogenous cell population; in fact, various subtypes have been identified. The classical distinction is between the so-called classically activated M1 subtype and the so-called alternative activated M2 subtype, each with a seemingly different metabolic response to activation (Galván-Peña and O'Neill, 2015; Rodríguez-Prados et al., 2010). M1 macrophages rely on glycolysis (Tannahill et al., 2013; Palsson-McDermott et al., 2015; Yang et al., 2014; Mills et al., 2016), whereas M2 macrophages obtain energy from OXPHOS (Vats et al., 2006; Haschemi et al., 2012). Interestingly, the switch to aerobic glycolysis in M1 macrophages is accompanied by alterations in mitochondrial tricarboxylic acid (TCA) cycle activity leading to metabolite accumulation and increase in mitochondrial reactive oxygen species (ROS) production. This metabolic phenotype is associated with activation of

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hypoxia inducible factor-1α (HIF-1α)-responsive gene expression including those encoding for pro-inflammatory cytokines such as interleukin (IL)-1β (Liu et al., 2016; Tannahill et al., 2013; Mills et al., 2016). However, why macrophages switch to aerobic glycolysis, an energetically less efficient mode of metabolism, is likewise incompletely understood.

Here we report that IFN-γ increases aerobic glycolysis within minutes, followed by a decline in OXPHOS after several hours. This metabolic reprogramming is reversible, independent of nitric oxide (NO) production and alterations in mitochondrial TCA cycle metabolite accumulations. The metabolic switch allows for M1 macrophages to tolerate a reduction in mitochondrial adenosine triphosphate (ATP) production, and even more, it allows for mitochondrial ROS production, which stabilizes HIF-1α and contributes to the inflammatory response. Furthermore, intracellular glycolytic ATP is critical for the phosphorylation and pro-inflammatory activity of Signal Transducer and Activator of Transcription 1 (STAT-1) in the activation loop of the Janus tyrosine kinase (JAK)-STAT-1 pathway after IFN-γ stimulation. Finally, glycolysis inhibition not only blocked the M1 phenotype in murine cell lines but also in human monocytes and macrophages.

2. Materials and Methods

2.1. Key Resources Table

Reagent or resource	Source	Identifier
Antibodies		
Rabbit monoclonal anti-Phospho-Stat1 (Tyr701)	Cell Signaling Technology	Cat# 7649
Rabbit polyclonal anti-stat1	Cell Signaling Technology	Cat# 9172
Rabbit monoclonal anti-Phospho-IKKα (Ser176)/IKKβ (Ser177)	Cell Signaling Technology	Cat# 2078
Rabbit monoclonal anti-Phospho-IκBα (Ser32)	Cell Signaling Technology	Cat# 2859
Mouse monoclonal anti-IκBα (Amino-terminal Antigen)	Cell Signaling Technology	Cat# 4814
Rabbit monoclonal anti-HIF-1α	Cell Signaling Technology	Cat# 14179
Rabbit monoclonal Anti-Hexokinase II	Cell Signaling Technology	Cat# 2867
Rabbit polyclonal anti-LDHa	Cell Signaling Technology	Cat# 2012
Rabbit monoclonal anti-PKM2	Cell Signaling Technology	Cat# 4053
Rabbit monoclonal anti-Phospho-NF-κB p65 (Ser536)	Cell Signaling Technology	Cat# 3033
Rabbit monoclonal anti-NF-κB p65	Cell Signaling Technology	Cat# 8242
Rabbit monoclonal anti- Phospho-Stat1 (Tyr701)	Thermo Fisher Scientific	Cat# 700349
Rabbit polyclonal anti- iNOS (C-terminal region)	ECM Biosciences	Cat# NP2131
Goat polyclonal anti-mouse IL-1 beta/IL-1F2	R&D systems	Cat# AF-401
Goat polyclonal anti-human IL-1 beta/IL-1F2	R&D systems	Cat# AF-201
Rabbit polyclonal anti-beta Actin	Abcam	Cat# ab8227
Rabbit monoclonal APC/Cy7 anti-mouse CD86	BioLegend	Cat# 105029
GAPDH	Cell Signaling Technology	Cat# 5174
Chemicals, peptides, and recombinant proteins		
Lipopolysaccharides from *Escherichia coli* O127:B8	Sigma-Aldrich	Cat# L4516
Recombinant Murine IFN-γ	PeproTech	Cat# 315-05
Recombinant Murine M-CSF	PeproTech	Cat# 315-02
Recombinant Mouse IFN-beta	R&D systems	Cat# 8234-MB-010
Recombinant Human M-CSF Protein	R&D systems	Cat# 216-MC
Recombinant Human IFN-γ	Peprotech	Cat# 300-02
2-Deoxy-D-glucose	Sigma-Aldrich	Cat# D8375
Lactate Dehydrogenase A Inhibitor, FX11	EMD Millipore	Cat# 427218
D-(+)-Galactose	Sigma-Aldrich	Cat# G0750
D-(+)-Glucose	Sigma-Aldrich	Cat# G8644
Sodium oxamate	Sigma-Aldrich	Cat# O2751
Nω-Nitro-L-arginine methyl ester hydrochloride	Sigma-Aldrich	Cat# N5751
ATP Solution	Thermo Fisher Scientific	Cat# R0441
Adenosine 5′-triphosphate, periodate oxidized sodium salt	Sigma-Aldrich	Cat# A6779
Adenosine 5′-[γ-thio]triphosphate tetralithium salt	Sigma-Aldrich	Cat# A1388
BzATP triethylammonium salt	Abcam	Cat# ab120444
Perchloric acid	Sigma-Aldrich	Cat# 311421
Potassium bicarbonate	Sigma-Aldrich	Cat# 60339
Potassium phosphate monobasic	Sigma-Aldrich	Cat# P5655
Potassium hydroxide	Sigma-Aldrich	Cat# 484016
Tetrabutylammonium hydrogensulfate	Sigma-Aldrich	Cat# 155837
Deuterium oxide	Sigma-Aldrich	Cat# 151882
Oligomycin A	Sigma-Aldrich	Cat# 75351
Oligomycin	Sigma-Aldrich	Cat# O4876
FCCP	Sigma-Aldrich	Cat# C2920
Antimycin A	Sigma-Aldrich	Cat# A8674
Rotenone	Sigma-Aldrich	Cat# R8875
Natural Streptolysin O (Hemolytic streptococcus) protein	Abcam	Cat# ab63978
JAK inhibitor I	EMD Millipore	Cat# 420097
7-AAD Viability Staining Solution	BioLegend	Cat# 420403
MitoSOX™ Red Mitochondrial Superoxide Indicator	Thermo Fisher Scientific	Cat# M36008
Fluoroshield Mounting Medium With DAPI	Abcam	Cat# ab104139
Ficoll-Paque PLUS	GE Healthcare Life Sciences	Cat# 17-1440-03
Critical commercial assays		
Proteome Profiler Mouse Cytokine Array Kit	R&D systems	Cat# ARY006
Proteome Profiler Human Cytokine Array Kit	R&D systems	Cat# ARY005B
Avidin/Biotin Blocking Kit	Vector Laboratories	Cat# SP-2001
ATP assay kit	MyBioSource	Cat# MBS841498

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