ORIGINAL RESEARCH

Dysregulation of Pyruvate Kinase M2 Promotes Inflammation in a Mouse Model of Obese Allergic Asthma

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Abstract

Obesity is a risk factor for the development of asthma and represents a difficult-to-treat disease phenotype. Aerobic glycolysis is emerging as a key feature of asthma, and changes in glucose metabolism are linked to leukocyte activation and adaptation to oxidative stress. Dysregulation of PKM2 (pyruvate kinase M2), the enzyme that catalyzes the last step of glycolysis, contributes to house dust mite (HDM)-induced airway inflammation and remodeling in lean mice. It remains unclear whether glycolytic reprogramming and dysregulation of PKM2 also contribute to obese asthma. The goal of the present study was to elucidate the functional role of PKM2 in a murine model of obese allergic asthma. We evaluated the small molecule activator of PKM2, TEPP46, and assessed the role of PKM2 using conditional ablation of the *Pkm2* allele from airway epithelial cells. In obese C57BL/6NJ mice,

parameters indicative of glycolytic reprogramming remained unchanged in the absence of stimulation with HDM. Obese mice that were subjected to HDM showed evidence of glycolytic reprogramming, and treatment with TEPP46 diminished airway inflammation, whereas parameters of airway remodeling were unaffected. Epithelial ablation of *Pkm2* decreased central airway resistance in both lean and obese allergic mice in addition to decreasing inflammatory cytokines in the lung tissue. Lastly, we highlight a novel role for PKM2 in the regulation of glutathione-dependent protein oxidation in the lung tissue of obese allergic mice via a putative IFN- γ -glutaredoxin1 pathway. Overall, targeting metabolism and protein oxidation may be a novel treatment strategy for obese allergic asthma.

Keywords: airway epithelial cells; obesity; S-glutathionylation; glycolysis; glutaredoxin

Asthma is a chronic inflammatory disease characterized by airway inflammation, remodeling, and hyperresponsiveness (AHR). Obesity is a prominent risk factor for asthma development, and patients with obesity and asthma are less responsive than

lean patients with asthma to available therapeutics (1–3). The molecular pathways that underlie obesity-associated allergic asthma remain incompletely understood. Increases in glycolysis, the utilization of glucose, have been reported in bronchiolar

epithelial cells from patients with obesity and asthma (4). Glycolytic reprogramming is important for immune cell activation and inflammation, and it contributes to the pathogenesis of multiple diseases (5–7). Moreover, a prominent role for glycolysis

(Received in original form November 9, 2020; accepted in final form February 7, 2021)

Supported by the following grants from the U.S. National Institutes of Health: R35HL135828 (Y.M.W.J.-H.), R01HL137268 (A.E.D. and A.v.d.V.), R01HL085646 (A.v.d.V.), R01HL138708 (A.v.d.V.), R21AG055325 (A.v.d.V.), T32HL076122 (M.E.P. and C.G.I.), 1S10OD025030-01 from the National Center for Research Resources (D.J.T.), R01HL122383 (V.A.), R01HL141364 (V.A.), and R01HL136917 (V.A.).

Author Contributions: A.M.M. designed and performed experiments, analyzed data, and wrote the paper. C.v.d.W., M.M., C.E., C.M., and R.A. performed experiments and analyzed data. J.v.d.V., A.E.D., M.E.P., C.G.I., D.J.T., A.v.d.V., and V.A. provided helpful discussion and edited the manuscript. Y.M.W.J.-H. designed experiments, analyzed the data, and wrote the paper.

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This article has a data supplement, which is accessible from this issue's table of contents at www.atsjournals.org.

Am J Respir Cell Mol Biol Vol 64, No 6, pp 709–721, June 2021

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Originally Published in Press as DOI: 10.1165/rcmb.2020-0512OC on March 4, 2021

Internet address: www.atsjournals.org

in asthma is emerging (4, 8–10). Our laboratory has demonstrated that IL-1 stimulates glycolytic reprogramming in airway epithelial cells in a lean mouse model of house dust mite (HDM)-induced allergic airways disease (9). We further showed that IL-1-driven glycolysis was associated with the activation of the IKK ϵ (inhibitory κ B kinase- ϵ) and TBK1 (TANK-binding kinase) pathways in airway epithelial cells (9).

PK (pyruvate kinase) catalyzes the final step in the glycolysis cascade, converting phosphoenolpyruvate (PEP) to pyruvate. PK exists in two distinct isoforms in the lung, M1 and M2, and both are able to catalyze the final step of glycolysis. The function of PKM2 is heavily regulated by its quaternary structure. The PKM2 tetramer performs its canonical glycolysis function. However, PKM2 can be post-translationally modified and can exist in a monomer/dimer form, allowing it to exert alternative “moonlighting” functions as a protein kinase and/or a transcriptional cofactor. Phosphorylation of PKM2 at Tyr105 (pPKM2) promotes the dimeric form of PKM2 to upregulate the expression of glycolytic enzymes, including LDHA (lactate dehydrogenase A) (11–13) and proinflammatory cytokines such as IL-1B (11, 14), thereby contributing to both glycolytic reprogramming and inflammation. TEPP46 is a small molecule that promotes the glycolysis-active tetramer configuration of PKM2, resulting in decreased glycolysis and inflammatory responses in immune cells (11, 15). Our laboratory demonstrated that treatment with TEPP46 reduced lung inflammation and airway remodeling in a lean model of HDM-induced allergic airways disease (16). We further showed that TEPP46 inhibited IL-1B-induced proinflammatory cytokine production by reducing phosphorylation of signal transducer and activator of transcription 3 at Tyr705 (pSTAT3) (16).

Obese asthma is associated with increases in oxidative stress (4, 17). Interestingly, increases in glycolysis play an important role in combating oxidative stress. The activation of the pentose-phosphate pathway (PPP), an offshoot of glycolysis, alleviates oxidative stress by promoting glutathione (GSH) redox homeostasis (18, 19). Oxidative stress leads to increases in PSSG (protein S-glutathionylation), the covalent linkage of GSH to the thiol group of protein cysteines.

The forward PSSG reaction can be catalyzed by GSTP (glutathione S-transferase P), whereas GLRX (glutaredoxins) catalyze the removal of GSH to restore the original protein thiol group (20). We have demonstrated that glycolytic reprogramming (9) and increases in PSSG (21) contribute to allergic airways disease pathology in lean mice.

A prior study reported that IL-1 signaling contributes to obesity-induced AHR (22). Based on our prior demonstration that IL-1 signaling promotes glycolytic reprogramming, we hypothesized that glycolytic reprogramming contributes to the pathophysiology of obese allergic asthma. Furthermore, we speculate that dysregulated activity of PKM2 and attendant oxidative changes contribute to the pathophysiology of obese allergic asthma. Herein, we used a mouse model of obese allergic airways disease involving a feeding regimen of a high-fat and high-sucrose (HF/HS) diet to model diet-induced obesity (DIO) superimposed with HDM exposures to induce allergic airways disease. We used two strategies to modulate PKM2 (by using treatment with TEPP46 or by conditional ablation of *Pkm2* in the airway epithelium). Our results show that both systemic administration of TEPP46 or conditional ablation of *Pkm2* from *Scgb1a1*-expressing epithelial cells decreased various parameters of inflammation, in association with decreased expression of IKK ϵ , IFN γ (IFNG), and the deglutathionylating enzyme GLRX1. These findings suggest that the alternative nonglycolysis functions of PKM2 exert a proinflammatory role in obese allergic lung disease and that targeting metabolic pathways may be an alternative therapeutic strategy for obese asthma.

Methods

Mouse Studies

C57BL/6NJ, *Scgb1a1-rtTA:TetOP-Cre* (control) (23), and *Scgb1a1-rtTA:TetOP-Cre:Pkm2^{loxP}* (Δ Epi-PKM2^{flx/flx}) (24) male and female mice were bred in house. AHR was analyzed after 20 weeks of being fed a control (low-fat and low-sucrose [LF/LS]) (10% kCal fat; 7% kCal sucrose; #D12450J; Research Diets, Inc.) diet or a HF/HS diet (45% kCal fat; 17% kCal sucrose; #D12491; Research Diets Inc.). After 22 weeks of LF/LS or HF/HS diet, the mice were

sensitized and challenged with HDM (catalog number XPB70D3A2.5; lot number 348718; endotoxin: 1,140 EU/vial; GREER) via intranasal administration. A total of 50 mg/kg of TEPP46, vehicle, or 2 mg/kg doxycycline (Dox) was given by intraperitoneal injections. All animal experiments were approved by the Institutional Animal Care and Use Committee at the University of Vermont. Additional details are provided in the data supplement.

BAL Fluid Processing

BAL fluid was processed as described in the data supplement.

Airway Hyperresponsiveness

Airway hyperresponsiveness was analyzed as described in the data supplement.

Assessment of Tissue Immune Cell Influx and Airway Remodeling

Tissue histopathology was assessed by hematoxylin and eosin, periodic acid Schiff, and Masson's trichrome staining as described in the data supplement.

Immunofluorescence Staining

Lung tissue sections were deparaffinized with xylene and rehydrated in ethanol. The slides were heated in 100 mM sodium citrate buffer for antigen retrieval, incubated with indicated antibodies, and counterstained with DAPI blue. Additional details are provided in the data supplement.

Total GSH and PSSG Measurements

Lung tissues were lysed in 100 mM potassium phosphate buffer with 0.6% sulfosalicylic acid and 0.1% Triton-X 100. Samples were incubated with 2.25 μ g/ml GSH reductase, 240 μ M nicotinamide adenine dinucleotide phosphate, and 3 μ M 5,5'-dithio-bis(2-nitrobenzoic acid), and kinetic absorbance at 412 nm was recorded (25). Additional details are provided in the data supplement.

Western Blotting

Lung tissue was lysed in radioimmunoprecipitation assay buffer with protease and phosphatase inhibitor cocktail (Thermo Fisher Scientific). IB was performed as described in the data supplement.

Real-Time Quantitative PCR

The mRNA was analyzed as described in the data supplement.

ELISA

Indicated cytokines were detected by specific ELISA kits (R&D Systems) in normalized lung tissue lysates according to manufacturer's instructions.

PK Activity

Total PK activity in the lung was assessed using the Pyruvate Kinase Activity Colorimetric/Fluorometric Assay Kit (BioVision, Inc.) according to the manufacturer's instructions.

Lactate Measurements

Lactate concentrations were measured in lung tissue and BAL fluid with L-lactate Assay Kit II (Eton BioScience Inc.) according to the manufacturer's instructions.

Statistical Analysis

Data are expressed as means $\pm$ SEM. Significant differences between groups were determined using the GraphPad Prism7 (GraphPad Prism Software, Inc.) by unpaired two-tailed *t* test and one-way or two-way ANOVA with a Tukey *post hoc* test for multiple comparisons. *P* values lower than 0.05 were accepted as significant. The Kruskal-Wallis test was used to evaluate histological staining.

Results

HF/HS Diet-induced Obesity Increases Airway Hyperresponsiveness without Changing Parameters of Glycolysis in the Lung Tissue

IL-1-driven glycolytic reprogramming in the lung promotes allergic airway disease in lean mice (9). To investigate whether DIO stimulates glycolytic reprogramming in the lung, male C57BL/6N mice were fed a normal control diet (LF/LS; lean) or an HF/HS (obese) diet. After 20 weeks of consuming a LF/LS or HF/HS diet, mice gained 65% or 125% of their starting body weight, respectively. Mice given the HF/HS diet accumulated more fat tissue mass compared with normal weight (lean) mice consuming the LF/LS diet (Figure 1A), confirming that the HF/HS diet is a representative diet to study obesity in mice.

Figure 1. Airway hyperresponsiveness is not accompanied by glycolytic reprogramming in the lung tissue of obese mice. (A) Percentage weight gain of male mice fed a normal (low-fat and low-sucrose [LF/LS]) or high-fat and high-sucrose (HF/HS) diet for 20 weeks. Epididymal fat tissue weight

Airway resistance, tissue resistance, and tissue elastance were increased in obese versus lean mice in response to 50 mg/ml methacholine (Figure 1B) (22, 26). A prior study demonstrated that *Il1b* mRNA expression is increased in the lungs of obese mice and that neutralization of IL1 signaling attenuated AHR in obese mice (22). However, we observed no statistically significant increases in either *Il1b* mRNA or IL1B protein in the lung tissue of obese mice (Figure 1C). We also observed no change in the concentrations of lactate in the BAL fluid or in the lung tissue of obese versus lean mice (Figure 1D). In addition, no alterations in PK activity, pPKM2, PKM2, PKM1, or LDHA protein concentrations were found between lung tissue from obese mice and that from lean mice (Figures 1E and 1F). Compared with lean mice, an increase in total cells was observed in BAL fluid from obese mice, reflected by increases in alveolar macrophages (Figure 1G). DIO did not enhance mucus metaplasia or subepithelial collagen deposition in the airways (Figures 1H and 1I and E1A and E1B). The α SMA (α smooth muscle actin) immunoreactivity tended to be more pronounced around the small airways of obese mice compared with lean mice (Figure 1J), potentially contributing to the small increases in AHR. These results show that although DIO increased AHR, it did not induce airway inflammation or mucus metaplasia, nor did it increase indices of glycolytic reprogramming in the lung.

Impact of the Small Molecule PKM2 Activator TEPP46 on Parameters of Glycolysis in Lungs of Lean or Obese Mice with HDM-induced Allergic Airways Disease

Although DIO alone did not promote glycolytic reprogramming in the lung, a recent study showed that glycolysis and mitochondrial bioenergetics were both augmented in airway epithelial cells from patients with obesity and asthma compared

with individuals with obesity without asthma (4), indicating that glycolytic reprogramming may contribute to the pathophysiology of obese allergic asthma. We therefore superimposed the HDM model of allergic airways disease on mice with DIO. Twenty-two weeks after feeding, mice were sensitized and challenged with saline or HDM during Weeks 23, 24, and 25 (Figures 2A–2C). We first investigated whether HDM increased parameters of glycolysis in these 8-month-old lean or obese mice. Indeed, HDM exposure increased lactate concentrations in the BAL fluid and the concentrations of glycolysis enzyme LDHA in the lungs from lean and obese mice compared with saline-treated control mice (Figures 2D and E1C). Notably, accumulation of lactate in the lung tissue was increased selectively in lean mice, but not obese mice, in response to HDM (Figure 2D). The pPKM2 disrupts the canonical glycolysis function of PKM2 and instead promotes PKM2's alternative role as a protein kinase and/or transcriptional coactivator, and this alternative function of PKM2 promotes glycolytic reprogramming and inflammation (11–13). We showed that pPKM2 was increased in lean and obese allergic mice compared with nonallergic control mice (Figure E1C). We next tested whether TEPP46, a small molecule that promotes PKM2 canonical function, attenuates HDM-induced glycolytic reprogramming in these lean and obese models. TEPP46 or vehicle was administered intraperitoneally once per day during the final 6 experimental days, and the mice were killed 24 hours after the final administration (Figure 2A). Total PK activity was unaltered in the lung tissue of both HDM groups compared with saline control mice, and PK activity was significantly increased in the lungs of obese allergic mice after TEPP46 treatment (Figure 2E). PKM1 and GAPDH protein concentrations remained unaltered between any of the groups (Figure 2F). Administration of TEPP46 decreased

lactate concentrations in the BAL fluid of lean allergic mice but did not affect concentrations of lactate or glycolytic enzymes in the lung tissue from these animals (Figures 2D and 2F). In contrast, in obese allergic mice, TEPP46 decreased pPKM2 (Figure 2F) but did not affect lactate concentrations in the BAL fluid or lung tissue (Figure 2D). Nonfasting serum glucose, lactate, and triglyceride concentrations were not changed between any of the groups (Figure E2A), suggesting that the changes in metabolism observed in the lung tissue are not merely reflecting alterations in the metabolic profile of the blood. Overall, these results demonstrate that TEPP46 disparately affected glycolytic reprogramming in lean allergic mice compared with obese allergic mice.

Administration of TEPP46 Reduces the Inflammatory Response in Lungs of Obese Mice with HDM-induced Allergic Airways Disease

Glycolytic reprogramming is strongly linked to enhanced inflammation (12, 27–29) in association with pPKM2 and PKM2's noncanonical functions (11, 15). We previously demonstrated that TEPP46 decreased allergic inflammation in 3-month-old lean mice in association with decreases in pSTAT3, cytokine production, and eosinophil recruitment (16). We hypothesized that TEPP46 would also reduce lung inflammation in obese mice with allergic airways disease. HDM led to increases in inflammatory cells in lung tissue and BAL fluid in both the lean and obese groups compared with the saline control group. Surprisingly, TEPP46 lowered inflammatory cell infiltrates in the lung tissue of obese allergic mice but did not lead to statistically significant decreases in BAL cells in this group. In the lean allergic group, TEPP46 decreased inflammatory cell infiltrates in the lung tissue but did not dampen total cell counts in the BAL fluid (Figures 3A and 3B). Macrophage percentages in the BAL fluid

Figure 1. (Continued). (in grams) after 20 weeks of LF/LS or HF/HS feeding. (B) Responsiveness to inhaled methacholine was calculated using the constant phase model that measures R_n , G, and H ($n = 5$ mice/group). (C) Measurement of *Il-1b* mRNA and IL1B protein in the lung tissue homogenate by quantitative PCR or ELISA. (D) Quantification of lactate in BAL fluid (BALF) and lung tissue homogenates. (E) Total PK (pyruvate kinase) activity in lung tissue of lean and obese mice. (F) Western blot analysis of LDHA (lactate dehydrogenase A), pPKM2, PKM2 (pyruvate kinase M2), and PKM1 protein concentrations in lung tissue. β -actin was used as a loading control. (G) Total and differential cell counts in the BALF. (H) Assessment of mucus metaplasia by PAS staining intensity, (I) subepithelial collagen via Masson's trichrome staining, and (J) α SMA (α smooth muscle actin) staining around the small airways ($n = 4$ –6 mice/group). Scale bars, 200 μ m. All data are expressed as means ($\pm$ SEM) with $n = 7$ –9 mice/group unless specified otherwise. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, and **** $P < 0.0001$ (unpaired two-tailed t test). Eosin. = eosinophils; G = tissue resistance; H = tissue elastance; Lymph. = lymphocytes; Macro. = macrophages; Neut. = neutrophils; PAS = periodic acid Schiff; pPKM2 = phosphorylation of PKM2 at Tyr105; RFU = relative fluorescence units; R_n = Newtonian resistance.

Figure 2. TEPP46 differentially influences glycolysis in the lungs of lean and obese allergic mice. (A) Schematic depicting 22 weeks of normal (LF/LS) or HF/HS diet feeding followed by intranasal sensitization with saline or 25 µg HDM during Weeks 23, 24, and 25 and the simultaneous administration of 50 mg/kg TEPP46 during Week 25. (B) The percentage body weight gain of male and female mice was recorded weekly throughout 25 weeks of LF/LS or HF/HS diet feeding. (C) Epididymal adipose tissue weight in grams at 25 weeks. (D) Lactate measurement in the BALF and lung tissue homogenates. (E) Total PK glycolysis activity in lung tissue homogenates. (F) Immunoblots probed with anti-LDHA, -pPKM2, -PKM2, -PKM1, and -GAPDH antibodies of lung tissue homogenates. β -actin shows equal loading between all samples. All data are expressed as means ($\pm$ SEM) with $n = 7$ –8 mice/group. * $P < 0.05$, ** $P < 0.01$, and **** $P < 0.0001$ (one-way ANOVA). HDM = house dust mite.

were decreased in all of the groups administered HDM versus saline (Figure 3C). Lean allergic mice exhibited higher percentages of eosinophils, whereas obese allergic mice displayed higher percentages of lymphocytes in the BAL fluid (Figure 3C). TEPP46 increased lymphocyte percentages in lean allergic mice but did not alter cell differentials in obese allergic mice (Figure 3C). CCL20 (C-C motif chemokine ligand 20), eotaxin, pSTAT3, IKK ϵ , and TBK1 concentrations were higher in both lean allergic and obese allergic mice compared with the lean saline control mice (Figures 3D and 3E) (9, 16). Analysis of proinflammatory cytokines IL-1A, TSLP (thymic stromal lymphopoietin), GM-CSF (granulocyte-macrophage colony-stimulating factor), and TGF β 1 (transforming growth factor β) concentrations in the lung tissue revealed no differences between any of the groups (Figure E2B). RANTES (regulated on activation normal T-cell expressed and secreted protein) was significantly increased in lean allergic mice and diminished after treatment with TEPP46, and a similar but not statistically significant trend was observed in obese allergic mice (Figure 3D). TEPP46 reduced pSTAT3, IKK ϵ , and TBK1 concentrations in the lung tissue from obese allergic mice (Figure 3E), suggesting a potential role for the nonglycolysis functions of PKM2 in these responses in obese allergic mice. In contrast, TEPP46 did not affect pSTAT3, IKK ϵ , and TBK1 in lean allergic mice (Figures 3E), which is possibly linked to the elevated concentrations of IL-1B that were observed in the lean allergic group compared with the obese group (Figure E2B).

We next addressed the impact of TEPP46 on airway remodeling in lean or obese allergic mice. Although *Col1a1* mRNA was increased to a similar extent in lean and obese allergic mice, TEPP46 decreased *Col1a1* mRNA only in the lean group and not the obese allergic group. Increases in *Muc5ac* and *Fn1* mRNA expression were observed in lean, but not obese, allergic mice, and TEPP46 significantly decreased *Fn1* mRNA expression in these mice (Figure E3A). Mucus metaplasia was increased in both lean and obese allergic groups compared with the saline control group but remained unaffected by TEPP46 (Figure E3B). No clear statistically significant differences in α SMA protein surrounding the small

Figure 3. Impact of TEPP46 on proinflammatory responses in lungs of lean or obese mice with HDM-induced allergic airways disease. (A) Representative hematoxylin and eosin (H&E) staining of the small airways in the left lung lobe. Scale bars, 200 μ m. (B and C) Total cell (B) and differential cell (C) counts in the BALF. (D) Measurements of CCL20 (C-C motif chemokine ligand 20), eotaxin, and RANTES (regulated on activation normal T-cell expressed and secreted protein) in lung tissue by ELISA. (E) Representative Western blots of lung tissue lysates probed with anti-pSTAT3 (phosphorylation of signal transducer and activator of transcription 3 at Tyr705), anti-STAT3, anti-IKK ϵ (inhibitory κ B kinase- ϵ), and anti-TBK1 (TANK-binding kinase) antibodies. An immunoblot probed with anti- β -actin antibody shows equal protein loading. All data are expressed as means ($\pm$ SEM) with $n = 6$ –8 mice/group. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, and **** $P < 0.0001$ (one-way ANOVA). ns = not significant.

airways or subepithelial collagen were observed between the obese groups (Figures E3C and E3D). Altogether, these data indicate that systemic administration of TEPP46 reduces some proinflammatory mediators in obese allergic mice but does not alleviate HDM-induced airway remodeling within the parameters of this study.

Conditional Ablation of PKM2 from Airway Epithelial Cells Does Not Alter Indices of Glycolysis in the Lung Tissue of Allergic Mice

We recently demonstrated that enhanced glycolysis in epithelial cells promotes proinflammatory signaling (9). As systemic administration of TEPP46 represents a nonspecific approach to modulate the function of PKM2 in lean or obese allergic airways disease, we next sought to address the importance of PKM2 specifically in airway epithelial cells in these models using specific deletion of the *Pkm2* gene. Although ablation of *Pkm2* is distinct from TEPP46-mediated PKM2 tetramer activation, ablation of *Pkm2* results in increased expression of *Pkm1*, the alternative splice variant of the PK gene. PKM1 only exists in a tetramer configuration to function as a glycolysis enzyme and does not share the alternative proinflammatory functions of PKM2 (12, 30, 31). Therefore, ablation of *Pkm2* and resultant enhanced expression of *Pkm1* could potentially mimic some of the effects observed in response to TEPP46. To address this question, we created triple transgenic male and female mice that expressed a *Pkm2^{loxP}*-floxed allele together with *Scgb1a1* (secretoglobin family 1A member 1)-rtTA, tetOp-CRE recombinase, to allow ablation of the *Pkm2* gene upon administration of Dox. *Scgb1a1-rtTA:tetOp-CRE:Pkm2^{loxP}* triple transgenic mice (Δ Epi-PKM2^{flx/flx}) or bi-transgenic mice expressing *Scgb1a1-rtTA*, tetOp-CRE and the wild-type *Pkm2* alleles (control) were subjected to LF/LS or HF/HS diets for 25 weeks. During the last 2 weeks, all of the mice were given Dox in addition to HDM (Figure 4A). This protocol caused mice to become obese, although some weight loss occurred during the time of Dox administration (Figures 4B and 4C). As anticipated, *Pkm2* mRNA and protein concentrations in lung tissues were decreased in Δ Epi-PKM2^{flx/flx} mice regardless of diet, whereas PKM1

Figure 4. Impact of epithelial ablation of PKM2 on parameters of glycolysis in lung tissue and airway hyperresponsiveness in mice fed a normal or HF/HS diet in conjunction with HDM-induced allergic airway disease. (A) *CCSP-rtTA:TetOp-Cre:Pkm2^{WT}* (control) and *CCSP-rtTA:TetOp-Cre:Pkm2^{loxP}* (Δ Epi-PKM2^{flx/flx}) were subjected to 22 weeks of normal (LF/LS) or HF/HS diet feeding followed by intranasal sensitization with 25 μ g HDM during Weeks 23, 24, and 25 and the administration of 2 mg/kg DOX during Weeks 24 and 25. (B) Percentage weight gain over 25 weeks of LF/LS or HF/HS diet feeding. (C) Epididymal adipose tissue fat pad weight (in grams) at euthanasia. (D) *Pkm2* mRNA expression quantified by quantitative PCR in whole lung tissue

concentrations were increased in the absence of airway epithelial PKM2 (Figure 4E). Immunofluorescence staining with anti-PKM2 antibody confirmed the ablation of PKM2 in airway epithelial cells in $\Delta\text{Epi-PKM2}^{\text{flx/flx}}$ mice (Figure E4). Assessment of AHR showed decreases in central airway resistance in both the lean and obese allergic groups (Figure 4F), whereas tissue resistance and elastance were increased in $\Delta\text{Epi-PKM2}^{\text{flx/flx}}$ mice compared with control allergic mice (Figure E5A). Evaluation of lactate, LDHA, and GAPDH protein concentrations in the lung tissue or BAL fluid revealed no changes between the groups (Figures 4G and 4H). These results suggest that non-SCGB1A1-expressing cells may contribute to HDM-induced glycolytic reprogramming in the lung or that the time frame of ablation of *Pkm2* was insufficient to dampen glycolytic reprogramming in secretory cells.

Conditional Ablation of PKM2 from Airway Epithelial Cells Attenuates Proinflammatory Mediators in Mice with Obese Allergic Airways Disease

We next examined the impact of ablation of epithelial PKM2 on HDM-induced inflammation in mice fed a LF/LS or HF/HS diet. Total BAL cell counts were unaffected by the ablation of PKM2 in the airway epithelial cells (Figure 5A), although an increased percentage of lymphocytes was detected in the BAL from $\Delta\text{Epi-PKM2}^{\text{flx/flx}}$ mice fed an HF/HS diet compared with those fed a LF/LS diet (Figure 5B). Qualitative assessment of tissue inflammation via hematoxylin and eosin staining revealed lower cellular infiltrates in the lung tissue from $\Delta\text{Epi-PKM2}^{\text{flx/flx}}$ allergic mice compared with control mice irrespective of diet (Figure 5C). Epithelial ablation of *Pkm2* resulted in lower concentrations of CCL20, RANTES, IL-1A in allergic mice on either diet (Figure 5D) and decreased concentrations of IKK ϵ and TBK1 in the lung tissue from allergic mice fed a HF/HS diet (Figure 5E) without consistently affecting pSTAT3 (Figure 5E). Assessment of airway remodeling markers

showed that epithelial deletion of PKM2 decreased *Fln1* expression only in lean mice (Figure E5B), whereas no statistically significant changes in *Muc5ac*, *Acta2*, or *Colla1* mRNA expression or hydroxyproline content were observed between the groups (Figures E5B and E5C). Altogether, these results demonstrate a role for airway epithelial PKM2 to promote cytokine production in association with increased expression of IKK ϵ and TBK1 in obese allergic mice.

Activation of PKM2 with TEPP46 and Epithelial-Specific Ablation of PKM2 Regulate GSH Redox Homeostasis

Obese asthma is associated with increases in oxidative stress in airway epithelial cells (4). Increases in glycolysis play an important role in combating oxidative stress through the activation of the PPP, which promotes GSH redox homeostasis (18, 19). We therefore addressed whether variations in redox homeostasis, specifically GSH and PSSG, were affected by TEPP46 treatment or the absence of epithelial PKM2 in the lungs of lean or obese allergic mice. The amount of total GSH remained similar in lean or obese allergic groups after exposure to TEPP46 or ablation of epithelial PKM2 (Figure 6A). In contrast, PSSG was significantly increased in the lung tissue from obese allergic mice compared with the saline group, whereas trends toward increases in PSSG were observed in lean allergic mice. TEPP46 did not affect PSSG in obese allergic mice but further augmented PSSG concentrations in lean allergic mice (Figure 6B). Conditional ablation of PKM2 in airway epithelial cells also led to increases in PSSG in the lung tissues, which were more pronounced in allergic mice fed an HF/HS diet compared with the LF/LS group (Figure 6B).

PSSG is controlled by the forward catalyst GSTP and the deglutathionylase GLRX1. Therefore, we evaluated whether PKM2 status affected GSTP and GLRX1 concentrations to explain the observed increases in PSSG. GLRX1 concentrations were elevated in lung tissue from lean and

obese groups exposed to HDM, whereas GSTP concentrations remained the same between all groups (Figures 6C and 6D). HDM significantly increased *Glrx1* mRNA in lean mice but not in obese mice (Figure 6E). *Glrx1* mRNA expression was significantly decreased after the ablation of epithelial *Pkm2* in allergic mice in both of the diet groups (Figure 6F). Although TEPP46 did not affect *Glrx1* mRNA (Figure 6E), it strongly decreased GLRX1 protein concentrations in the lungs of obese allergic mice (Figure 6C), and similar decreases in GLRX1 protein were observed after the ablation of epithelial *Pkm2* in allergic mice in the HF/HS diet group (Figures 6D).

IFNG is a gene target of PKM2 (15, 30) and is also a potent inducer of GLRX1 in primary mouse tracheal epithelial cells (32). Therefore, we investigated whether the decreases in *Glrx1* expression observed after the ablation of epithelial *Pkm2* in both diet groups were associated with changes in *Ifng*. Indeed, *Ifng* expression was significantly decreased in lung tissues after the ablation of epithelial *Pkm2* in both of the diet groups, mirroring the observed decreases in *Glrx1* (Figure 6F). *Ifng* mRNA was increased in lungs of lean and obese allergic mice compared with the saline control group. Although *Ifng* mRNA trended to be lower in mice receiving TEPP46, these trends did not reach statistical significance (Figure 6E). All together, these findings demonstrate that the ablation of epithelial PKM2 leads to a decrease in expression of *Ifng*, *Glrx1*, and GLRX1 protein concentrations. The precise molecular details and potential intermediate pathways that connect PKM2, IFNG, and GLRX1 will require additional investigation. As GLRX1 is a critical deglutathionylase, decreases in GLRX1 provide an explanation for the increases in PSSG that are most notable in mice with obese allergic airway disease. These results highlight a putative role for epithelial PKM2 in the regulation of GSH redox homeostasis in lean and obese allergic airway disease.

Figure 4. (Continued). homogenates. (E) Representative Western blots depicting PKM2 and PKM1 protein concentrations in the whole lung. β -actin was used as a protein loading control. (F) Responsiveness to inhaled methacholine was calculated using the constant phase model to measure R_n . (G) Representative immunoblots using the whole lung homogenate show LDHA and GAPDH protein concentrations. β -actin was used as a protein loading control. (H) Quantification of lactate concentrations in the BALF and lung tissue from control and $\Delta\text{Epi-PKM2}^{\text{flx/flx}}$ mice. Note that all mice in this study received HDM. All data are expressed as means ($\pm$ SEM) with $n = 7-9$ mice/group. ** $P < 0.01$, *** $P < 0.001$, and **** $P < 0.0001$ (two-way ANOVA). DOX = doxycycline

Figure 5. Limiting PKM2 expression in airway epithelial cells mitigates HDM-induced inflammatory responses in mice fed a normal or HF/HS diet. (A and B) Total (A) and differential (B) cell counts in the BALF of control and Δ Epi-PKM2^{flx/flx} mice. (*n* = 4–7/group). (C) Representative hematoxylin and eosin staining of the small airways in the left lung lobe. Scale bars, 200 μ m. (D) CCL20, eotaxin, RANTES, IL-1A, and IL-1B protein concentrations in the lung were evaluated by ELISA. (E) Whole lung tissue homogenates were analyzed via immunoblotting with anti-pSTAT3, -STAT3, -TBK1, and -IKK ϵ antibodies. β -actin was used as a protein loading control. All data are expressed as means ($\pm$ SEM) with *n* = 7–9 mice/group. **P* < 0.05, ***P* < 0.01, and ****P* < 0.001 (two-way ANOVA). Note that all mice in this study received HDM.

Discussion

Glycolytic reprogramming is emerging as a key parameter of allergic airways disease and is important in the development of mucus metaplasia, airway inflammation, and AHR (9, 16). The extent to which enhanced glycolysis affects obesity-associated allergic airways disease remains unclear. In the current study, we demonstrated that enhanced AHR in obese mice did not correspond with increased indices of glycolytic reprogramming and that additional stimulation with HDM was necessary to increase glycolysis in the lungs of obese mice. We showed herein that pharmacological or genetic approaches to attenuate glycolysis by altering the function of PKM2 were associated with decreased inflammatory mediators, decreases in expression of GLRX1, and elevated PSSG. Altogether, these findings suggest that targeting glycolytic reprogramming and associated redox changes could be an alternative therapy for obese allergic airways disease.

Obesity-associated asthma includes multiple phenotypes, including late-onset asthma that arises as a consequence of obesity and existing asthma phenotypes and irritant-induced asthma that is complicated by obesity (33, 34). DIO is positively correlated with AHR and enhanced IL1 signaling in the lung (22, 26, 35). Given that IL1 induces glycolytic reprogramming–stimulated AHR (9) in obese mice, we rationalized that glycolysis would also be enhanced in obese lung disease. Contrasting with this expectation, 20 weeks of consuming an HF/HS diet did not result in increases in parameters of glycolysis. These findings are in line with a previous study that demonstrated that obesity alone does not alter glycolytic flux in human airway epithelial cells from individuals without asthma but that both glycolysis and mitochondrial bioenergetics were augmented in epithelial cells from patients with obesity and asthma compared with lean patients with asthma (4). This study highlights that glycolytic reprogramming in the lung is an important component of allergic airways disease and not DIO *per se* based on the confines of the present study design.

We therefore superimposed the DIO model with the allergen HDM to investigate whether glycolytic reprogramming contributes to the pathophysiology of obese

Figure 6. TEPP46 and epithelial ablation of PKM2 decreases *Glrx1* expression and increases PSSG (protein S-glutathionylation) in the lung tissue of allergic mice fed a normal or HF/HS diet. (A) Quantification of total reduced glutathione (GSH) concentrations in lung tissue homogenates. (B) Quantification of total protein glutathionylation in the lung tissue from each group. (C and D)

allergic airways disease. Indeed, we observed evidence of glycolytic reprogramming in the lungs of obese mice in response to HDM in accordance with the development of allergic airways disease in these mice. However, HDM-stimulated increases in lactate in the BAL fluid were dampened in obese as compared with lean allergic mice. The small molecule activator of PKM2, TEPP46, significantly decreased lactate concentrations in lean allergic mice but did not affect lactate concentrations in obese allergic mice. The dichotomy between lean and obese allergic mice regarding changes in glycolysis may be due to the different immune cell profile in the BAL fluid. Glycolytic reprogramming is required for T-cell effector function (36, 37), and lactate concentrations are significantly decreased in the airways and lungs from lean allergic *Rag*^{-/-} mice, which lack mature B and T lymphocytes (9, 38). A high-fat diet alone is sufficient to shift the immune response in the lungs of obese mice. Attenuated lactate production in the BAL fluid and lung tissue in obese versus lean allergic mice may be the result of reduced T-cell activation in obese mice. Eosinophils also rely on glycolytic metabolism as a source of energy (39, 40), and high-fat diet feeding was shown to reduce eosinophil recruitment to the lung after ovalbumin challenge (41). The trending decrease in eosinophils in the BAL fluid of obese compared with lean allergic mice could also explain the observed decrease in lactate production. Alternatively, higher lactate concentrations in the BAL fluid of obese allergic mice compared with those in saline control mice may be the result of increased glycolysis in the airway epithelial cells (4) without the contribution of enhanced glycolysis from activated immune cells.

Noncanonical functions of PKM2 have been implicated in inflammatory diseases, and dimeric PKM2 can act as a protein kinase and/or transcriptional coactivator, leading to the activation of a number of hypoxia inducible factor 1 α and STAT3 target genes, including *Il1b* (11, 12, 28, 42). pSTAT3 contributes to allergic inflammation, and ablation of airway epithelial cell STAT3 significantly decreases AHR and eosinophil recruitment into the airways and lung tissue (43). Systemic administration of TEPP46 reduced pSTAT3 and IL-1 signaling in the lungs of lean allergic mice (16). The decreases in pSTAT3 observed in the lungs of obese allergic

Figure 7. Graphical representation depicting a link between dysregulation of PKM2 and lean or obese allergic lung inflammation in mice. (A) Pathways affected by treatment with TEPP46 and their impact for lean (green) or obese (red) allergic airways disease. (B) Pathways affected by epithelial ablation of PKM2 and their impact for lean (green) or obese (red) allergic lung disease.

mice treated with TEPP46 therefore could be a potential consequence of diminished pPKM2. Both TEPP46 and the ablation of epithelial PKM2 decreased IKKε and TBK1 concentrations in allergic mice fed an HF/HS diet compared with the respective

control animals, indicating a putative role for PKM2 in the activation of IKKε and TBK1 signaling pathways in obese allergic airways disease, although additional studies will be required to solidify this possibility. Ablation of *Ikbke* and pharmacologic

inhibition of IKKε and TBK1 were previously shown to improve parameters of allergic inflammation (44) and mucus metaplasia *in vivo* (9). These studies indicate that prolonged activation of the canonical glycolysis function of PKM2 and abrogated IKKε and TBK1 signaling may be a beneficial treatment strategy for obese asthma but that additional times beyond the 6-day regimen used herein may be required to observe beneficial responses. Contrary to prior results (16), TEPP46 had no effect on inflammatory parameters in the lungs from lean allergic mice. These discrepant data possibly reflect the age of the mice (8 mo vs. 3 mo), purified nutrient diet versus chow diet, or differences between HDM doses and lots used in the present study.

The production of oxidants is a byproduct of glucose flux through oxidative phosphorylation (4). Enhanced expression of glycolysis inactive PKM2 shunts glucose into the PPP to generate nicotinamide adenine dinucleotide phosphate to combat oxidative stress. As TEPP46 reestablishes glucose flux through oxidative phosphorylation (11), the PPP shunt is attenuated in association with an increase in oxidants (45). Similarly, the ablation of epithelial PKM2 results in the upregulation of PKM1 in the lung (Figure 4E). In contrast to PKM2, the PKM1 isoform does not have alternative functions (it has no protein kinase or transcriptional activity) and is a stable tetramer with high constitutive glycolytic activity that promotes glucose use via oxidative phosphorylation (46). This scenario provides a putative explanation for the increases in PSSG observed in response to the epithelial ablation of PKM2 and the increases in PSSG in response to TEPP46 observed in the lean allergic mice. Moreover, the observed decreases in GLRX1 that were particularly notable after the ablation of epithelial *Pkm2* in the HF/HS group provide an additional mechanism to allow the increases in PSSG in these mice. Increases in PSSG in response to changes in PKM2 expression or

Figure 6. (Continued). Representative Western blots for GSTP (GSH S-transferase P) and GLRX1 (glutaredoxin 1) protein concentrations in the whole lung tissue. β-actin is used as a protein loading control. Bottom, Densitometric evaluation of GLRX1. Results shown are normalized to β-actin. (E) *Glrx1* and *Ifng* mRNA expression in the whole lung tissue were measured by quantitative PCR. (F) *Glrx1* and *Ifng* mRNA expression in the whole lung tissue from control or ΔEpi-PKM2^{flx/flx} allergic mice were measured by quantitative PCR. All data are expressed as means (±SEM) with *n* = 7–9 mice/group. **P* < 0.05, ***P* < 0.01, and ****P* < 0.001 (one-way or two-way ANOVA).

activity are associated with reduced proinflammatory responses in allergic mice fed an HF/HS diet. NF- κ B (nuclear factor κ B) is a transcription factor family critical in HDM-induced inflammation in the lung. Glutathionylation of multiple proteins involved in signaling pathways leading to activation of NF- κ B prevents NF- κ B-driven inflammation (47). Specifically, glutathionylation of I κ B (inhibitory NF- κ B) kinase α and I κ B α was shown to occur in parallel with decreased secretion of CCL20 and RANTES from airway epithelial cells and α smooth muscle cells, respectively (48, 49), and may account for the reduction in CCL20 and RANTES in Δ Epi-PKM2^{flx/flx} allergic mice.

Altogether, our results demonstrate that HDM-induced glycolytic reprogramming in the lung displays characteristics that differ between lean and obese mice but are nonetheless responsive to modulation of PKM2 (Figure 7). Increases in PSSG mediated by altering the expression or activity of PKM2 were associated with reduced proinflammatory mediators in obese allergic mice, suggesting a protective role for PSSG. Future studies will be necessary to identify target proteins of glutathionylation and the mechanistic role of these targets in obese asthma. Moreover, the exact mechanism by which PKM2

disrupts obese allergic airways disease requires further research. Overall, the dysregulation of PKM2 and attendant effects on *Ifng*, IKK ϵ , and GLRX1 signaling may offer new platforms for the management of obese allergic airways disease. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

Acknowledgment: The authors thank Nirav Daphtary and Minara Aliyeva for technical expertise toward airway hyperresponsiveness measurements and analysis. Imaging was performed in the Microscopy Imaging Center at the University of Vermont (Research Resource Identification #SCR_018821).

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