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The role of low-level lactate production in airway inflammation in asthma

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Ostroukhova M, Goplen N, Karim MZ, Michalec L, Guo L, Liang Q, Alam R. The role of low-level lactate production in airway inflammation in asthma. *Am J Physiol Lung Cell Mol Physiol* 302: L300–L307, 2012. First published November 11, 2011; doi:10.1152/ajplung.00221.2011.—Warburg and coworkers (Warburg O, Posener K, Negelein E. *Z Biochem* 152: 319, 1924) first reported that cancerous cells switch glucose metabolism from oxidative phosphorylation to aerobic glycolysis, and that this switch is important for their proliferation. Nothing is known about aerobic glycolysis in T cells from asthma. The objective was to study aerobic glycolysis in human asthma and the role of this metabolic pathway in airway hyperreactivity and inflammation in a mouse model of asthma. Human peripheral blood and mouse spleen CD4 T cells were isolated by negative selection. T cell proliferation was measured by thymidine incorporation. Cytokines and serum lactate were measured by ELISA. Mouse airway hyperreactivity to inhaled methacholine was measured by a FlexiVent apparatus. The serum lactate concentration was significantly elevated in clinically stable asthmatic subjects compared with healthy and chronic obstructive pulmonary disease controls, and negatively correlated with forced expiratory volume in 1 s. Proliferating CD4 T cells from human asthma and a mouse model of asthma produced higher amounts of lactate upon stimulation, suggesting a heightened glycolytic activity. Lactate stimulated and inhibited T cell proliferation at low and high concentrations, respectively. Dichloroacetate (DCA), an inhibitor of aerobic glycolysis, inhibited lactate production, proliferation of T cells, and production of IL-5, IL-17, and IFN- γ , but it stimulated production of IL-10 and induction of Foxp3. DCA also inhibited airway inflammation and hyperreactivity in a mouse model of asthma. We conclude that aerobic glycolysis is increased in asthma, which promotes T cell activation. Inhibition of aerobic glycolysis blocks T cell activation and development of asthma.

aerobic glycolysis; T cell function; pyruvate dehydrogenase kinase; airway hyperreactivity

THE METABOLISM OF GLUCOSE through mitochondrial oxidative phosphorylation produces a total of 36 ATP molecules. Under hypoxemic conditions this metabolism switches from oxidative phosphorylation to anaerobic glycolysis. The latter process produces two molecules of ATP, NADH, and pyruvate each. Many cancer cells preferentially metabolize glucose through glycolysis even under normoxic conditions. This aerobic glycolysis of cancer cells was first reported by Warburg et al. (32). Hence this phenomenon is known as the Warburg effect. The preferential usage of a less-energy-generating metabolic pathway by cancer cells is superficially counterintuitive since the energy requirement of these rapidly proliferating cells is high.

There are number of reasons why aerobic glycolysis is preferred (22). The p53 gene is a major regulator of mitochon-

drial respiration (2, 18, 19). The p53 gene is inactivated in highly proliferative cells. It is also frequently mutated in cancer cells. This is one of the mechanisms for switching mitochondrial respiration to aerobic glycolysis. The activation of Myc, Ras, Akt, and hypoxia-inducible factor 1 (HIF-1) in cancer cells also contributes to the Warburg effect. Myc activates many glycolytic enzymes (7). HIF-1 increases the expression of glucose transporters and glycolytic enzymes (24). Furthermore, it induces pyruvate dehydrogenase kinase 1 (PDK1), which phosphorylates and inactivates pyruvate dehydrogenase and thus suppresses the TCA cycle and oxidative phosphorylation. Increased expression of glucose transporters leads to its increased uptake, which may also influence its metabolism in the cell. Akt increases glucose uptake and metabolism (8). A recent study has shown that AKT induces the endoplasmic reticulum UDPase ENTPD5, which promotes protein glycosylation, reduces the cytosolic ATP-to-AMP ratio, and thereby derepresses PFK and triggers aerobic glycolysis (9). An alternative mechanistic view of the Warburg effect is that a part of the lactate in tumor cells comes from an increase in glutamine metabolism.

Activated T cells mimic many properties of tumor cells including increase in cell size, rapid replication, and activation of the signaling molecules-Akt, Ras, and HIF1 α that upregulate aerobic glycolysis. Indeed, an increase in the expression of the glucose transporters and key glycolytic enzymes as well as a switch from oxidative phosphorylation to aerobic glycolysis has been reported in proliferating lymphocytes (1, 5, 11). In this article we ask whether lactate production, the final product in aerobic glycolysis, was increased in T cells from a chronic inflammatory such as allergic asthma. We studied the effect of increased lactate level on T cell proliferation. Furthermore, we critically evaluated the role of pyruvate dehydrogenase kinase in lactate generation, T cell cytokine production, and airway inflammation in asthma.

METHODS AND MATERIALS

Patients. Patients were recruited from the Allergy and Immunology Clinic at National Jewish Health (NJH). Healthy controls were recruited from the National Jewish Hospital staff. The study was approved by the NJH institutional review board. A written consent was obtained from all study subjects. The diagnosis of asthma and chronic obstructive pulmonary disease (COPD) was made based on the National Asthma Education and Prevention Program (NAEPP) and GOLD (Global Initiative for Chronic Obstructive Lung Disease) criteria, respectively. We recruited 28 asthmatic patients (ages 23–75, mean 47.4 yr, 11 men and 17 women) and 28 healthy controls (age 20–61, mean 38.4 yr, 10 men and 18 women). The COPD patients (14 men and 8 women) were of age 45–70, mean 57.2 yr. Table 1 shows the clinical features of the asthmatic patients. Peripheral venous blood for serum and CD4 T cells was drawn from the antecubital fossa after overnight fasting. The patients abstained from taking all medications

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Table 1. *Characteristic features of the study patients*

Parameter (number of subjects measured/total patients)	Asthmatic Patients (N = 28)	Healthy Controls (N = 28)	COPD (N = 21)
BMI	31 ± 1.2	28 ± 3.6	29 ± 3.9
Skin test	Positive 24 (92%), negative 2 (8%)	Negative	Negative
Eosinophil count	463 ± 116/μl	245 ± 61/μl	275 ± 71/μl
FEV ₁	69.6 ± 5%	93 ± 5%	64.6 ± 5%
IgE	156 ± 45 kIU/l	Not measured	Not measured
Blood random glucose	95 ± 9 mg/dl	88 ± 7 mg/dl	91 ± 6 mg/dl
Asthma severity	Intermittent: 4 patients (14 %) Mild persistent: 6 (21%) Moderate persistent: 7 (25%) Severe: 11 (40%)	NA	NA
Therapy	SABA only: 4 patients (14%), ICS + SABA: 4 (14%) ICS + LABA: 20 (72%) Oral steroids: 4 (14%) Omalizumab: 1 (3%)	NA	SABA only: none ICS + LABA (100%) Oral steroids: none

Values are means ± SD. COPD, chronic obstructive pulmonary disease; BMI, body mass index; FEV₁, forced expiratory volume in 1 s; NA, not applicable; SABA, short-acting bronchodilator; LABA, long-acting bronchodilator; ICS, inhaled corticosteroid.

except short-acting β -agonists before blood sampling. Serum was stored at -80°C before assay. CD4⁺ T cells were isolated from the fresh blood.

Isolation and culture of human CD4⁺ T cells from the peripheral blood. Mononuclear cells were isolated by density centrifugation by using Histopaque (Sigma-Aldrich, St. Louis, MO) according to the manufacturer's protocol. CD4⁺ T cells were purified by negative selection by using the Miltenyi CD4⁺ T Cell Isolation Kit according to the manufacturer's recommendations (Miltenyi Biotec, Auburn, CA) as described previously (16). CD4⁺ T cells were more than 97% pure, as determined by flow cytometry. CD4⁺ T cells were stimulated with anti-CD3/anti-CD28 antibodies (BD Pharmingen, San Diego, CA) at 2 $\mu\text{g}/\text{ml}$ each in RPMI 1640 (Irvine Scientific, Santa Ana, CA) supplemented with 2 mM L-glutamine, 50 μM 2-mercaptoethanol, 1.3 g/l sodium pyruvate, and 50 mg/l gentamycin and 10% FBS. At the indicated time points cells were lysed in TRIzol for RNA isolation or culture supernatant was collected for lactate and cytokine measurement by ELISA. For induction of FoxP3 we stimulated CD4⁺ T cells with anti-CD3/CD28 antibodies with and without the addition of increasing concentrations of DCA. FoxP3 was detected by flow cytometry upon staining with a mouse anti-FoxP3 antibody (clone 236A/E7) from eBioscience.

Mouse CD4⁺ T cell isolation from spleen. CD4⁺ T cells were purified by negative selection by using the Miltenyi CD4⁺ T Cell Isolation Kit according to the manufacturer's instructions (Miltenyi Biotec) and as described previously (13). Mouse CD4⁺ T cells were stimulated and cultured as described for human T cells.

Measurement of lactate. Lactate was measured in cell culture supernatants and in human serum by use of the Lactate Assay Kit (Eton Bioscience, San Diego, CA). Results are presented as means ± SE from triplicate wells after subtracting the background for culture medium (for culture supernatant) and dialyzed control serum (for serum). The latter was prepared by dialyzing healthy normal serum against 1,000 volumes of PBS overnight.

Cell proliferation assays. CD4⁺ T cell proliferation was assessed in an [³H]thymidine incorporation assay. Briefly, cells were cultured in 96-well flat-bottom culture plates at $0.2 \times 10^6/\text{well}$ in a total volume of 250 $\mu\text{l}/\text{well}$ RPMI 1640 plus 10% FBS. Cells were stimulated cells with anti-CD3/CD28 antibodies (BD Scientific) at 2 $\mu\text{g}/\text{ml}$ each (13, 15). In some experiments the following inhibitors or a relevant vehicle were added to the cultures: dichloroacetic acid (DCA), 2-deoxy-D-glucose (2DG) and sodium lactate (all from Sigma-Aldrich, St. Louis, MO). After 2 days of culture, [³H]thymidine (1 mCi/well; NEN Life Science Products) was added and cells were cultured for an additional 16 h. Results are presented as means ± SE of triplicate wells after subtracting the background.

Expression of mRNA and protein for select glycolytic enzymes in CD4⁺ T cells. RNA was isolated and real-time PCR was performed according to the method described previously (14). Primers for 6-phosphofructo-2-kinase/fructose-2-bisphosphatase (PFKFB), fructose 1,6-bisphosphatase 1 (FBP1), and pyruvate dehydrogenase kinase 1 (PDK1) were designed with use of the Primer Express software and purchased from Eurofins MWG Operon. We used the following oligonucleotide sequences: PFKFB Forward GGGACCTAACCCGCTCATG, Reverse CGCAATAGTGTCACCCCGTTAGCC; FBP1 Forward GGAAAGCT-GAGAACTGCTGTACGA, Reverse TCCCCCAGCCTT CTCCAT; PDK1 Forward CCTCCTGCCTGAGTCTCTGAA, Reverse ACTTGT-GCA ATAGGCCATGTGT. GAPDH mRNA expression was used as a reference. The expression of the corresponding proteins was measured by Western blotting as described previously (13, 14).

Cytokine assays. Cytokine concentration was measured by ELISA using antibody pairs for human and mouse IL-5 and IFN- γ (BD OptEIA), IL-10 and IL-17 from eBioscience, and mouse IL-13 (R&D Systems) as described previously (13, 14). The detection limits for ELISA were as follows: 7 pg/ml for IL-5, 2 pg/ml for IL-10, 4 pg/ml for IL-17, and 2 pg/ml for IFN- γ .

Mouse asthma protocol. BALB/C mice between 6 and 8 wk of age were purchased from Jackson Laboratory. The mice were housed and used in a pathogen-free facility at the NJH in accordance with all applicable guidelines. Animal studies were performed according to institutional guidelines of animal use and care. All procedures used on animals were reviewed and approved by the Institutional Animal Care and Use Committee of NJH.

Mice were immunized subcutaneously twice with the ragweed allergen, *Ambrosia artemisiifolia* (Greer Laboratories, Lenoir, NC), in alum and then intranasally exposed to ragweed for 3 consecutive days 2 wk later according to the previously described protocol (12). Mice were pretreated intraperitoneally with DCA at 5 mg/mouse or vehicle 1 h before the intranasal exposure. Airway hyperreactivity, bronchoalveolar lavage, and lung histology were assessed 72 h after the last allergen exposure. Inflammation was quantified by using the Meta-morph image acquisition and analysis software on hematoxylin and eosin-stained lung sections (5 μm) at $\times 200$ magnification. Airway inflammation was measured as the area of inflammatory infiltrates per micrometer of basement membrane in a minimum of seven airways per mouse and three mice per group. Airway hyperreactivity was assessed as the total lung resistance in response to nebulized methacholine as described previously (12).

Statistical analyses. Mann-Whitney U-test for nonpaired samples and Pearson's correlation coefficient were used to analyze human samples. Student's t-test and ANOVA were used for statistical analyses of mouse experiments and human T cell studies.

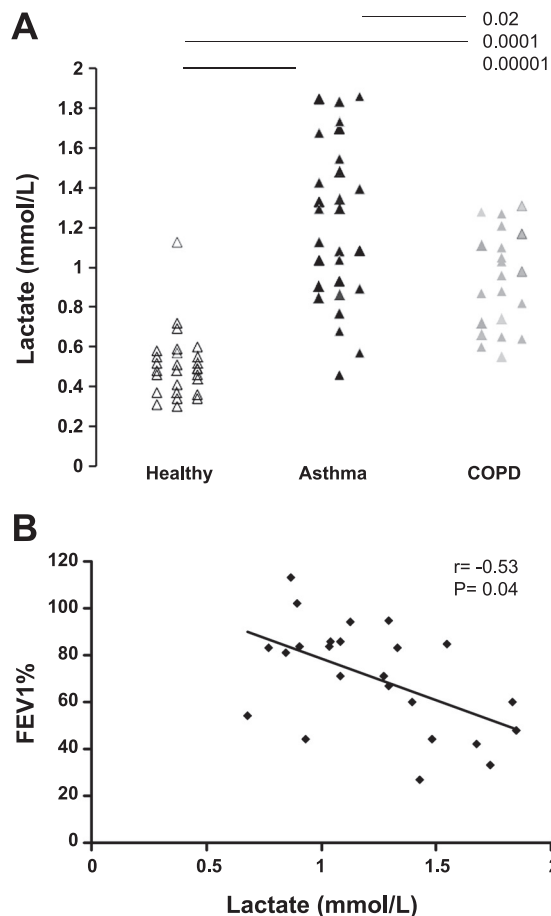


Fig. 1. Lactate level in the serum from asthmatic patients and control subjects. A: serum from 28 asthmatic patients, 28 healthy controls, and 21 chronic obstructive pulmonary disease (COPD) patients was used to measure the lactate level by ELISA. Shown are individual measurements in each group. Statistical difference between 2 groups was calculated by the Mann-Whitney *U*-test for nonpaired samples. B: correlation between forced expiratory volume in 1 s (FEV₁) and the serum lactate level in asthmatic patients. Pearson's correlation coefficient *R* is -0.53 ($P = 0.04$).

RESULTS

Serum lactate level in asthma. Glucose is metabolized to pyruvate before its entry into mitochondria for oxidative phosphorylation. If not metabolized through oxidative phosphorylation, pyruvate is converted to lactate by lactate dehydrogenase. A measure of glycolysis in the absence of oxidative phosphorylation is a rise in lactate concentration. To test whether aerobic glycolysis is an important mechanism of glucose metabolism in asthmatic patients we measured the serum level of lactate in 28 allergic asthmatic patients, 28 healthy controls, and 21 patients with COPD. Serum samples were taken at a time when the patients were clinically stable on medications. Their oxygen saturation as measured by pulse oximetry was at or above 92%. The characteristics of asthmatic patients are shown in Table 1. The lactate level was significantly higher in asthmatic and COPD patients (Fig. 1A). The difference between asthmatic and COPD patients was also significant. Note that this increased level does not fall in the lactic acidosis range, which is usually above 4 mmol/l. The serum lactate level negatively correlated ($r = -0.53$, $P = 0.04$) with forced expiratory volume in 1 s (FEV₁) (Fig. 1B) but

not with PC₂₀ (concentration that induces 20% decline in FEV₁) for methacholine ($r = -0.42$), eosinophils ($r = -0.27$) or body mass index ($r = -0.02$) in asthmatic patients. Excessive short-acting β -agonist usage (multiple doses within an hour or so) in acute asthma (status asthmaticus) has been reported to be associated with increased serum lactate levels (6, 23, 26). We recruited clinically stable asthmatic patients. Sixty percent of our patients had mild to moderate asthma and did not take any short-acting β -agonists within 12 h before blood sampling. Some patients with severe asthma took their morning dose of a short-acting β -agonist, which was within 3–6 h of blood sampling. None reported excessive (more than one dose in 6 h) usage of a short-acting β -agonist.

Lactate production by T cells. The serum lactate level may reflect its production by blood cells and by the tissue. We examined whether T cells from asthmatic patients produced increased levels of lactate. To this goal we isolated CD4 T cells and stimulated them with anti-CD3 and anti-CD28 antibodies for 48 h. The culture supernatant was assayed for lactate. In parallel, we examined their proliferation in a thymidine uptake assay. Lactate was detectable in the culture supernatant from stimulated but not nonstimulated cells. The lactate production was sixfold higher in asthmatic patients (Fig. 2A). The CD4 T cell proliferation was 3.1-fold higher in asthmatic patients (Fig. 2B). Thus there was a disproportionate production of lactate in T cells from asthmatic patients.

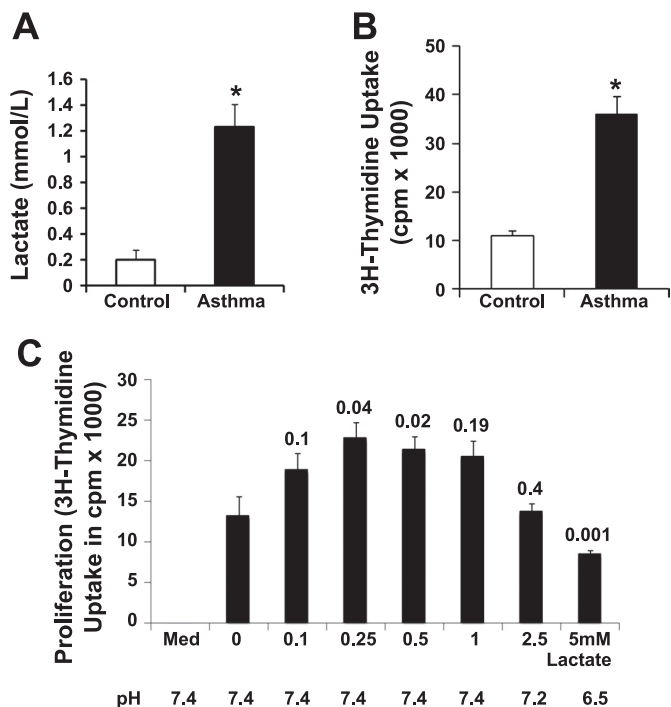


Fig. 2. Lactate production and its effect on T cell proliferation. Purified CD4⁺ T cells from allergic asthmatic patients and healthy controls ($N = 5$) were stimulated with anti-CD3 and anti-CD28 antibodies. Lactate secretion (A) and [³H]thymidine incorporation (B) were measured at 48 and 64 h, respectively. The volume of culture supernatant for lactate measurement was normalized to 1 ml per 10^6 cells at the end of the culture. * $P < 0.04$, paired *t*-test. C: CD4⁺ T cells from healthy controls ($N = 6$) were cultured with anti-CD3/CD28 antibodies in the absence or presence of sodium lactate for 64 h. [³H]thymidine incorporation in the last 16 h of culture was measured (* $P < 0.05$, paired *t*-test). The pH of the culture medium after the addition of lactate was shown below the corresponding bar graph.

Effect of lactate on T cells. Next we asked whether lactate could secondarily affect T cell proliferation. We stimulated CD4 T cells with anti-CD3/28 antibodies in the presence or absence of increasing concentrations of lactate. We observed a bimodal response. At a low concentration lactate stimulated whereas at a high concentration, which borders with the lactic acidosis range, it inhibited T cell proliferation (Fig. 2C). The pH did not change at these concentrations of lactate.

PDK1 expression in activated T cells. We examined the expression of mRNA for select key enzymes for glycolysis and lactate generation (5, 8). These enzymes include PFKFBF, FBP1, and PDK1. The basal expression level did not show any difference (Fig. 3, A–C). However, following CD3/CD28 stimulation the expression level of PDK1 modestly declined in normal subjects but significantly increased in asthmatic subjects. Conversely, the expression of FBP1 was increased in healthy subjects but not in asthmatic patients. The expression of PFKFBF was increased both groups, but there was no significant difference between the groups. We also examined protein expression at 48 h by Western blotting following stimulation of CD4 T cells with anti-CD3/CD28 antibodies. The expression of the proteins largely paralleled the expression of mRNA (Fig. 3D). PDK1 expression was higher in stimulated CD4 T cells from asthmatic patients.

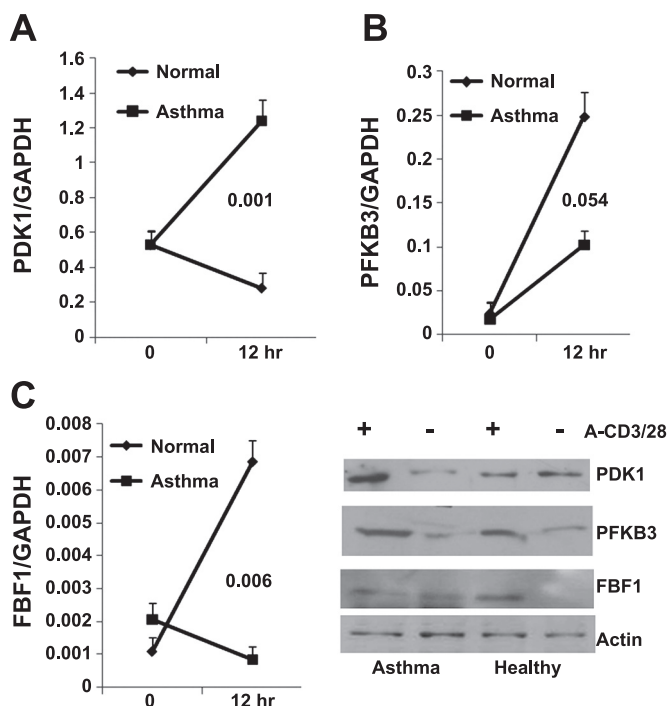


Fig. 3. Expression of mRNA (A–C) and protein (D) for pyruvate dehydrogenase kinase 1 (PDK1), 6-phosphofructo-2-kinase/fructose-2-bisphosphatase 3 (PFKFBF3), and fructose 1,6-bisphosphatase 1 (FBP1) in CD4 T cells from asthmatic subjects and healthy controls. Messenger RNA was isolated from CD4 T cells obtained from asthmatic patients and healthy controls ($N = 5$ each) at the baseline (0) and 12 h after anti-CD3/CD28 stimulation. GAPDH mRNA expression was used as a reference control and the results are presented as the ratio of the expression of mRNA for the enzyme to GAPDH. The P value for difference between the groups at 12 h is shown. The basal expression level did not show any difference. The protein expression was measured by Western blotting (D) at 24 h with (+) and without (–) stimulation with anti-CD3 and anti-CD28 antibodies (A-CD3/28). The FBP1 membrane was reprobbed for actin as a control for protein loading. One of 3 representative experiments is shown.

Effect of a PDK inhibitor on T cell proliferation, cytokine production, and Foxp3 induction. Lactate is converted to pyruvate by the pyruvate dehydrogenase complex. PDK1 inhibits this action by phosphorylating pyruvate dehydrogenase. The increase in PDK1 expression in stimulated CD4 T cells suggests that this enzyme is likely to play a role in the heightened level of serum lactate in asthmatic patients. To test this possibility we used DCA, an inhibitor of PDK1. This inhibitor promotes pyruvate generation from lactate and metabolism of pyruvate through oxidative phosphorylation (4, 20, 31). As a positive control we used 2DG, which is an inhibitor of hexokinase (HK) and blocks glycolysis at an early stage (17). We confirmed a dose-dependent inhibition of lactate production by DCA in CD4 T cells (Fig. 4A). DCA also caused a dose-dependent inhibition of T cell proliferation and production of IL-5, IFN- γ , and IL-17 (Fig. 4, B–D and F). Interestingly, DCA stimulated IL-10 production at the highest concentration (Fig. 4E). In accordance with the induction of IL-10, DCA also stimulated the induction of Foxp3 in CD4 T cells (Fig. 4F). As anticipated, 2DG potently inhibited both T cell proliferation and cytokine production including IL-10. Note that the proliferation and the production of lactate and IL-5 were higher in asthmatic patients whereas the production of IFN- γ was higher in healthy controls. The production of IL-10 and IL-17 and the induction of Foxp3 were studied in healthy controls only. We checked the viability of cells upon culture with DCA and 2DG. The viability (propidium iodide-negative cells) was 96 ± 2 , 92 ± 3 , and $89 \pm 3\%$ in control, DCA (20 mM)-containing, and 2DG-containing cultures, respectively.

Effect of PDK inhibition in a mouse model of asthma. To elucidate the role of aerobic glycolysis and lactate production in airway inflammation we studied a mouse model of acute asthma. BALB/C mice were immunized and challenged with ragweed as described previously. Like those from the human asthma, T cells from the mouse model of asthma produced higher levels of lactate (Fig. 5A). This production was inhibited by DCA in a dose-dependent manner. DCA also inhibited T cell IL-5 production (Fig. 5B) in immunized mice. Next we examined the effect of DCA on airway inflammation and airway hyperreactivity in vivo. Pretreatment of mice with DCA inhibited airway inflammation (Fig. 5C). This was associated with a reduction in IL-5, IL-13, and lactate levels recovered from the bronchoalveolar lavage fluid (Fig. 5, D–F). The pretreatment with DCA also inhibited airway hyperreactivity in response to methacholine (Fig. 5G).

DISCUSSION

We show that the serum lactate level is increased in clinically stable asthmatic patients. Activated T cells from asthmatic patients produce increased amounts of lactate and express higher levels of the lactate generating enzyme PDK1. Lactate exerts a bimodal effect on T cell proliferation. It stimulates T cell proliferation in the concentration range that is observed in the serum from asthmatic patients. On the other hand it inhibits T cell proliferation in the concentration range that is typically observed during lactic acidosis. The inhibition of lactate generation by a PDK inhibitor blocks T cell proliferation and cytokine production. T cells from a mouse model of asthma manifest similar lactate-generating activity and re-

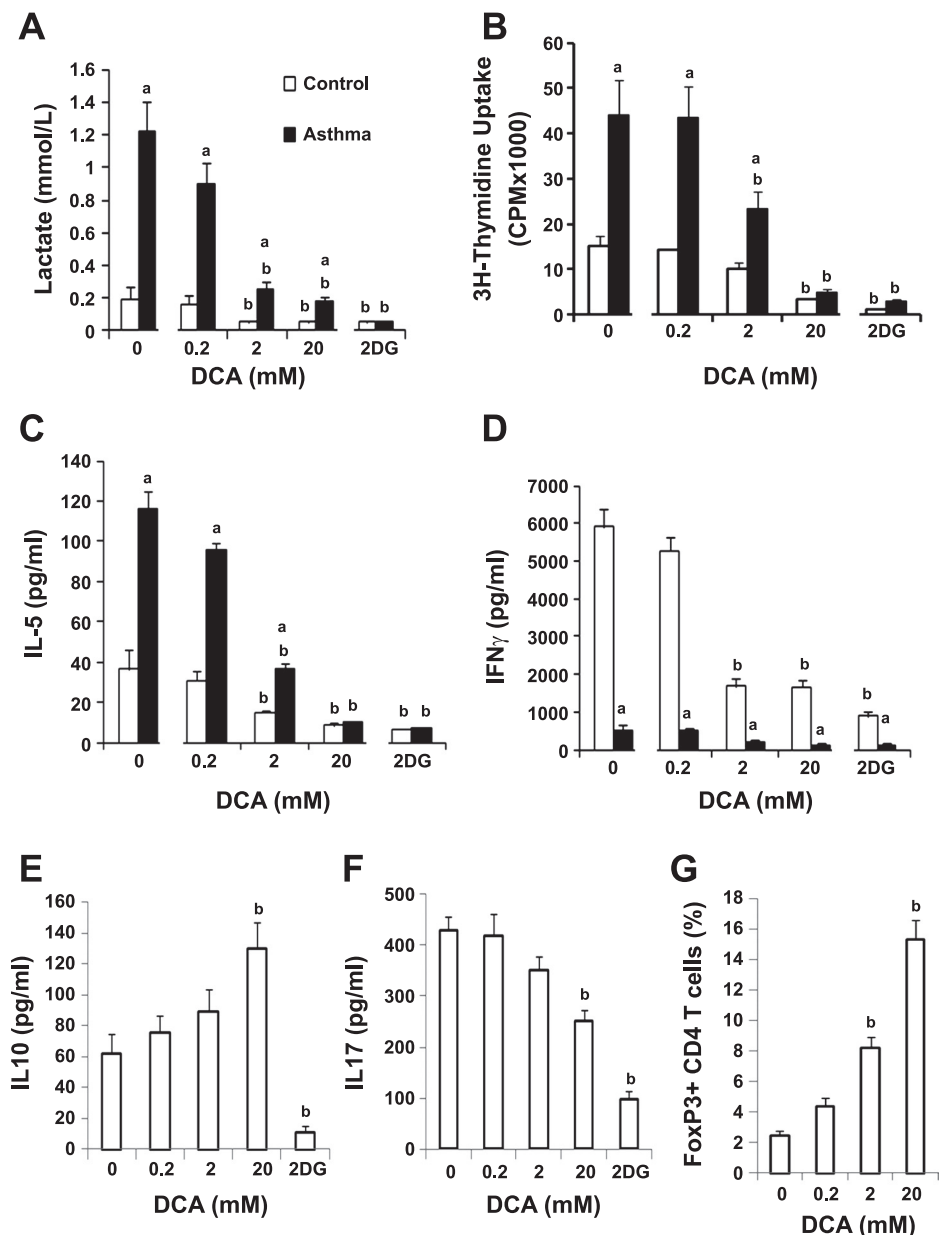


Fig. 4. Inhibition of aerobic glycolysis through PDK inhibition affects CD4 T cell proliferation, FoxP3 induction, and lactate and cytokine production. Dichloroacetic acid (DCA), a PDK inhibitor, or vehicle (RPMI 1640) were added to CD4⁺ T cells from the asthma patients and healthy controls that were stimulated with anti-CD3 and anti-CD28 antibodies. The hexokinase inhibitor 2-deoxy-D-glucose (2DG, 5 mM) was used as positive control. Cell proliferation was assessed in a [³H]thymidine uptake assay (B). Culture supernatants were collected at 48 h for lactate (A), IL-5 (C), IFN- γ (D), IL-10 (E), and IL-17 (F) assay by ELISA. The induction of Foxp3 was analyzed by flow cytometry (G). Note that IL-10 and IL-17 were measured in healthy controls only. All experiments were done in triplicates and results are means $\pm$ SE of 3–4 experiments. ^a $P < 0.05$, comparison between the study groups (asthma vs. control); ^b $P < 0.05$, comparison with the sham-treated (0 mM) sample from the same study group.

sponse to the PDK inhibitor. Finally, the inhibition of PDK blocks airway inflammation and hyperreactivity in vivo.

High serum lactate levels (>4 mmol/l) have been observed in patients with status asthmaticus and acidosis, although the incidence is low (27, 33). Excessive β -agonist administration in the setting of acute asthma without hypoxemia has also been occasionally associated with increased lactate (6, 23, 26). This normoxic lactemia is known as type B lactic acidosis. The levels of lactate in these reports are typically in the range of 3–20 mmol/l. The basal serum lactate level in our study patients is still below what is considered an abnormal lactate concentration (>2 mmol/l). Nonetheless, the median and mean levels are still higher in asthmatic than control subjects. Eighteen of the total 28 asthmatic patients had mild to moderate asthma (NAEPP guidelines). These patients did not take short-acting bronchodilators for more than 12 h before serum sampling. Although many of our patients are on combination

therapy, we are not aware of any reports linking long-acting β -agonists with lactemia. We believe it is unlikely that short-acting β -agonist contributed to the increased serum lactate level in our patients. To further address this point we cultured isolated CD4 T cells in vitro. T cells from asthmatic patients produced much higher levels of lactate under in vitro culture conditions in the absence of any β -agonists. Finally, CD4 T cells from the mouse model of asthma also produced higher levels of lactate. These mice were never exposed to β -agonists.

We recognize that mild lactemia or relative functional impairment of the pyruvate dehydrogenase (PDH) complex is not the cause but rather a feature of asthma. Congenital PDH mutation or deficiency does not predispose the affected patients to asthma. Mutations have been described in the genes (*PDHA1*, *PDHB*, *DLAT*, *DLD*, *PDX1*, and *PDP1*) encoding the E1 α , E1 β , E2, E3, E3BP, and PDP1 proteins of the PDH complex (25). There is a gradation of phenotype with decreas-

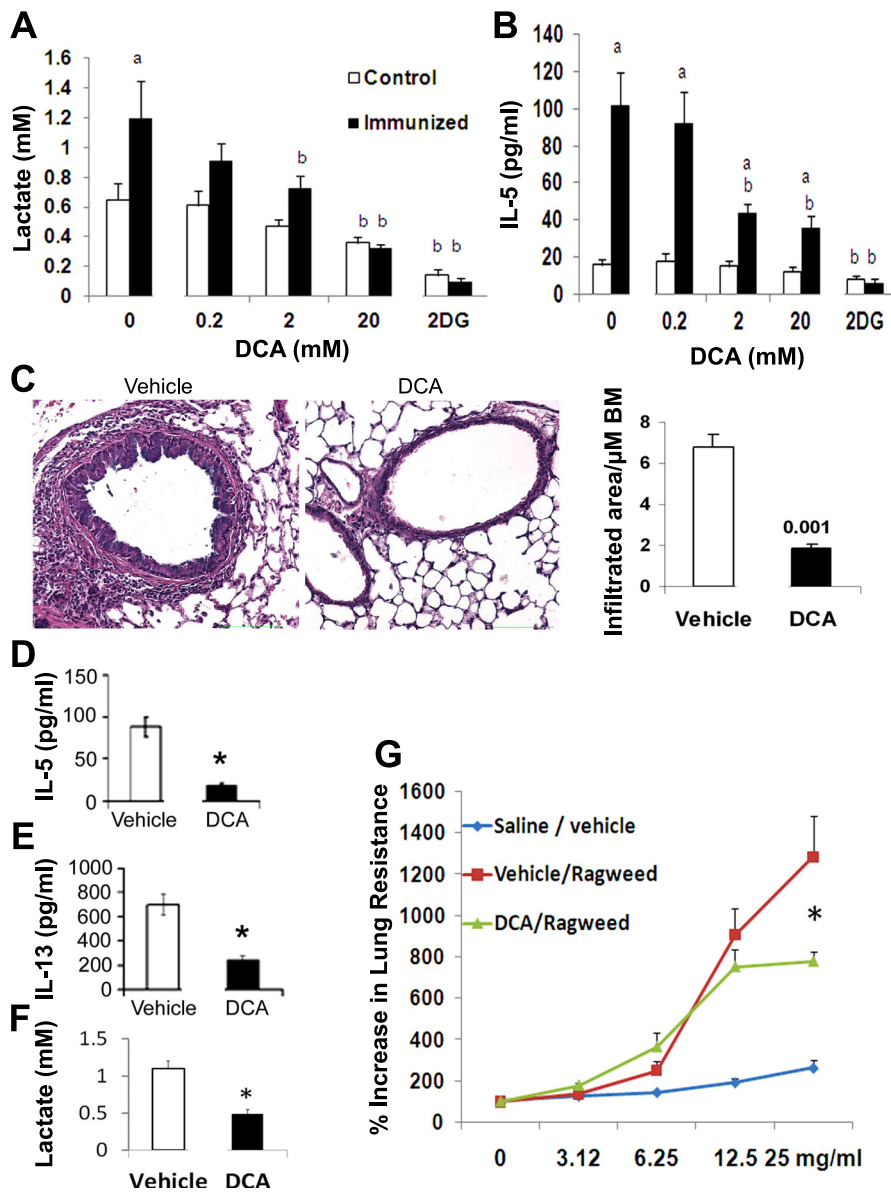


Fig. 5. Inhibition of PDK downregulates mouse T cell function, airway hyperreactivity and inflammation. **A** and **B**: CD4 T cells were isolated from the spleen of immunized and control mice and stimulated with anti-CD3 and anti-CD28 antibodies for 48 h. Culture supernatants were assayed for lactate (**A**) and IL-5 (**B**) by ELISA. $N = 5$, $^aP < 0.05$, comparison between the study groups; $^bP < 0.05$, comparison within the sham-treated (0 mM) sample with the same study group. **C**: ragweed-immunized mice were treated intraperitoneally with DCA (5 mg per mouse) or vehicle (PBS) 1 h prior to intranasal exposure to ragweed on 3 consecutive days after immunization. The mice were euthanized 72 h later. Lung tissue was examined for inflammation by hematoxylin and eosin staining ($N = 5$ per group). Representative images (left, $\times 20$ magnification, scale bars = 100 μ m) and morphometric analysis of airway inflammation (right) are shown. BM, basement membrane. Bronchoalveolar lavage (BAL) fluid from same mice was analyzed for IL-5 (**D**), IL-13 (**E**), and lactate (**F**) by ELISA ($N = 5$, $^*P < 0.05$). **G**: airway hyperreactivity in DCA- or vehicle-treated mice. Airway hyperreactivity to increasing concentrations (3.125, 6.25, 12.5, and 25 mg/ml) of methacholine inhalation was assessed by flexiVent. A statistical difference ($^*P = 0.04$, $N = 4$) was observed at the 25-mg dose of methacholine.

ing PDH complex activity. The phenotypes vary from fatal infantile lactic acidosis at the lower end of PDH activity to more ataxia cases at the higher end with Leigh syndrome and cerebral atrophy taking up the middle range.

Aerobic glycolysis in proliferating T cells has previously been studied. Cytokine (IL-2, IL-3, and IL-7)-activated T cells showed higher glycolysis rate and lactate production (1, 5). This was associated with increased HK and phosphofructokinase (PFK1/PFKBF) activity. Cytokines are known to activate Akt. The latter positively regulates HK and PFK1/PFKBF (8). In agreement we have observed increased expression of PFKBF in activated T cells from healthy as well as asthmatic patients. In contrast, PDK1 expression was higher in activated T cells from asthmatic patients, which may explain their increased lactate production. However, the basal expression of PDK1, PFKBF, and FBP1 was largely similar in both groups. We speculate that the basal production of lactate, as reflected by its increased serum level in asthma, is driven by the increased availability of the substrate but not of the studied

enzymes, although we cannot rule the increased expression of other enzymes in the lactate production pathway in asthma.

The exact reason why proliferating lymphocytes switch their energy metabolism from oxidative phosphorylation to aerobic glycolysis remains unclear. It has been shown that a balanced combination of oxidative phosphorylation and aerobic glycolysis is the most efficient and fastest way to meet the energy need of proliferating cells. This is especially true under high glucose flux rates. An alternative explanation is that aerobic glycolysis provides cells with macromolecular precursors and NADH at a level that cannot be met by oxidative phosphorylation. This is suggested by the observation that inhibiting glycolysis with 2DG in the presence of alternative Krebs cycle substrates (aspartate plus acetate) blocks PHA-induced proliferation, despite maintaining sufficient energy-generating capacity (10).

Increased lactate production by T cells promotes not only cell proliferation but also cytokine production. Inhibition of lactate production by DCA blocks IL-5, IL-17, and IFN- γ

production. However, DCA stimulates production of IL-10 and induction of Foxp3 in CD4 T cells. Basal aerobic glycolysis has previously been shown to be highest in differentiated T effector cells, especially in Th2 and Th17 cells, and lowest in regulatory T cells (21). Furthermore, inhibition of aerobic glycolysis with 2DG increases the induction of FoxP3+ T cells in vitro (28), which is in agreement with our results. Thus DCA inhibition of T cell proliferation and cytokine production could, in part, be mediated by the induced FoxP3+ T cells. This possibility needs to be investigated in a future study. Interestingly, 2DG inhibited IL-10 in our experiments. Since these inhibitors act at two different levels of the glycolytic pathway, our results suggest that the upstream and downstream enzymes of this metabolic pathway have differential effects on T cell cytokine production.

This is the first demonstration of inhibition of airway inflammation by DCA. The effect of DCA is unlikely to be specific for Th2 inflammation. Previously, DCA was shown to inhibit collagen-induced arthritis in a mouse model (3). The effect of lactate on T cells and macrophages has previously been studied. One study reported an inhibition of T cell proliferation at high concentrations of lactate (10). In contrast, other studies reported a stimulatory effect of on macrophages and T cells. Lactate promoted IL-23 secretion by macrophages and IL-17 secretion by T cells (30). Furthermore, it augmented TLR4-induced NF- κ B-dependent gene transcription through the induction of MD2 (29). Our results are in agreement with both types of effect of lactate on T cells. We observed a stimulatory effect at low concentrations and an inhibitory effect at high concentrations of lactate. The stimulatory effect occurred at a physiologically relevant concentration. Since proliferation increases aerobic glycolysis and lactate production, this could establish a positive feedback mechanism for heightened T cell proliferation in asthma.

GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise are declared by the author(s).

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