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T Cell Metabolism Is Dependent on Anatomical Location within the Lung

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ABSTRACT
The metabolic shift from oxidative phosphorylation to glycolysis is universally accepted as a necessary step for immune cells to mount effector functions. However, it is unknown if this paradigm holds true for T cells regardless of anatomical location. In this study, we compared metabolic responses among distinct mouse pulmonary CD4+ effector T cell (Teff) pools following intranasal vaccination with either *Francisella tularensis* or *Bordetella pertussis*. Surprisingly, in contrast to circulating CD4+ Teff, upon ex vivo stimulation, resident CD4+ Teff did not shift to glycolysis. This impairment in the resident pool was modestly overcome following in vivo infection. However, consistent with an ex vivo triggered shift toward glycolysis, circulating CD4+ Teff remained superior compared with resident CD4+ Teff after in vivo infection. These data indicate differences in lung T cell metabolism is associated with anatomic location, a feature which may be exploited to enhance or dampen pulmonary T cell responses. *ImmunoHorizons*, 2019, 3: 433–439.

INTRODUCTION
Pulmonary infection or vaccination can elicit parenchymal-resident T cells that are retained in the tissue after the pathogen is cleared. These resident T cells are spatially poised to respond rapidly to a subsequent infection and, in some instances, do not require assistance from circulating T cells for effective control of a secondary challenge (1–4). Our understanding of how T cells optimally aid in control of infection is linked to their relative metabolic state. Specifically, it has been shown that cells must rapidly shift their metabolism from oxidative phosphorylation to glycolysis to become optimally activated. Glycolysis not only provides key metabolic intermediates for generation of cytokines but also the rapid production of ATP. However, it is not known if this paradigm is true for all subsets of T cells in different tissues, including the lung.

In this study, we used two Gram-negative bacterial pathogens that elicit pulmonary-resident T cell responses dominated by the CD4+ T cell subset to examine the glycolytic capability of resident and circulating T cells present in the lung (1, 5). By comparing responses in these immune T cell compartments, we determined whether there were pathogen-intrinsic metabolic properties of resident T cells or if there are features of T cell metabolism in the lung that are independent of the pathogen that elicited them.

MATERIALS AND METHODS

**Bacteria**
The following bacteria were used for this study: *Francisella tularensis* subsp. *holarctica* live vaccine strain (LVS; Jean Celli, Washington State University, Pullman, WA), *F. tularensis* subsp.
tularensis strain SchuS4 (Ftt; Jeannine Peterson, Centers for Disease Control and Prevention, Fort Collins, CO), and Bordetella pertussis D420 (Tod Merkel, Center for Biologics Evaluation and Research, Food and Drug Administration, Bethesda, MD).

**Infection of mice**

Five- to six-week-old specific-pathogen free C57BL/6J (B6) female mice were purchased from The Jackson Laboratory (Bar Harbor, ME). To establish immune animals, mice were intranasally (i.n.) inoculated with 150 CFU of LVS or 5 × 10⁵ CFU of B. pertussis in 25 μl of PBS to a single nare after anesthesia with ketamine/xylazine. Where indicated, LVS-immune mice were i.n. challenged with 25 CFU of Ftt 28 d after vaccination and treated i.p. with 1 mg/kg FTY720 (Sigma-Aldrich) or vehicle on day −3 and every other day to restrict recruitment of circulating cells into the pulmonary tissue. Bacterial inocula were confirmed by serial dilution and plating on Modified Mueller Hinton (LVS and Ftt) or Bordet–Gengou blood agar (B. pertussis). Animal studies were approved by and conducted in accordance with Rocky Mountain Laboratories’ Animal Care and Use Committee.

**i.v. labeling of circulating cells and tissue harvest**

Mice were i.v. injected with 2.5 μg of anti-CD45.2 FITC or PE (BioLegend) in 100 μl of PBS. Three minutes later, mice were euthanized, and spleens and lungs were aseptically isolated and processed into single-cell suspensions as previously described (6). The total number of viable cells was determined by trypan blue exclusion using a TC20 Automated Cell Counter (Bio-Rad).

**Flow cytometry**

Cells were stained with the following directly conjugated Abs: CD3 BV510 or BV395 (clone 145-2C11), CD4 AF700 (clone GK1.5), CD8a BV605 (clone 53-6.7), CD44 PE-Cy7 (clone IM7), and CD62L Pacific Blue (clone MEL-14). All Abs were purchased from Life Technologies, BD Biosciences, or BioLegend. Data acquisition was performed on an LSRII or Symphony flow cytometer (BD Biosciences), and data analysis was completed using FlowJo 10 (Tree Star). The gating strategy for data analysis is shown in Supplemental Fig. 1.

**T cell purification**

Lung resident (IV⁻) and circulating cells (IV⁺) in immune mice were discriminated from each other using i.v. staining as described above. Lungs were processed into a single-cell suspension, and IV⁻ or IV⁺ CD4⁺ effector T cells (Teff) were sorted using a BD Aria sorter (BD Biosciences) by gating on CD4⁺ CD44⁺ events within the IV⁻ or IV⁺ gate. Total naive CD4⁺ T cells were purified from the lung by first depleting CD11c⁺ cells using CD11c microbeads and then positively selecting CD4⁺ T cells with CD4 microbeads according to the manufacturer’s instructions (Miltenyi Biotech). The viability of purified cells was >98% at the time of use; each enrichment process resulted in >80% purity of each cell population as determined by flow cytometry.

**2-NBDG uptake**

Total lung cells in RPMI 1640 supplemented with 0.2 mM l-glutamine, 1 mM HEPES buffer, and 0.1 mM nonessential amino acids (all from Life Technologies) and 10% heat-inactivated FCS (Atlas Biologicals) were seeded in a round-bottom 96-well plate at 1.5 × 10⁶ cells per well. Cells were stimulated with anti-CD3/CD28 beads (4:1), paraformaldehyde-killed LVS (5:1), or vehicle (mock) overnight at 37°C. During the last 2 h of culture, 20 μM 2-NBDG (Sigma) was added. Samples from Ftt-infected mice were fixed with 2% paraformaldehyde for 30 min. 2-NBDG uptake by CD4⁺ T cells was determined by flow cytometry.

**Statistical analysis**

Statistically significant differences between two groups were determined using an unpaired two-tailed t test. For three or more comparisons, a one-way ANOVA with Holm–Sidak posttest was used. Statistical significance was set at p < 0.05.

**RESULTS**

**Pulmonary IV⁻ CD4⁺ T cells do not shift to glycolysis after activation**

i.n. vaccination elicits pulmonary T cells that reside in anatomically distinct compartments and are discriminated by i.v. staining (1, 5, 7). Nearly all IV⁻ CD4⁺ T cells have an effector (Teff) phenotype (CD44hi), therefore, we purified Teff from the IV⁻ pool to compare the glycolytic shift in these distinct T cell pools within the lung. Less than 10% of CD4⁺ T cells in IV⁻ in nonimmune animals (5), which precludes obtaining adequate numbers of these cells for comparison with Teff isolated from immune animals. Therefore, we used total CD4⁺ T cells from the lungs of nonimmune animals as internal controls to confirm that the assay was performing as expected. Importantly, nonimmune pulmonary CD4⁺ T cells were...
>80% pure after enrichment and contaminating cells are metabolically active; therefore, they contribute to measured metabolic parameters. Regardless of vaccinating bacterium, IV− and IV+ CD4+ T eff had similar levels of basal glycolysis (Fig. 1A and data not shown). Independent of the vaccinating agent, IV− CD4+ T eff failed to increase their ECAR in response to either bead activation or oligomycin (Fig. 1B, IC). Similarly, both LVS- and B. pertussis–immune IV− CD4+ T eff had significantly greater glycolytic capacity compared with IV− CD4+ T eff in response to anti-CD3/CD28 beads and oligomycin (Fig. 1D, 1E). To confirm that the inability of IV− CD4+ T eff from the lung to undergo a glycolytic response was not attributable to the assay itself, we measured ECAR among immune splenic CD4+ T cells following activation with anti-CD3/CD28 beads. As expected, splenic immune CD4+ T cells readily shifted to glycolysis, as measured by an increase in ECAR following activation (Supplemental Fig. 2). Together, these data suggest that there are distinguishable metabolic signatures among CD4+ T eff in the lung that are different from peripheral tissues and are dependent on their location in the pulmonary compartment.

IV− CD4+ T eff do not increase oxidative phosphorylation to compensate for decreased glycolysis after activation

The energetic demands of a cell are met through either oxidative phosphorylation or glycolysis. The inability of IV− CD4+ T eff to shift toward glycolysis upon activation suggested that they, instead, may increase their rates of oxidative phosphorylation to meet their energetic needs following activation. However, regardless of the vaccinating bacterium, IV− and IV− CD4+ T eff had similar OCR levels after bead injection (Fig. 2A–C and data not shown). All cells were responsive to mitochondrial uncoupler FCCP and mitochondrial electron transport chain inhibitors rotenone and antimycin, as indicated by an increase or decrease, respectively, in OCR following exposure to these compounds. However, similar to their superior glycolytic capacity, the maximal respiration of IV− CD4+ T eff was significantly higher than that of IV− CD4+ T eff in LVS-vaccinated mice, whereas cells from B. pertussis–immune animals exhibited a similar trend (Fig. 2D, 2E). Together, these results indicate that IV− CD4+ T eff do not increase oxidative phosphorylation to compensate for the absence of a glycolytic shift after activation.

Differences in glycolytic usage are maintained with Ag stimulation

Although anti-CD3/CD28 beads are a useful tool to study polyclonal T cell responses, it is possible that IV− CD4+ T eff require the integration of Ag stimulation through the TCR and the infection-associated cytokine milieu to use glycolysis. To test this possibility, we established an in vitro culture system in which lung single-cell suspensions were stimulated with anti-CD3/CD28 beads or killed bacteria (Ag) and measured changes in glycolysis using uptake of fluorescent glucose (2-NBDG). Consistent with changes in ECAR, IV− CD4+ T eff from LVS- and B. pertussis–vaccinated mice had significantly higher uptake of 2-NBDG following addition of anti-CD3/CD28 beads or killed bacteria compared with IV− CD4+ T eff (Fig. 3). These data suggest that differences in IV− and IV− CD4+ T eff glycolytic capacity are maintained even when cells are stimulated with Ag in the presence of the complex cellular mixture of the lung.

Ftt infection changes the metabolic state of IV+ and IV− CD4+ T eff

Pulmonary infection induces complex changes within the lung. Consequently, the metabolic response of T cells combating a secondary infection in situ might differ from those in resting animals examined ex vivo. Therefore, we next assessed whether there were differences in basal glycolysis among IV− and IV− CD4+ T eff during in vivo infection. Mice were treated with FTY720 to

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FIGURE 2. IV− CD4+ Teff do not use oxidative phosphorylation to compensate for the lack of a glycolytic shift.

Mice were nonimmune or i.n. vaccinated with 150 CFU of LVS (A, B, and D) or 5 × 10^5 CFU of B. pertussis (Bp) D420 (C and E). Twenty-eight (LVS) or forty-two (Bp) D420 days later, IV staining was performed, and IV+ and IV− CD4+ T eff (CD44hi) were sorted from vaccinated mice. Total CD4+ T cells were purified from naive lung. Cells were pooled from 8–10 mice per group with three to six technical replicates per group. Cells were seeded into a 96-well Seahorse plate, and (A) the OCR was measured after activation in real time with anti-CD3/CD28 beads and subsequent injection of oligomycin, FCCP, and rotenone/antimycin. (B and C) The respiration after anti-CD3/CD28 activation and (D and E) maximal respiration were calculated. (A) is representative of three independent experiments, and (B)–(E) are pooled from three independent experiments for each vaccination. Error bars represent SD. *p ≤ 0.05.

DISCUSSION

The metabolic shift from oxidative phosphorylation to glycolysis is a hallmark of innate and adaptive immune cell activation. Engagement of TLRs in macrophages and dendritic cells induces glycolysis, and this metabolic shift is required for optimal phagocytosis, cytokine production, and Ag presentation (8–10). Similarly, the ability to use glycolysis is required for CD4+ and CD8+ T cells to become optimal effectors (i.e., produce IFN-γ) (11–14). To date, our understanding of T cell metabolism has been gleaned from the studies using splenic and lymph node–derived T cells. However, the environment surrounding a T cell in a lymphoid organ is very different from that of one residing within nonlymphoid tissues. These differences may contribute to their metabolic profile and/or potential. Given the important function of pulmonary-resident T cells in combating infection and their unique anatomical niche, we sought to understand their metabolic state after vaccination and during secondary challenge.

Using two different assays to assess glycolysis in IV− CD4+ T eff purified from resting LVS- and B. pertussis–immune mice, we made the surprising finding that their ability to shift toward glycolysis following activation was significantly reduced compared with IV+ CD4+ T eff. Furthermore, IV− CD4+ T eff did not compensate for this absence in shifting to glycolysis by increasing oxidative phosphorylation upon activation. Together, these data indicated IV− CD4+ T eff from resting animals are not prone to alter their metabolic profile upon activation ex vivo. Immune IV− CD4+ T cells undergo proliferation and produce IFN-γ and TNF-α after Ftt challenge (5). However, we previously reported that IV+ CD4+ T eff control intracellular Ftt replication significantly better than the equivalent number of IV− CD4+ T eff (5). Data presented in this study suggest that this disparity in effector capabilities is consistent with the failure of IV− CD4+ T eff to readily shift to glycolysis to the same extent as IV+ CD4+ T eff ex vivo. There are a couple of possible explanations for the failure of IV− CD4+ T eff to shift to glycolysis upon ex vivo activation. First, IV− T cells express higher levels of...
the inhibitory receptor PD-1 than their IV+ counterparts (4, 5, 7), and signaling through PD-1 renders CD4+ T cells poorly glycolytic (15). Alternatively, IV-2 T cells may require the integration of multiple signals coming from the inflammatory milieu to shift to glycolysis that were not recapitulated ex vivo. We tested whether PD-1 blockade could improve IV-2 CD4+ T effector function or whether treatment with a mixture of cytokines previously associated with promoting pulmonary T cell activity could improve their glycolytic shift. In all cases, the improvement observed was modest at best (data not shown). These data support the hypothesis that multiple signals specific to the pulmonary compartment converge to drive the appropriate metabolic shift in IV-2 CD4+ T effector. Because of the unique environment of the lung, which requires tight regulation of inflammatory responses to properly respond to infection without causing significant damage, it would not be unexpected that tissue-resident T cells would be resistant to immediate activation. Rather, optimal activation would require a sophisticated sequence and combination of events to appropriately trigger their effector functions, which may also be dependent on the infecting pathogen. For example, given the differences in the rates of clearance of Ftt and B. pertussis, the flavor and complexity of in vivo activation of T cells is likely to profoundly impact the function of IV- T cells. For example, B. pertussis elicits a greater inflammatory response soon postinfection, whereas Ftt fails to trigger detectable inflammation for up to 3 d after inoculation (16, 17). Therefore, although ex vivo stimulation results in minor changes in the metabolic profile of IV- CD4+ T eff independent of the vaccinating bacterium, our data suggest that the nature of secondary infection may contribute to the overall efficacy of the resident T cell response. This in turn may impact the relative need for circulating T cells in control and clearance of infection.

In addition to establishing that there are likely a complex series of signals unique to in vivo infection that promote a shift in glycolysis among IV- CD4+ T effector, we also observed that these cells began to lose this capability at later time points postinfection compared with IV+ CD4+ T effector. It is possible that this change in rates of glycolysis among IV- CD4+ T effector over time is tuned for appropriate generation of effector cytokines and metabolic intermediates by IV- T cells without causing overt immunopathologic conditions as the host battles the infection. This is particularly important in settings in which pathogens like Ftt are not cleared within the first week of infection. A persistent, robust T cell response could result in a significant pathological condition that would be detrimental to the host and, therefore, must be appropriately tempered. Alternatively, the drop in glycolytic capacity of IV- T effector may reflect exhaustion of these cells over time and highlights the requirement for a pool of effective circulating cells to enter the tissue in support of the pre-exiting resident pool.

Together, our data underscore the importance of understanding the metabolic capacity of T cells that reside in unique microenvironments such as the lung. By defining these characteristics, we may gain the ability to appropriately tune

FIGURE 3. T cell activation with Ag drives glucose uptake in IV+ but not IV- CD4+ T effector.
Mice were nonimmune (NI) or i.n. vaccinated with 150 CFU of LVS or 5 × 10^5 CFU of B. pertussis (Bp) D420. Twenty-eight (LVS) or forty-two (B. pertussis) days later, IV staining was performed, lungs were digested into a single-cell suspension, and cells were seeded into a 96-well round-bottom plate. Lung cells were stimulated overnight with anti-CD3/CD28 beads or Ag or mock-treated. During the last 2 h of stimulation, 2-NBDG was added, and uptake was measured by flow cytometry for the indicated cell population in (A and C) LVS- or (B and D) B. pertussis–vaccinated mice. Data are representative of two independent experiments for each vaccination with n = 4–5 mice per group. Error bars represent SD. *p ≤ 0.05.
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REFERENCES

14. Menk, A. V., N. E. Scharping, R. S. Moreci, X. Zeng, C. Guy, S. Salvatore, H. Bae, J. Xie, H. A. Young, S. G. Wendell, and...

