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*J Immunol* 2016; 196:2004-2009; Prepublished online 29  
January 2016;  
doi: 10.4049/jimmunol.1501557  
<http://www.jimmunol.org/content/196/5/2004>

**Supplementary Material** <http://www.jimmunol.org/content/suppl/2016/01/29/jimmunol.1501557.DCSupplemental>

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*The Journal of Immunology* is published twice each month by  
The American Association of Immunologists, Inc.,  
1451 Rockville Pike, Suite 650, Rockville, MD 20852  
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Immunologists, Inc. All rights reserved.  
Print ISSN: 0022-1767 Online ISSN: 1550-6606.



# Cutting Edge: Critical Role of Glycolysis in Human Plasmacytoid Dendritic Cell Antiviral Responses

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Plasmacytoid dendritic cells (pDCs) are vital to antiviral defense, directing immune responses via secretion of huge concentrations of IFN- $\alpha$ . These cells are critical in protecting the lung against clinically relevant respiratory viruses, particularly influenza (Flu), a virus responsible for substantial worldwide morbidity and mortality. How pDC responses to such viral pathogens are regulated, however, is poorly understood in humans. Using an unbiased approach of gene chip analysis, we discovered that Flu significantly affects metabolism in primary human pDCs. We demonstrate that Flu and RV, another common respiratory virus, induce glycolysis in pDCs and that this metabolic pathway regulates pDC antiviral functions, including IFN- $\alpha$  production and phenotypic maturation. Intranasal vaccination of human volunteers with live influenza virus also increases glycolysis in circulating pDCs, highlighting a previously unrecognized potential role for metabolism in regulating pDC immune responses to viral infections in humans. *The Journal of Immunology*, 2016, 196: 2004–2009.

**I**nfluenza viruses (Flu) cause significant morbidity and mortality worldwide, imposing an enormous public health burden (1). Plasmacytoid dendritic cells (pDCs) are critical in directing antiviral responses against many viruses (2) and are recruited to the sites of viral replication during respiratory Flu infections (3, 4). In mice, pDCs promote viral clearance and are essential for protecting the lung against Flu infection (5).

pDCs direct immune responses through their unique ability to produce massive concentrations of type I IFNs upon viral

encounter (6). Type I IFNs promote an antiviral state, limiting viral replication while also activating multiple immune cells including macrophages, DCs, as well as B and T lymphocytes (7). pDCs are thus crucial for both innate and adaptive antiviral responses (8).

The regulation of pDC responses to Flu is incompletely understood in humans. In mice, TLR7 and MyD88 are essential for Flu-induced secretion of type I IFN (9). TLR7 is also required for pDC IFN- $\alpha$  responses to Flu in humans (10), and a role for PI3K in pDC IFN- $\alpha$  production has also been demonstrated (11). In addition, several signaling pathways including IL-18R, Fc $\epsilon$ R1, and Ig-like transcript 7 have been shown to negatively regulate Flu-induced pDC IFN- $\alpha$  production in humans (12–14). Whether additional pathways regulate human pDC responses to Flu is unknown. We used an unbiased approach of gene chip analysis of Flu-exposed primary human pDCs to address this question. We discovered that metabolism, and specifically glycolysis, plays a critical, previously unrecognized role in human pDC antiviral responses. Flu and RV, both ssRNA viruses, and the TLR7-agonist gardiquimod (Gard) all induce glycolysis in human pDCs, and this metabolic pathway is essential for pDC functions, including viral-induced IFN- $\alpha$  secretion and phenotypic maturation. The discovery that live attenuated influenza vaccine (LAIV) promotes increased glycolytic rates in circulating human pDCs highlights the potential role of glycolysis in human pDC responses to viral infections in vivo.

## Materials and Methods

### Human subjects

For ex vivo studies, enriched leukocyte packs were obtained from a blood bank. For in vivo studies, informed consent was obtained from 16 healthy adults (7 males and 9 females with no recent history of Flu infection or vaccination). Blood was drawn before, 1 and/or 3 d and 10 d (in a subset) after administration of LAIV (Flumist Quadrivalent; MedImmune). Nasal samples were

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Received for publication July 13, 2015. Accepted for publication December 28, 2015.

This work was supported by National Institutes of Health/National Institute of Allergy and Infectious Diseases Grant R01AI098077 (to M.A.G.) and grant awards from Children's Medical Center Foundation (Children's Clinical Research Advisory Council; to M.A.G.) and the Crystal Charity Ball of Dallas, TX (to M.A.G. and J.D.F.). Support was also provided by National Institutes of Health National Center for Advancing Translational Sciences Grant UL1TR001105.

The microarray data presented in this article have been submitted to GenBank (<http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?token=qfarmocyrvcvdsb&acc=GSE68849>) under accession number GSE68849.

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The online version of this article contains supplemental material.

Abbreviations used in this article: cRPMI, complete RPMI 1640 medium; 2-DG, 2-deoxyglucose; ECAR, extracellular acidification rate; Flu, influenza virus; Gard, gardiquimod; HIF, hypoxia-inducible factor; IRF7, IFN regulatory factor 7; LAIV, live attenuated influenza vaccine; OCR, oxygen consumption rate; OXPHOS, oxidative phosphorylation; pDC, plasmacytoid dendritic cell; RV, rhinovirus; XF, extracellular flux.

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collected by instilling saline (0.9%; two sprays) into each nare and collecting material expelled by forcible nasal exhalation (15). Samples were then mixed with PBS, centrifuged ( $1300 \times g$  for 10 min), and stored for PCR analysis. All procedures were performed according to an Institutional Review Board–approved protocol.

#### *Purification of blood pDCs, culture conditions, and reagents*

pDCs were purified from PBMCs by negative selection using Ab-coated magnetic beads (StemCell Technologies); purity (Lineage<sup>−</sup>HLA-DR<sup>+</sup>CD11c<sup>−</sup>CD123<sup>+</sup>) was >90%. pDCs were cultured in complete RPMI 1640 medium (cRPMI) with 10 ng/ml IL-3 (R&D Systems) (14). The conditions evaluated were as follows: control, Flu (A/PR/8/34 [H1N1], 0.1–0.25 PFU/cell; Charles River Laboratories), RV (RV-16,  $10^6$ – $10^7$  PFU/ml; a gift from J. Gern, University of Wisconsin), and Gard (a TLR7 agonist, 1  $\mu$ g/ml; InvivoGen). Cells were harvested by centrifugation, and supernatants were stored at  $-80^\circ\text{C}$  for IFN- $\alpha$  ELISA and lactate assays. In some experiments, pDCs were harvested for RNA isolation or flow cytometry. For glycolysis inhibition studies, pDCs were cultured with 10 mM 2-deoxyglucose (2-DG, a glycolytic inhibitor; Sigma-Aldrich) and 10 mM D-glucose (Sigma-Aldrich). pDC viability was assessed using trypan blue (Sigma-Aldrich).

#### *Extracellular acidification rate and oxygen consumption rate measurements*

To determine extracellular acidification rate (ECAR) and oxygen consumption rate (OCR), stimulated pDCs were seeded onto Cell-Tak (BD Biosciences)–coated 24-well plates in extracellular flux (XF) medium (with 2 mM L-glutamine [pH 7.4]) for 1–2 h at  $37^\circ\text{C}$ . ECAR and OCR were measured in real time using the XF-24 Analyzer (Seahorse Biosciences). The following treatments were evaluated over 135 min: 1) no glucose, 2) 10 mM D-glucose, 3) 1 mM oligomycin (to induce maximal glycolysis), and 4) 100 mM 2-DG. Basal ECAR and OCR were evaluated simultaneously in XF medium with 25 mM glucose.

#### *Lactate quantification by gas chromatography/mass spectrometry*

To quantify glucose-derived [ $^{13}\text{C}$ ] lactate, pDCs were cultured in glucose-free cRPMI medium, 10% dialyzed FBS (Invitrogen) and 10 mM D-[1,6- $^{13}\text{C}$ ]-glucose (Cambridge Isotope Laboratories). Lactate was extracted from supernatants using methanol, chloroform, and water. Samples were evaporated and derivatized by trimethylsilylation (Tri-Sil HTP reagent; Thermo Scientific) and injected into an Agilent 6970 gas chromatograph networked to an Agilent 5973 mass selective detector. Fragment ions  $m/z$  117–119 were used to monitor lactate enrichment, and the [ $^{13}\text{C}$ ] lactate amount was calculated based on [ $^{13}\text{C}$ ] lactate enrichment and total lactate abundance.

#### *IFN- $\alpha$ quantification*

IFN- $\alpha$  was measured using the Human IFN- $\alpha$  (pan-specific) ELISA kit (MabTech) and analyzed on an ELISA reader DTX 880 Multimode detector (Beckman Coulter). For LAIV experiments, the IFN- $\alpha$ 2a Ultra-Sensitive Kit was used, and data were analyzed by MESO SECTOR S 600 (Meso Scale Discovery).

#### *Flow cytometry*

pDCs were stained (14) with the following anti-human Abs: HLA-DR-allophycocyanin-Cy7, CD80-FITC, and CD86-PeCy5 (BD Biosciences); acquired on a BD LSR II flow cytometer (BD Biosciences); and analyzed using FlowJo software (Tree Star). The Molecules of Equivalent Soluble fluorochrome values of HLA-DR, CD80, and CD86 were measured using the Ultra Rainbow Calibration kit (BD Biosciences). For mitochondrial staining, MitoTracker Red CMXRos (200 nM; Invitrogen) was used.

#### *Protein and RNA analysis*

Total RNA was extracted from pDC pellets using Arcturus Picopure RNA isolation kit (Applied Biosystems) and quantified by NanoDrop 2000 (Thermo Scientific). Total protein was measured using the BCA protein assay kit (Thermo Scientific). Quantitative real-time PCR for IFN- $\alpha$ , CD80, CD86, HLA-DR,  $\beta$  glucuronidase (*GUSB*), IL-3 $\alpha$ , CD2-associated protein (*CD2AP*), CD317 (tetherin),  $\beta$ -actin (*ACTB*), hexokinase 2 (*HK2*), lactate dehydrogenase A (*LDHA*), and IFN regulatory factor 7 (*IRF7*) was performed using TaqMan probe/primers (Applied Biosystems) and results normalized to peptidylprolyl isomerase A (*PPIA*).

#### *ELISPOT assay*

ELISPOT assay was used to measure the frequency of H1N1 Flu peptide-specific IFN- $\gamma$ -producing T cells. PBMCs ( $10^5$ ) were placed in 96-well

IFN- $\gamma$ -coated plates and stimulated with or without H1N1 Flu peptide (20  $\mu$ g/ml) or anti-CD3 (5  $\mu$ g/ml) for 24 h at  $37^\circ\text{C}$ . After washing, plates were incubated with biotinylated anti-IFN- $\gamma$  Ab (1  $\mu$ g/ml) and developed using streptavidin-HRP and 3-amino-9-ethyl carbazole. Plates were analyzed using a Bio-Sys-Bioreader 5000 ELISPOT reader. Data was normalized to the number of spots obtained  $\pm$  anti-CD3 stimulation.

#### *Statistical analysis*

Statistical analysis was performed using Graph Pad Prism version 6 and included Student *t* test, one- and two-way ANOVA. Pearson correlation analysis was performed in select experiments. Data are presented as mean  $\pm$  SEM. A *p* value  $\leq$  0.05 indicated significant differences.

## Results and Discussion

### *Viral exposure enhances glycolysis and activates HIF-1 $\alpha$ in human pDCs*

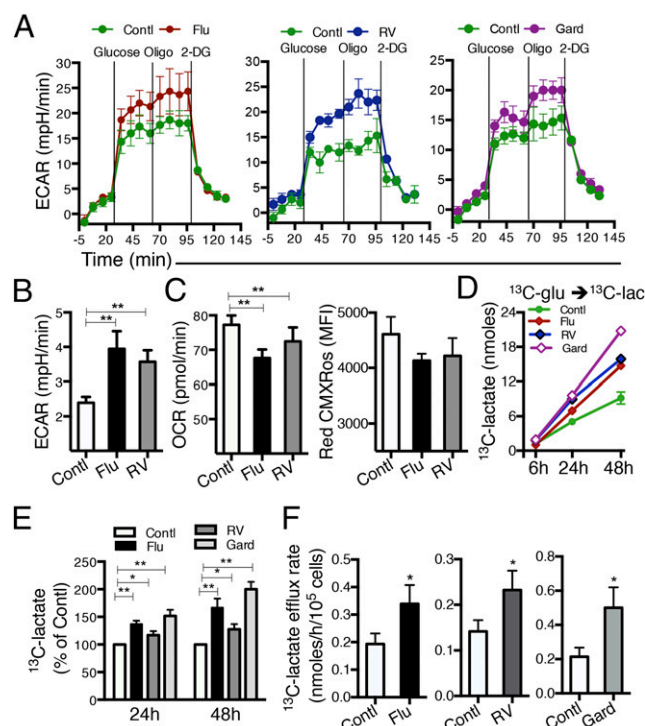
We used gene chip analysis to identify novel pathways involved in pDC antiviral responses, using Flu exposure as our model. Functional annotation analysis of ~2600 differentially regulated genes revealed 75 biological pathways impacted by Flu. In addition to expected pathways related to antiviral defense and cytokine production (16), Flu significantly influenced metabolism in pDCs; 17 pathways related to metabolism were affected (Supplemental Fig. 1A). Glycolysis and oxidative phosphorylation (OXPHOS), the major energy producing pathways, were differentially affected by Flu. Although most genes related to glycolysis were upregulated, OXPHOS pathway genes were not. Expression of key glycolytic genes *HK2* and *LDHA* as well as hypoxia-inducible factor 1 $\alpha$  (*HIF-1 $\alpha$* ), a transcription factor known to regulate cellular metabolism, was significantly increased in Flu-exposed pDCs (Supplemental Fig. 1B). These findings were confirmed by quantitative real-time PCR and similar effects were observed with RV, another ssRNA virus and the TLR7 agonist Gard (Supplemental Fig. 1C). Flu and RV also induced significant increases in ATP in pDCs (Supplemental Fig. 1D). Collectively, these data indicate that these respiratory viruses significantly impact pDC metabolism.

HIF-1 $\alpha$  is a principal regulator of metabolism, controlling most genes encoding glycolytic enzymes in mammalian cells (17). A central role for HIF-1 $\alpha$  has also been observed during inflammation and viral infections (18, 19). Flu infection in macaques promotes HIF-1 $\alpha$  expression in blood leukocytes and tissue macrophages, highlighting a potential role for HIF-1 $\alpha$  in respiratory viral infections (20). Our data reveal that Flu induces significant increases in both HIF-1 $\alpha$  protein expression and nuclear translocation in pDCs (Supplemental Fig. 1E–G), suggesting that viral-induced metabolic changes are regulated in part by HIF-1 $\alpha$  in these cells.

### *Flu, RV, and Gard induce glycolysis in human pDCs*

To demonstrate the impact of viral stimulation on the glycolytic pathway in pDCs, we measured ECAR, an indicator of glycolysis in real time. pDCs were first exposed to Flu, RV, or Gard followed by the various treatments depicted in Fig. 1A. Flu-, RV-, and Gard-exposed pDCs generated elevated ECAR upon addition of glucose, and this persisted after treatment with oligomycin, an ATP synthase inhibitor added to induce maximal glycolysis (Fig. 1A). In contrast to the viral-mediated increase in ECAR (Fig. 1B), OCR, a reflection of mitochondrial OXPHOS, was reduced in pDCs exposed to Flu and RV (Fig. 1C, *left panel*). Mitochondrial membrane potential was also unaffected by Flu or RV exposure (Fig. 1C,





**FIGURE 1.** Flu, RV, and Gard induce glycolysis in human pDCs. **(A)** pDCs were cultured in cRPMI with no stimulant (Contl), Flu, RV, or Gard for 24 h, washed, and reincubated in nonbuffered medium. Real-time ECAR was measured in supernatants of pDC cultures using an XF-24 analyzer. Vertical lines indicate addition of glucose (glycolysis substrate), oligomycin (ATP synthase inhibitor), and 2-DG (glycolysis inhibitor). One of three independent experiments shown. Summary of pDC basal ECAR **(B)** and basal OCR **(C, left panel)** measured simultaneously in the same well. **(C, right panel)** Red CMXRos mean fluorescence intensity (MFI) was quantified by flow cytometry in pDCs cultured  $\pm$  Flu for 48 h. Data represent mean  $\pm$  SEM,  $n = 3$  for **(B)** and **(C)**. **(D)** pDCs were exposed to Flu, RV, or Gard for 6, 24, and 48 h and glucose-derived- $^{13}\text{C}$  lactate measured in supernatants by mass spectrometry. One of three experiments shown. **(E)** Mean  $\pm$  SEM values of  $^{13}\text{C}$  lactate normalized to control in Flu-, RV-, and Gard-treated pDCs after 24 and 48 h;  $n = 4$ –6. **(F)** Lactate efflux rate (nmoles/h/ $10^5$  cells) in pDCs exposed to Flu, RV, or Gard;  $n = 4$ –6. \* $p \leq 0.05$ , \*\* $p \leq 0.01$ , paired  $t$  test, control versus treatment.

*right panel*). A longer-term assessment of glycolysis was accomplished by quantifying extracellular lactate, a product of glycolysis, in supernatants of pDCs exposed to viruses over 48 h. Time-dependent production of  $^{13}\text{C}$  glucose-derived  $^{13}\text{C}$  lactate was significantly increased by Flu, RV, and Gard exposure compared with control conditions (Fig. 1D, 1E). Flu-, RV-, and Gard-activated pDCs also exhibited elevated rates of lactate production (Fig. 1F). These results demonstrate that viral exposure enhances glycolytic flux but not mitochondrial OXPHOS in pDCs and provide evidence that pDCs preferentially induce glycolysis to potentially meet biosynthetic demands during viral infections.

#### Inhibition of glycolysis impairs pDC antiviral responses

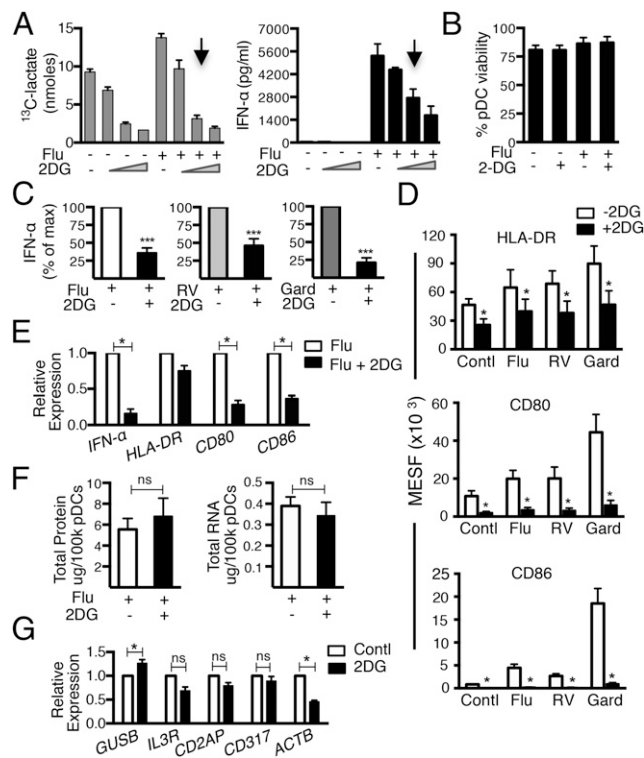
To investigate the role of glycolysis in regulating pDC antiviral responses, we used 2-DG, a glycolysis inhibitor. Addition of 2-DG significantly reduced  $^{13}\text{C}$  lactate efflux by pDCs and concomitantly impaired Flu-induced IFN- $\alpha$  secretion in a dose-dependent manner (Fig. 2A) without decreasing pDC viability (Fig. 2B). Inhibition of glycolysis also interrupted RV- and Gard-induced pDC IFN- $\alpha$  secretion (Fig. 2C). Viral

and Gard-induced pDC maturation, assessed as upregulation of surface HLA-DR, CD80, and CD86, was similarly impaired by 2-DG (Fig. 2D). In addition, Flu-induced expression of IFN- $\alpha$ , CD-80, and CD-86 mRNA was also significantly inhibited by 2-DG (Fig. 2E), suggesting that glycolysis may regulate these antiviral genes at the transcriptional level. Taken together, these data highlight a critical role for glycolysis in pDC antiviral functional responses including IFN- $\alpha$  production and upregulation of costimulatory molecules.

We next evaluated the impact of glycolysis inhibition on total protein and total RNA concentrations in Flu-exposed pDCs. No differences were induced by 2-DG treatment (Fig. 2F), suggesting that glycolysis is not a global regulator of all transcription and protein synthesis in these cells. To assess whether glycolysis impacted transcription of pDC genes not induced by viral exposure, we measured the effect of 2-DG on transcription of the following genes for which expression was unaltered by Flu in our DNA microarray experiments (GenBank accession number GSE68849; <http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?token=qfarmocyrvcvdsb&acc=GSE68849>): *GUSB* (encodes an essential lysosomal enzyme), *ACTB* (maintains cell motility), *IL-3R* (a receptor that maintains pDC viability), *CD2AP* (regulates pDC migration), *CD317* (a receptor that negatively regulates proinflammatory cytokine secretion), and *PPIA* (encodes cyclophilin A, a regulator of cellular protein folding and trafficking) (21–25). Results confirmed that glycolysis is not required for global pDC gene expression (Fig. 2G); *GUSB* expression was actually increased, whereas *IL-3R*, *CD2AP*, and *CD317* were unaltered by inhibition of glycolysis. *PPIA* expression was similarly unaffected by glycolysis inhibition and was used to normalize these data. Conversely, 2-DG treatment resulted in decreased actin mRNA expression in pDCs, suggesting that some cellular processes, in addition to viral-induced pDC IFN- $\alpha$  and maturation responses, are likely also regulated by glycolysis in these cells.

Because IFN- $\beta$  has been shown to directly regulate glucose metabolism (26), it seemed possible that viral-induced glycolysis in pDCs could be mediated by IFN- $\alpha/\beta$ . To test this, we exposed pDCs to IFN- $\alpha$  and measured  $^{13}\text{C}$  lactate production. pDC lactate efflux was not induced by IFN- $\alpha$  treatment (Supplemental Fig. 2A). Furthermore, IFN- $\alpha/\beta$  receptor blockade did not inhibit Flu-induced increases in lactate, suggesting that type I IFN does not regulate Flu-induced glycolysis in pDCs. To evaluate the role of TLR7 activation, we used chloroquine to disrupt endosomal acidification, a required component of TLR7 signaling (27). Chloroquine inhibited Flu- and Gard-induced increases in both  $^{13}\text{C}$  lactate and IFN- $\alpha$  production, providing evidence that TLR7 signaling is involved in viral-induced upregulation of glycolytic pathways in pDCs (Supplemental Fig. 2B, 2C).

Recent discoveries indicate that metabolic pathways regulate mDC and T cell responses to activation signals, including TLR agonists, growth factors, and Ags (28–30). Our results reveal that viral exposure promotes metabolic activation in pDCs and that these metabolic changes regulate critical pDC antiviral functions. Why viruses such as Flu and RV specifically induce glycolysis in human pDCs is unclear. Because immune cells face substantial bioenergetic challenges to generate immune responses, it is possible that rapid generation of ATP,



**FIGURE 2.** Inhibiting glycolysis impairs viral-induced pDC antiviral responses without disrupting viability. (A) [ $^{13}\text{C}$ ] Lactate and IFN- $\alpha$  in supernatants of pDCs exposed to Flu in media containing 10 mM D-glucose with or without 2-DG (1, 10, and 20 mM). One of three experiments shown. Arrows indicate 2-DG concentration (10 mM) used in all subsequent experiments. (B) Effects of 2-DG on pDC viability. (C) IFN- $\alpha$  (picograms per milliliter) in supernatants of pDCs exposed to Flu, RV, or Gard for 24 h  $\pm$  2-DG. (D) Surface expression of HLA-DR, CD80, and CD86 on pDCs cultured for 48 h in conditions shown in (C). (E) Relative expression of IFN- $\alpha$ , HLA-DR, CD80, and CD86 to *PPIA* in pDCs exposed to Flu  $\pm$  2-DG for 24 h. (F) Total protein and total RNA in pDCs exposed to Flu  $\pm$  2-DG for 24 h. (G) Impact of 2-DG on expression of *GUSB*, *IL3R*, *CD2AP*, *CD317*, and *ACTB*. Data represent mean  $\pm$  SEM,  $n = 3$ –8 in (B)–(G), \* $p \leq 0.05$ , \*\*\* $p \leq 0.001$ , paired  $t$  test.

one advantage of glycolysis over OXPHOS (31), could provide energy required for immediate secretion of IFN- $\alpha$  by pDCs. Increased glycolysis could also support the anabolic demands of viral-exposed pDCs by providing intermediate precursors for biosynthesis of nucleotides, nonessential amino acids and lipids as demonstrated in TLR agonist-induced murine DCs (32). We were unable to detect such precursor macromolecule intermediates in our experimental system, however, because of the limited number of pDCs available from human donors.

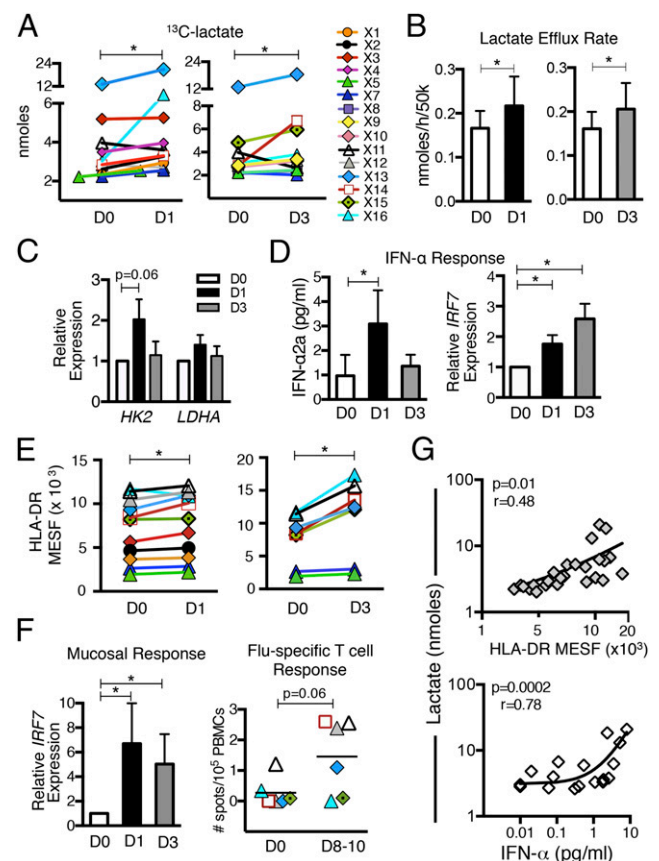
Inflammation and infection deplete tissue oxygen and nutrient levels, providing optimal conditions for glycolysis and not OXPHOS. pDCs are known to be recruited to such sites (3). Preferential induction of glycolysis upon pathogen encounter could thus promote generation of pDC antiviral responses in these anaerobic microenvironments.

#### LAIV results in increased glycolysis in human pDCs

To evaluate the impact of in vivo viral infection on pDC metabolism, we used LAIV. Blood pDCs were purified from healthy human donors (median,  $3 \times 10^5$ ; range,  $1$ – $7 \times 10^5$  pDCs/donor) before and after intranasal LAIV administration, and ex vivo pDC lactate efflux was measured. Despite substantial variation in baseline rates of pDC lactate production

among donors, pDCs isolated after LAIV demonstrated significant increases in ex vivo lactate secretion and lactate efflux rates (elevated glycolysis) (Fig. 3A, 3B). Transcription of *HK2* was also increased in pDCs isolated after LAIV, although the increase not significant (Fig. 3C). LAIV also significantly increased pDC IFN- $\alpha$  secretion, *IRF7* mRNA expression (Fig. 3D), and surface HLA-DR expression (Fig. 3E), further demonstrating a systemic pDC response to the locally administered LAIV-induced Flu infection.

To confirm that LAIV induced a local innate response, we also measured *IRF7* expression in nasal samples. Expression of *IRF7* mRNA was significantly increased at both 1 and 3 d after vaccination (Fig. 3F, left panel), providing evidence of LAIV-induced antiviral response in the nasal mucosa. Although beyond the scope of our study, we also measured adaptive responses to LAIV in a subset of participants. Increased Flu-specific T cell IFN- $\gamma$  responses, although not significant ( $p = 0.06$ ), were detected after LAIV (Fig. 3F, right panel), providing evidence that LAIV induced both innate and



**FIGURE 3.** In vivo viral infection (LAIV) increases glycolysis in human pDCs and correlates with pDC antiviral responses. Blood pDCs were purified from healthy donors before (D0), 1 (D1), and/or 3 (D3) days after LAIV and pDCs ( $5 \times 10^4$ ) were cultured ex vivo for 24 h. [ $^{13}\text{C}$ ] lactate quantification (A) and lactate efflux rate (B) in pDC supernatants. (C) Relative pDC mRNA expression of *HK2* and *LDHA*. (D) IFN- $\alpha$ 2a secretion (left panel) and relative *IRF7* expression (right panel) in pDCs cultured ex vivo for 24 h. (E) Effect of LAIV on pDC HLA-DR expression. (F) Nasal cell *IRF7* expression normalized to *PPIA* (left panel); Flu-specific IFN- $\gamma$ -secreting T cells measured by ELISPOT before (D0) and after (days 8–10) LAIV (right panel). (G) Correlations between pDC glycolysis (lactate production) and HLA-DR expression (upper panel) and IFN- $\alpha$  secretion (lower panel). Data from three time points (D0, D1, and D3) displayed. Data in histograms represent mean  $\pm$  SEM. In (A) and (E), lines connect data from individual donors.  $n = 6$ –11, \* $p \leq 0.05$ , paired  $t$  test.

adaptive immune responses. More importantly, significant associations between glycolysis and two critical components of innate antiviral pDC responses were revealed by our study. Positive correlations between pDC HLA-DR expression (Fig. 3G, *upper panel*) and pDC IFN- $\alpha$  secretion (Fig. 3G, *lower panel*) versus ex vivo lactate production were observed, supporting the notion that activation state and metabolism may be linked in pDCs. Results from this in vivo viral infection model thus complement the ex vivo findings and highlight a potential regulatory role for glycolysis in human pDC early responses to viral infections.

To our knowledge, these data provide the first indication that pDCs use glycolysis, a key energy producing metabolic pathway, to perform critical antiviral functions. Our findings offer new insight into the regulation of pDC function and provide a foundation for potential development of novel strategies to augment immune responses in humans. Production of IFN- $\alpha$ , a potent antiviral cytokine, represents a primary pDC function. Considering the extensive biologic effects of IFN- $\alpha$ , glycolysis in pDCs—now linked to IFN- $\alpha$  production—could thus affect multiple components of antiviral responses extending beyond the pDC itself. IFN- $\alpha$  promotes pathogen clearance and also augments Ag presentation and cytokine production of other innate immune cells (7). The concept of manipulating glycolysis, a model already under investigation in cancer (33) to potentially impact pDC IFN- $\alpha$  production, represents one potential application of our findings. This could be applied toward potential development of treatment strategies for viral infections such as chronic hepatitis C virus and HIV (34, 35), where deficient pDC IFN- $\alpha$  responses are associated with clinical disease. Augmenting pDC IFN- $\alpha$  secretion through manipulation of pDC glycolysis could theoretically be evaluated in any clinical scenario associated with aberrant pDC IFN- $\alpha$  responses. TLR7 agonists, known to activate pDCs, have already been evaluated to treat infections associated with human papilloma and hepatitis C virus (36). In our study, the TLR7 agonist Gard induced pDC glycolysis and IFN- $\alpha$  secretion and could thus also be examined as a potential method to enhance antiviral immune responses. This strategy, however, is not pDC specific and could also activate other TLR7-expressing cells. It is important to note that as new approaches are developed to specifically target genetic pathways in distinct cell types, manipulation of the pDC glycolysis–IFN- $\alpha$  axis could provide an avenue to enhance general antiviral responses.

Despite the challenges of working with primary human pDCs, our study demonstrates a critical role for glucose metabolism in the regulation of pDC responses to common respiratory viral pathogens responsible for considerable morbidity and mortality in humans. Development of strategies to enhance pDC metabolic pathways to potentially improve antiviral responses represents one possible application of this discovery.

## Acknowledgments

We thank J. Gern and Y. Bochkov for providing rhinovirus, A. Ali for help with metabolic flux experiments, R. Kong for assistance with imaging flow cytometry, and J. Seidel for assistance with obtaining LAIV.

## Disclosures

R.J.D. is a member of the scientific advisory boards of Peloton Therapeutics and Agios Pharmaceuticals. The other authors have no financial conflicts of interest.

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