

Critical role of fatty acid metabolism in ILC2-mediated barrier protection during malnutrition and helminth infection

Christoph Wilhelm,^{1,4*} Oliver J. Harrison,^{1*} Vanessa Schmitt,⁴ Martin Pelletier,³ Sean P. Spencer,^{1,5} Joseph F. Urban Jr.,⁶ Michelle Ploch,³ Thirumalai R. Ramalingam,² Richard M. Siegel,³ and Yasmine Belkaid¹

¹Mucosal Immunology Section and ²Immunopathogenesis Section, Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892

³Immunoregulation Section, Autoimmunity Branch, National Institute of Arthritis and Musculoskeletal and Skin Diseases, National Institutes of Health, Bethesda, MD 20892

⁴Institute of Clinical Chemistry and Clinical Pharmacology, University Hospital Bonn, University of Bonn, 53127 Bonn, Germany

⁵Department of Pathology and Laboratory Medicine, Institute for Immunology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA 19104

⁶Diet, Genomics, and Immunology Laboratory, Beltsville Human Nutrition Research Center, Agricultural Research Service, U.S. Department of Agriculture, Beltsville, MD 20705

Innate lymphoid cells (ILC) play an important role in many immune processes, including control of infections, inflammation, and tissue repair. To date, little is known about the metabolism of ILC and whether these cells can metabolically adapt in response to environmental signals. Here we show that type 2 innate lymphoid cells (ILC2), important mediators of barrier immunity, predominantly depend on fatty acid (FA) metabolism during helminth infection. Further, in situations where an essential nutrient, such as vitamin A, is limited, ILC2 sustain their function and selectively maintain interleukin 13 (IL-13) production via increased acquisition and utilization of FA. Together, these results reveal that ILC2 preferentially use FAs to maintain their function in the context of helminth infection or malnutrition and propose that enhanced FA usage and FA-dependent IL-13 production by ILC2 could represent a host adaptation to maintain barrier immunity under dietary restriction.

Innate lymphoid cells (ILC) have recently emerged as an important component of tissue immunity with several subsets described both in mice and humans (Spits and Di Santo, 2011; Spits et al., 2013). Type 1 ILC (ILC1) produce the cytokine IFN- γ and are implicated in protection against intracellular pathogens (Fuchs et al., 2013; Klose et al., 2014). ILC3 limit gut microbial translocation and promote antibacterial responses (Spits et al., 2013), whereas ILC2 can produce IL-5, IL-9, IL-13, and amphiregulin (Spits et al., 2013), a cytokine profile that underlies the diverse functions of these cells, ranging from control of host metabolism to immunity to helminths, tissue repair, and mucus production (Eberl et al., 2015).

Recently, a rapidly growing body of literature has described the metabolic requirements of classical T cells at various stages of activation. This collective work revealed that

naive T cells generate energy through mitochondrial oxidative phosphorylation (OXPHOS; MacIver et al., 2013; Pearce and Pearce, 2013). Long-lived memory T cells predominately use mitochondrial fatty acid oxidation (FAO) for persistence and function (van der Windt et al., 2012, 2013; O'Sullivan et al., 2014; Cui et al., 2015). Upon antigen encounter and activation, the metabolic requirements of naive T cells rapidly adapt to match a high energy demand required for proliferation, growth, and cytokine production. This metabolic change is achieved by a switch to aerobic glycolysis (Pearce et al., 2013), a metabolic process that generates fewer ATP molecules than OXPHOS but is performed at a much faster rate (Pearce and Pearce, 2013). Although this line of investigation in T cell biology represents a growing field of research, little is known about the metabolic requirements of ILC at steady state or during infections and, more particularly, how these cells metabolically adapt to environmental stressors. Recent findings highlight arginase-1 as a cell-intrinsic regulator of ILC2 proliferative capacity and effector function during acute lung inflammation (Monticelli et al., 2016). Although ILC-intrinsic arginase-1 bolsters arginine catabolism and aerobic glycolysis, another study also identified genes involved in fatty acid (FA)

*C. Wilhelm and O.J. Harrison contributed equally to this paper.

Correspondence to Yasmine Belkaid: ybelkaid@niaid.nih.gov; or Christoph Wilhelm: christoph.wilhelm@uni-bonn.de

M. Pelletier's present address is Dept. of Microbiology and Immunology, Centre de Recherche du CHU de Quebec, Université Laval, Quebec City G1V 4G2, Canada.

S.P. Spencer's present address is Dept. of Medicine, Massachusetts General Hospital, Boston, MA 02114.

Abbreviations used: 2-DG, 2-deoxy-D-glucose; FA, fatty acid; FAO, fatty acid oxidation; FCCP, trifluoromethoxy phenylhydrazine; ILC, innate lymphoid cell; OCR, oxygen consumption rate; OXPHOS, oxidative phosphorylation; RA, retinoic acid; RAI, retinoic acid inhibitor; SRC, spare respiratory capacity; VAI, vitamin A insufficient.

This article is distributed under the terms of an Attribution-Noncommercial-Share Alike-No Mirror Sites license for the first six months after the publication date (see <http://www.rupress.org/terms>). After six months it is available under a Creative Commons License (Attribution-Noncommercial-Share Alike 3.0 Unported license, as described at <http://creativecommons.org/licenses/by-nc-sa/3.0/>).

metabolism as a defining feature of ILC2 (Robinette et al., 2015). How FAO contributes to ILC2 function and cellular metabolism remains unknown.

Vitamin A deficiency is one of the most common and severe nutrient deficiencies, affecting an estimated 250 million preschool age children worldwide (WHO, 2009). This essential nutrient deficiency is associated with profound impairment in adaptive immunity as well as a selective defect in ILC3 (Hall et al., 2011; Veldhoen and Brucklacher-Waldert, 2012; Spencer et al., 2014). Paradoxically, vitamin A deficiency is characterized by an increase in ILC2 and enhanced production of the cytokine IL-13 by these cells (Spencer et al., 2014). This innate adaptation to malnutrition allows the host to sustain barrier immunity and to maintain immune responses to helminth infection in the face of malnutrition (Spencer et al., 2014). However, whether the expansion of ILC2 and subsequent changes to their function observed during vitamin A deficiency are also associated with a metabolic adaptation remains unknown.

Here we demonstrate that ILC constitutively take up high amounts of FA. Although abrogation of FAO under homeostatic conditions does not impinge on ILC2 homeostasis, this axis is key to ILC2-mediated intestinal barrier protection during helminth infection. Furthermore, in the absence of vitamin A, the accumulation of ILC2 and associated IL-13 production is fueled by enhanced acquisition of long-chain FA and their efficient metabolism through FAO. Together, our findings uncover the basic metabolic requirements of ILC2 during helminth infection and reveal dynamic metabolic adaptation of ILC2 to maintain barrier immunity under nutritional stress.

RESULTS AND DISCUSSION

ILC acquire long-chain FAs from the environment

To investigate the metabolic requirement of ILC under steady-state conditions, we injected mice with the fluorescently-labeled long-chain FA palmitate (Bodipy FL C₁₆). Under homeostatic conditions, ILC2 and ILC3 efficiently acquired FA at all barrier sites analyzed, as well as in the mesenteric adipose tissue (Fig. 1, A–C), with the highest uptake observed in the mesenteric adipose tissue (Fig. 1 C). Uptake of Bodipy FL C₁₆ was higher in ILC compared with regulatory T cells (T reg cells), which, like ILC, comprise a substantial cell population present at barrier sites and were previously shown to acquire free FA from the environment and use FA as cellular fuel (Michalek et al., 2011; Berod et al., 2014; Fig. 1, A and B). Within the ILC compartment, ILC2 showed the highest level of FA uptake, followed by ILC3 and ILC1 (Fig. 1, D and E). Naive T cells showed substantial uptake of glucose, as assessed by uptake of fluorescent glucose analogue 2-NBDG (Fig. 1, F and G; O'Sullivan et al., 2014). However, glucose uptake was less pronounced in ILC when compared with naive T cells and no major differences were observed in this property between intestinal T reg cells, ILC2, and ILC3 (Fig. 1, F and G). To assess the requirement for FAO in ILC2

homeostasis, we treated mice for 7 d with etomoxir, an inhibitor of CPT1a, an enzyme that catalyzes the addition of carnitine to long-chain FAs essential for further transport into the mitochondria. Assessment of ILC2 numbers in various compartments showed no reduction in ILC2 in comparison to vehicle-treated control mice (Fig. 1 H). These results suggest that although ILC2 take up high amounts of FAs, their basal number can be maintained in the context of FAO blockade.

FAs but not glucose are essential for the expansion of ILC2 during helminth infection

Although maintenance of ILC2 at steady state may not be uniquely dependent on FAO, their metabolic requirements may change upon activation. Previous work highlighted the importance of FAO in the regulation of M2 macrophages and showed that blocking lipolysis was associated with impaired helminth expulsion (Vats et al., 2006; Huang et al., 2014). Although these studies focused on the regulation of M2 macrophages by FAO, these findings support the idea that this pathway may be generally important for the promotion of type 2 immunity, including ILC2 responses. To test this hypothesis, we assessed ILC2 behavior during helminth infection while blocking glycolysis (using 2-deoxy-D-glucose [2-DG]) or FAO (using etomoxir). Mice treated with 2-DG expelled *Trichuris muris* in a manner comparable to animals treated with vehicle alone (Fig. 2 A). In agreement with this observation, 2-DG treatment had no impact on ILC2 numbers or ILC2-derived cytokine production (Fig. 2, B and C). Thus, inhibition of glucose utilization during helminth infection had no impact on the accumulation of ILC2. In contrast, in vivo blockade of FAO during *T. muris* infection led to a significant reduction in ILC2 accumulation and prevented infection-driven increases in IL-5 and IL-13 (Fig. 2, D–F). Despite impaired ILC2 responses, no increase in worm burden was observed at this time point (Fig. 2 G), likely a direct effect of etomoxir on helminth survival (Fig. S1; Taylor et al., 2013). To circumvent this experimental limitation we adopted a complementary approach to inhibit FA utilization during infection. WT mice were treated with orlistat, a lipase inhibitor that limits intestinal absorption of dietary FAs (Heck et al., 2000). Such treatment significantly compromised anti-helminth immunity as indicated by increased worm burden compared with vehicle-treated control mice (Fig. 2 H). Orlistat treatment was also associated with a significant reduction in the accumulation of ILC2 and ILC2-derived cytokines (IL-5 and IL-13; Fig. 2 H). As orlistat-mediated blockade of FA uptake could also influence several facets of adaptive immunity during helminth infection, we sought to further link the requirement for FA uptake to ILC2 effector function by using *Rag1*^{−/−} mice. Mirroring our observations in WT mice, treatment of *Rag1*^{−/−} mice with orlistat resulted in elevated helminth burden (Fig. 2 I) with reduced ILC2 accumulation and production of IL-5 and IL-13 compared with vehicle-treated controls (Fig. 2 I). These results support the idea that, in the context of a helminth infection, ILC2 accumulation and protective

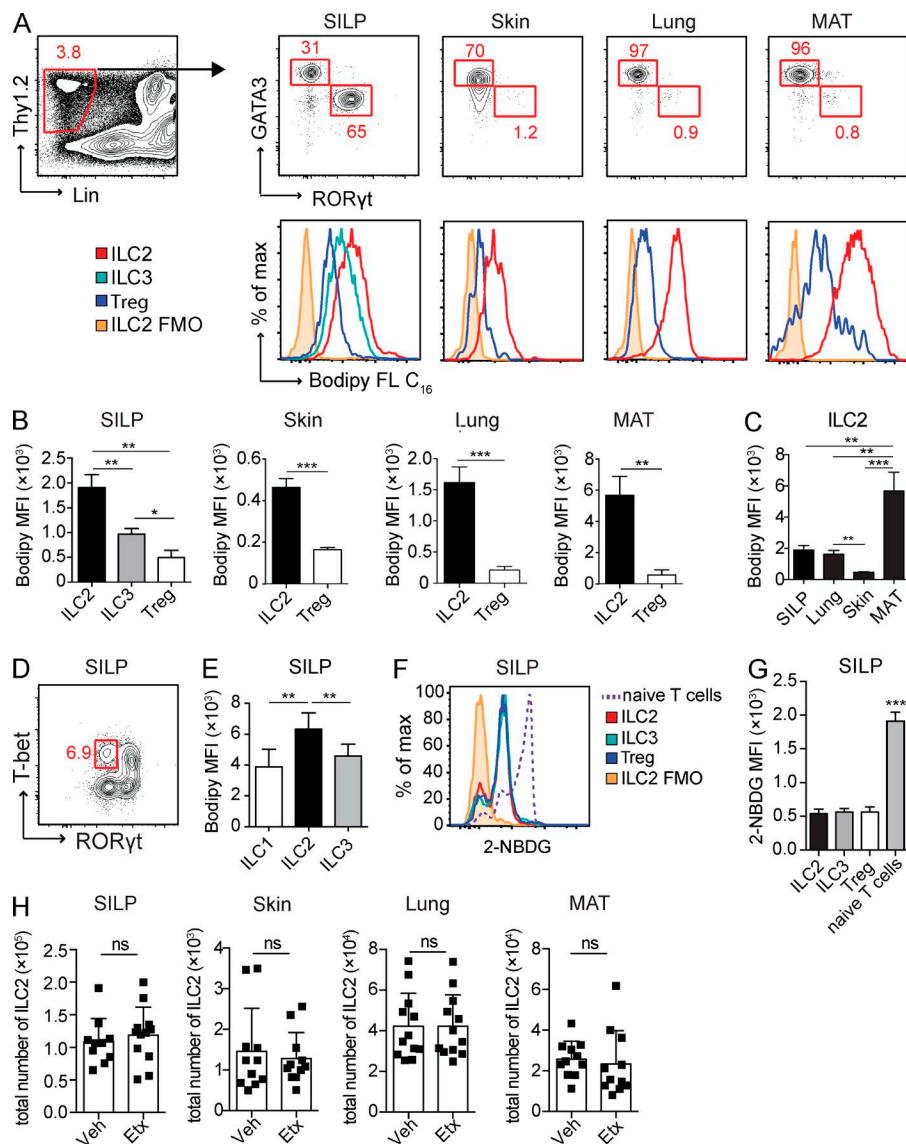


Figure 1. ILC acquire high amounts of extracellular FA. (A) Flow cytometric analysis of cells isolated from small intestinal lamina propria (SILP), skin, lung, and mesenteric adipose tissue (MAT) of naive C57BL/6 mice. Panels represent live CD45⁺ cells stained with Thy1.2 and lineage (Lin) markers (left). ILC gated on Lin[−] and Thy1.2 expression (ILC) were stained for RORγt (ILC3) and GATA3 (ILC2; top), and histograms of Bodipy FL C₁₆ median fluorescence intensity (MFI) in ILC2, ILC3, and Foxp3⁺ T reg cells (bottom) are shown. For fluorescence minus one (FMO), mice were not injected with fluorescent analogues. (B) Bodipy FL C₁₆ MFI of ILC2, ILC3, and Foxp3⁺ T reg cells from indicated tissues. (C) Bodipy FL C₁₆ MFI of ILC2 from indicated tissues. (D) Gating strategy to identify ILC1. Panel represents live CD45⁺ Thy1.2⁺ lineage cells stained for RORγt and T-bet expression. (E) Bodipy FL C₁₆ MFI of ILC1, ILC2, and ILC3. Representative histogram (F) and MFI values (G) of 2-NBDG uptake in small intestinal lamina propria ILC2, ILC3, T reg cells, and naive T cells (isolated from the mesenteric lymph node). (H) Total number of ILC2 in the SILP, skin, lung, and MAT of C57BL/6 mice treated with vehicle control (Veh) or etomoxir (Etx). Results are representative of at least two independent experiments with three to four mice in each experimental group (A–C and F–G) or pooled results from two experiments with two to four mice in each experimental group (D and E) or three experiments with three to four mice in each experimental group (H). ns, not significant; *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$, unpaired Student's *t* test (mean \pm SEM).

function is independent of glycolysis but directly dependent on FA availability and FA oxidation.

Increased acquisition of FAs by ILC2 in the absence of vitamin A

A major consequence of dietary restriction and malnutrition is alteration in the nutrient source available to fuel cellular immunity. Under physiological settings, glucose, FAs, and amino acids can all be readily used (Pearce and Pearce, 2013), whereas in times of dietary restriction, glucose becomes sparse and the body starts to use and mobilize FA (Viscarra and Ortiz, 2013). We previously demonstrated that in the absence of vitamin A, ILC2 were significantly enhanced both numerically and functionally (Spencer et al., 2014). Therefore, we postulated that during vitamin A deficiency, ILC2 might also increase their acquisition and use of FA. To test this possibility, we treated mice with a pan retinoic acid (RA) receptor signaling inhibitor (RAi) previously

shown to recapitulate the effect of vitamin A deficiency (Spencer et al., 2014). Inhibition of RA signaling had no impact on the capacity of either ILC or T reg cells to uptake the glucose analogue 2-NBDG (Fig. 3, A and B). In contrast, treatment of mice with RAi significantly increased the acquisition of FA in ILC2, ILC3, and T reg cells compared with mice receiving vehicle control (Fig. 3, C and D). Notably, under these settings, the most dramatic increase in FA uptake was observed in ILC2 (Fig. 3, C and D). We next confirmed these findings under conditions in which vitamin A deficiency is gradually established during postnatal development by placing mice on a diet lacking vitamin A. In agreement with results obtained in mice treated with RAi, ILC from vitamin A insufficient (VAI) mice, and more specifically ILC2, increased their uptake of FA compared with ILC isolated from mice fed a control diet (Fig. 3, E and F). Hence, vitamin A deficiency and impaired RA signaling is associated with a significant increase in FA uptake by ILC2.

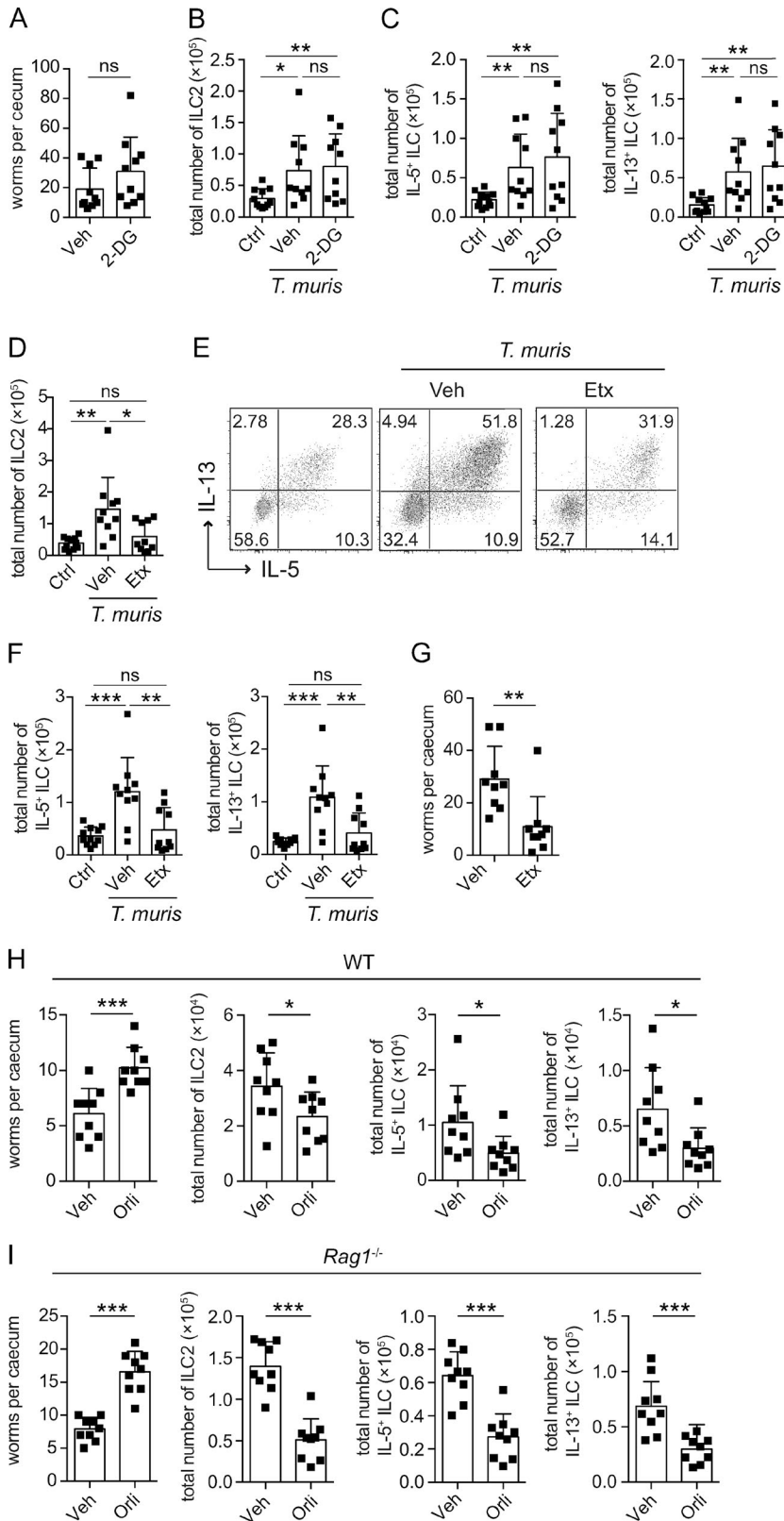


Figure 2. FAO and FA are important for ILC2 response to helminth infection. WT mice were infected with *T. muris* and treated with vehicle (Veh) or 2-DG. (A) Cecal worm burden of vehicle (Veh) or 2-DG-treated WT mice, 18 d after infection. Total number of ILC2 (B) or of IL-5 and IL-13 producing ILC (C) from uninfected mice (Ctrl), vehicle (Veh), or 2-DG-treated mice 18 d after infection. WT mice were infected with *T. muris* and treated with vehicle (Veh) or Etomoxir (Etx). (D) Total number of ILC2 (E) and intracellular cytokine expression from ILC2, and total number of IL-5 and IL-13 producing ILC from uninfected (Ctrl), vehicle (Veh), or Etomoxir (Etx)-treated WT mice (F) 18 d after infection. (G) Cecal worm burden of vehicle (Veh) or Etomoxir (Etx)-treated WT mice 18 d after infection. (H) Total number of ILC2 and of IL-5 and IL-13 producing ILC from WT mice treated with vehicle (Veh) or Orlistat (Orli) 15 d after infection. (I) *Rag1*^{-/-} mice treated with vehicle (Veh) or Orlistat (Orli) 15 d after infection. Data represents pooled results from two experiments with four to five mice in each experimental group. *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$, unpaired Student's *t* test (mean \pm SEM).

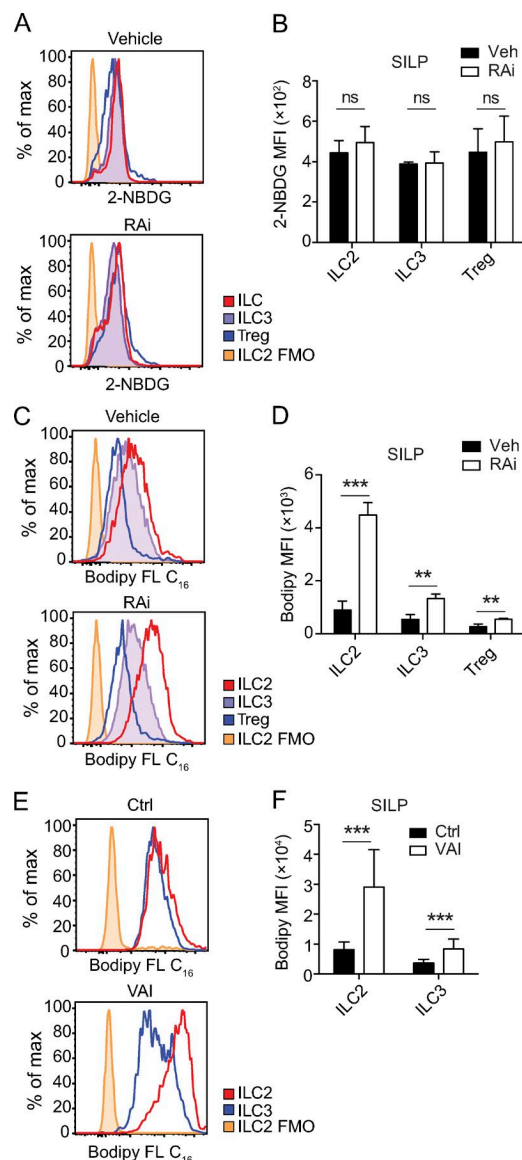


Figure 3. ILC2 increase the acquisition of extracellular FA in the absence of vitamin A or RA signaling. Flow cytometric plots of small intestinal lamina propria (SILP) cells of naive C57BL/6 mice treated with vehicle (Veh) or RAI and with 2-NBDG or Bodipy FL C₁₆. Representative histograms (A) and median fluorescence intensity (MFI) values (B) of 2-NBDG uptake in ILC2 (Lin⁺, Thy1.2⁺, and KLRG1⁺), ILC3 (Lin⁺, Thy1.2⁺, IL-7R⁺, and KLRG1⁺), and T reg cells (TCR β ⁺, CD4⁺, and CD25⁺) from mice treated with vehicle (Veh) or RAI. Representative histograms (C) and MFI values (D) of Bodipy FL C₁₆ uptake by ILC2, ILC3, and T reg cells from mice treated with vehicle (Veh) or RAI. Representative histograms (E) and MFI values (F) of Bodipy FL C₁₆ uptake in ILC2 and ILC3 isolated from control (Ctrl) and VAI mice. Results are representative of one (A and B) or two (C and D) independent experiments with three to five mice in each experimental group, or pooled results from two experiments with three to five mice (E and F). **, $P \leq 0.01$; ***, $P \leq 0.001$, unpaired Student's t test (mean \pm SEM).

Accumulation of ILC2 in the absence of RA is dependent on increased mitochondrial FAO

These results suggest that, to sustain their cellular functions, ILC2 may adapt to nutritional stress by increasing FA usage for the generation of ATP. To address this possibility, we investigated the expression profile of genes known to regulate glycolysis or FA metabolism. Indeed, disruption of RA signaling in ILC2 either in vivo or in vitro resulted in decreased expression of genes controlling key and rate-limiting enzymes required for glycolysis (hexokinase [*hk2*] and pyruvate dehydrogenase kinase 1 [*pdh1*]; Fig. 4, A and B). Furthermore, we observed a coordinate increase in genes encoding PPAR α (*ppara*; a gene not detected in the presence of RA) and FATP-6 (*slc27a6*), components of FA sensing, metabolic adaptation (Contreras et al., 2013), and FA transport (Fig. 4, A and B; Contreras et al., 2013). As such, reduced RA signaling as a consequence of malnutrition results in an altered gene expression profile by ILC2, which parallels their metabolic adaptation to FA uptake and FAO. To directly assess how RA signaling influences mitochondrial OXPHOS, FAO, and cellular metabolism of ILC2, we performed extracellular flux analysis. In this assay, the rate of extracellular O₂ consumption (oxygen consumption rate [OCR]) can be used as a readout for OXPHOS. Inhibition of RA signaling in ILC2 resulted in increased basal OCR (Fig. 4, C and D). Further, the spare respiratory capacity (SRC), the quantitative difference between maximal OCR (after trifluoromethoxy phenylhydrazine [FCCP]) and basal OCR, was increased in ILC2 in the presence of RAI compared with conditions where RA signaling was active (Fig. 4 C). SRC can be considered as the extra capacity available to cells to produce energy under stress and has been linked to cellular survival (van der Windt et al., 2012). Consistent with increased dependence on FAO in the absence of vitamin A metabolites, SRC was reduced upon FAO blockade by etomoxir in the presence of RAI (Fig. 4 E). Similarly, the addition of RA to cultured ILC2 reduced basal OCR and SRC compared with cells cultured in media alone (Fig. 4 F). Together, these observations suggest that in the absence of vitamin A metabolites, ILC2 may rely on FAO for increased accumulation and function. Indeed, intestinal ILC2 proliferate and accumulate during vitamin A deficiency both in vivo and in vitro (Spencer et al., 2014). To directly test if FA usage is required for the accumulation of ILC2 in the absence of RA signaling, we cultured ILC2 isolated from the small intestinal lamina propria in the presence or absence of etomoxir. As previously reported (Spencer et al., 2014), in vitro blockade of RA signaling in ILC2 cultures resulted in ILC2 accumulation (Fig. 4 G). Such accumulation was independent of glucose usage, as inhibition of glycolysis with 2-DG did not impact the increase in ILC2 numbers (Fig. 4 G). In contrast, inhibition of FAO with etomoxir limited RAI-mediated ILC2 expansion (Fig. 4 G). In agreement with our in vitro observations, enhanced ILC2 accumulation after RAI treat-

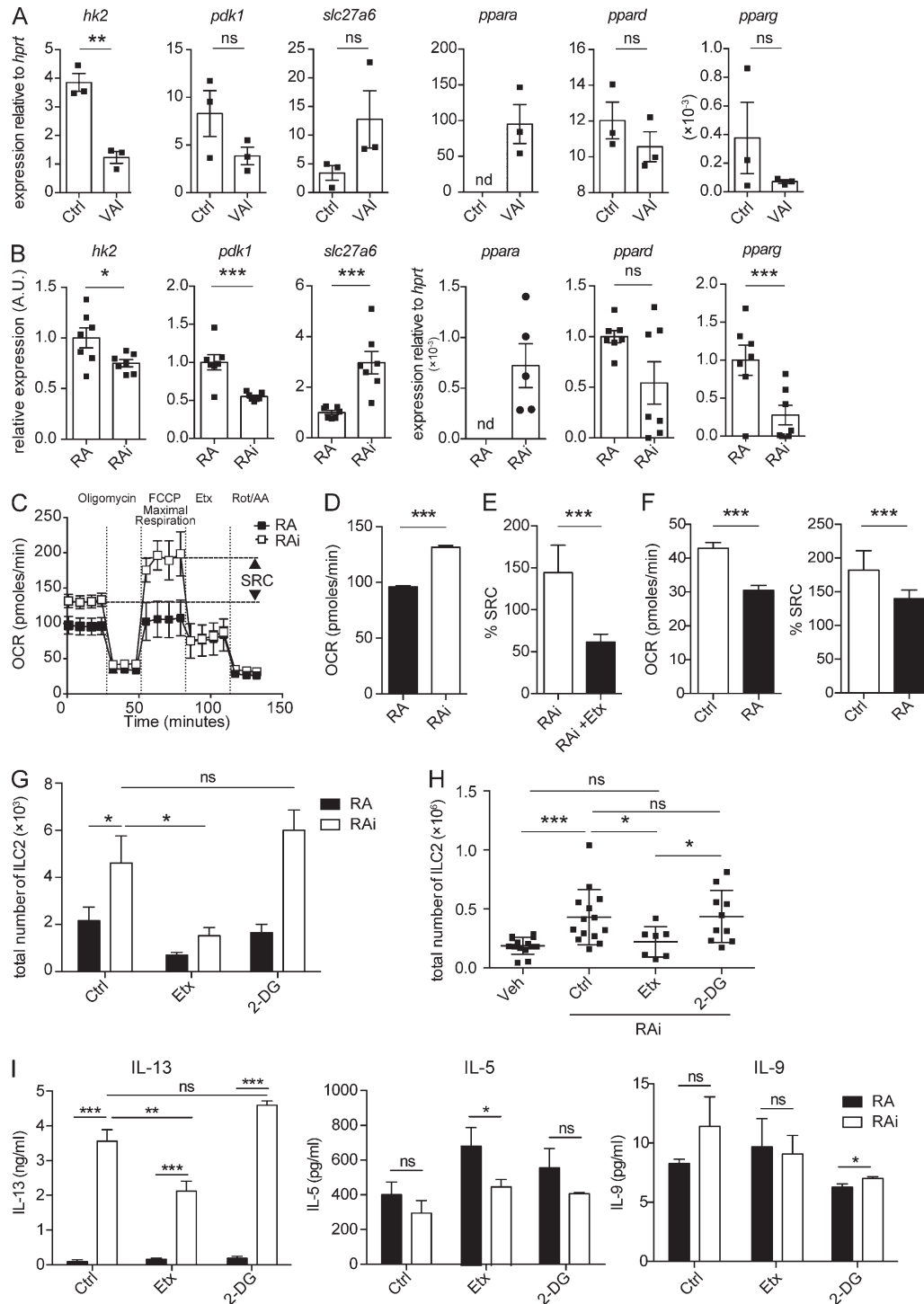


Figure 4. ILC2 increase FAO in the absence of RA signaling. Relative messenger RNA expression of indicated genes in intestinal ILC2 isolated from control or VAI mice (A) and relative messenger RNA expression of indicated genes in purified ILC2 cultured with RA or RAi (B). A.U., arbitrary units. (C) Extracellular flux analysis of ILC2 cultured in the presence of RA or RAi followed by sequential addition of oligomycin, FCCP, etomoxir (Etx), and rotenone in combination with antimycin (Rot/AA). (D) Basal OCR of ILC2 cultured with RA or RAi. (E) SRC of RAi-treated ILC2 in the presence or absence of etomoxir. (F) Basal OCR and SRC of ILC2 cultured with DMSO (Ctrl) or RA. (G) Total numbers of purified ILC2 cultured with IL-7 in the presence of RA or RAi and either vehicle (Veh), 2-DG, or etomoxir (Etx) for 7 d. (H) Total numbers of ILC2 in the small intestinal lamina propria (SILP) of WT mice treated with vehicle (Veh) or RAi and either 2-DG or etomoxir (Etx) for 8 d. (I) Production of IL-13, IL-5, and IL-9 in cell culture supernatant of purified small intestinal ILC2 cultured in

ment in vivo was largely independent of glucose, as blocking glycolysis with 2-DG did not impair ILC2 accumulation in the intestinal tissue (Fig. 4 H). In contrast, in vivo treatment with etomoxir resulted in a significant reduction in the number of intestinal ILC2 (Fig. 4 H). These results suggest that in the absence of RA, accumulation of intestinal ILC2 is independent of glucose and mainly driven by the usage of FA.

Vitamin A deficiency or inhibition of RA signaling both in vivo and in vitro leads to a substantial increase in IL-13 production by ILC2 (Spencer et al., 2014; Fig. 4 I). We next assessed if such increase was also dependent on FA usage. Blockade of FAO with etomoxir partially reduced the enhanced production of IL-13 afforded by impaired RA signaling (Fig. 4 I). Conversely, inhibition of glycolysis had no effect on IL-13 production under this setting (Fig. 4 I). In contrast to IL-13, other ILC2-derived cytokines including IL-5 and IL-9 were not increased after RA blockade and the basal production of these cytokines was not reduced after etomoxir treatment (Fig. 4 I). These findings reveal a differential metabolic requirement for the production of ILC2-derived cytokines and support the idea that IL-13 may be preferentially sustained during defined settings of malnutrition because of its fundamental role in barrier protection.

Considering gastrointestinal anti-helminth immunity as a fundamental aspect of mucosal barrier immunity, such immune responses may have evolved to function in a glucose-deprived environment, a result of the competition between host and parasite for local nutritional resources (Budischak et al., 2015). Throughout evolution, the organism also had to adapt to successive changes in nutrition and periods of unstable nutritional uptake. Therefore, it is likely that immune functions need to be coupled to the nutritional status of the host. Notably, in a vitamin A-deprived environment, metabolically costly immunity such as T cell responses are impaired (Hall et al., 2011). In contrast, absence of RA promotes the specific amplification of ILC2 and IL-13 that we now show to be dependent on FAO. Here, differential metabolic control of ILC2-derived cytokines and specific up-regulation of IL-13 versus IL-5 and IL-9 may underlie posttranscriptional regulation as previously shown for the regulation of IFN- γ production by T cells, mediated by binding of the glycolysis enzyme GAPDH to the 3'UTR of *Ifng* messenger RNA (Chang et al., 2013). Given the pleiotropic role of ILC2 and associated cytokines, such as IL-13, in the maintenance of barrier immunity (Cliffe et al., 2005; Spencer et al., 2014; Karo-Atar et al., 2016), the preferential maintenance of these responses through FAO may have represented an important evolutionary advantage in the context of nutritional and infectious challenges.

MATERIALS AND METHODS

Mice. C57BL/6 (WT) and *Rag1*^{-/-} mice were purchased from Taconic. All mice were bred and maintained under specified pathogen-free conditions at an American Association for the Accreditation of Laboratory Animal Care-accredited animal facility at the National Institute of Allergy and Infectious Diseases (NIAID) and housed in accordance with the procedures outlined in the Guide for the Care and Use of Laboratory Animals under an animal study proposal approved by the NIAID Animal Care and Use Committee. All mice were used between 6 and 13 wk of age.

Diet studies. Vitamin A-deficient (TD.10991) and -sufficient (20,000 IU vitamin A/kg; TD.10992) diets were purchased from Envigo. At day 14.5 of gestation, pregnant females were administered either a vitamin A-deficient or -sufficient diet and maintained on the diet until weaning of litter. Weanlings were maintained on an appropriate diet throughout the study.

Cell isolation from tissues and flow cytometry. Cells from small and large intestinal lamina propria were prepared as previously described (Sun et al., 2007). Skin and lung tissues were diced and subsequently digested with 0.25 mg/ml Liberase TL (Roche) at 37°C for 45 min (lung) or Liberase TL and 1 mg/ml DNase I at 37°C for 1 h and 15 min (skin). Isolated lung cells were further purified using a 37.5% Percoll gradient followed by lysis of red blood cells with ACK buffer. Single-cell suspensions were stained with anti-CD16/32 (eBioscience) and with fluorochrome-conjugated antibodies against any combination of the following surface antigens: CD4, CD8 α , CD11b, CD11c, DX5, CD49b, TCR β , TCR $\gamma\delta$, CD19, Ter119, NK1.1, Gr-1, and Thy1.2. Before fixation, Live/Dead Fixable Blue Cell Stain kit (Invitrogen) was used to exclude dead cells. For examination of transcription factors and cellular proliferation, cells were subsequently treated with the Foxp3 fixation/permeabilization kit (eBioscience) in accordance with the manufacturer's instructions and stained for 20 min at room temperature with fluorochrome-conjugated antibodies against ROR γ t, GATA3, and T-bet.

Re-stimulation of cells for intracellular cytokine staining. Cells isolated from the lamina propria were stimulated for 3 h with 50 ng/ml phorbol 12-myristate 13-acetate and 2.5 μ g/ml ionomycin in the presence of 1 μ g/ml brefeldin A (GolgiPlug; BD). Stimulated cells were stained for surface markers, fixed with formaldehyde, permeabilized, and stained with antibodies to IL-5 and IL-13.

Fluorescence-activated cell sorting and in vitro culture of ILC. ILC2 were sorted by flow cytometry from small intestine

the presence of RA or RAI and either vehicle, 2-DG, or etomoxir (Etx) after 7 d of culture. Results are representative of two or more independent experiments with ILC2 sorted from one to two mice (A), two to three mice (B-F and I), or three to five individual mice (H) per experimental group. ns, not significant. *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$, unpaired Student's *t* test (mean \pm SEM).

lamina propria cells from WT and VAI mice based on the absence of lineage markers (CD4, CD8 α , CD11b, CD11c, DX5, CD49b, TCR- β , TCR- $\gamma\delta$, CD19, Ter119, NK1.1, and Gr-1) but on the expression of Thy1.2 and IL-7R. ILC2 were sorted as lineage negative, Thy1.2⁺, or KLRG1⁺. ILC2 (5×10^3 cells) were cultured in 50 μ l RPMI 1640 supplemented with 10% FBS, penicillin, streptomycin, Hepes, glutamine, nonessential amino acids, 50 mM β -mercaptoethanol (Complete Media), and 20 ng/ml IL-7 in the presence of 1 μ M of all-trans RA (Sigma-Aldrich) or 1.8 μ M of RA inhibitor BMS 493 (Tocris Bioscience) in combination with either 2 mM 2-DG (Sigma-Aldrich) or 200 μ M Etomoxir (R&D Systems) for 7 d. Cytokine expression in the cell-free supernatant was assessed using a bead-based cytokine detection assay (FlowCytomix [eBioscience] or CBA [BD]). For metabolic assays and gene expression analysis, ILC2 were expanded with IL-33 and IL-7 for 5 (gene expression) or 7 d (metabolic assays) followed by culture with IL-7 in combination with RA or BMS 493 for 24 h (metabolic assays) or an additional 4 d (gene expression).

Infection with *T. muris*. 6–10-wk-old mice were inoculated with 30 or 150 infective *T. muris* eggs on day 0 by oral gavage. Mice were treated with 2-DG, etomoxir, or appropriate vehicle controls as described below. Infected mice were killed 15–18 d after infection. Worm burden in the cecal epithelial cell layer was assessed and intestinal tissue was processed for analysis of immune cells. For treatment with 2-DG, 1 g/kg 2-DG in PBS or PBS only as a control was supplied every day starting upon infection with *T. muris* for 7 d and every other day thereafter. For treatment with etomoxir, 15 mg/kg etomoxir in 30 μ l DMSO or DMSO only as control was supplied every other day. WT or *Rag1*^{-/-} mice were inoculated with 30 infective *T. muris* eggs and treated with DMSO or 200 mg/kg orlistat (Cayman Chemicals) every other day starting 7 d after infection. Infected mice were killed 15 d later and their worm burden was assessed in the cecal epithelial cell layer.

Culture of *T. muris*. *T. muris* larvae were isolated from the cecum of infected mice 18 d after inoculation. Between four and eight larvae were cultured in 200 μ l RPMI 1640 supplemented with 10% FBS, penicillin, streptomycin, Hepes, L-glutamine, nonessential amino acids, and 50 mM β -mercaptoethanol in the presence or absence of 200 μ M etomoxir for 2 wk.

In vivo inhibitor treatment. To block RA signaling, a total of 220 mg of the pan RA receptor inverse agonist BMS 493 (R&D Systems; RAi) was resuspended in 30 μ l of biotechnology performance-certified DMSO (Sigma-Aldrich) and administered intraperitoneally to C57BL/6 WT mice every day for 8 d as previously described (Kastner et al., 2001). To block FAO, mice were administered 15 mg/kg etomoxir (Tocris Bioscience) every other day throughout the course of infection or for 7 d under steady-state conditions. To block gly-

colysis, mice were administered 1 g/kg 2-DG (Cayman Chemicals) every other day starting upon infection with *T. muris* for 7 d and then every day thereafter. For simultaneous treatment, mice were treated with the indicated combination in a total volume of 30 μ l DMSO or DMSO only as a vehicle control. To block FA uptake, mice were treated with 200 mg/kg orlistat every other day starting 7 d after infection. Control mice received DMSO only.

Bodipy and 2-NBDG treatment. Mice were injected intraperitoneally with 50 μ g Bodipy FL C₁₆ (Thermo Fisher Scientific) in 50 μ l DMSO or with 100 μ g 2-NBDG (Cayman Chemicals) in PBS, 60 or 20 min, respectively, before sacrifice and cell isolation.

Real-time PCR. RNA was extracted from purified or cultured ILC2 using RNAeasy Mini kit (QIAGEN) or Trizol (Thermo Fisher Scientific) and reverse transcribed with Omniscript (QIAGEN) according to the manufacturer's instructions. The cDNA served as a template for amplification of genes of interest. For analysis of genes expressed by ILC2, TaqMan probes for *hk2*, *ppar α* , *ppar β* , *ppar γ* , *slc27a6*, and *pdk1* (Thermo Fisher Scientific) were used and gene expression was normalized to *hprt1*.

Metabolic assays. For real-time measurement of OCR, ILC2 were cultured in XF media supplemented with 10% FCS and 10 mM glucose and analyzed with an XF-96 Extracellular Flux Analyzer (Seahorse Bioscience). Three or more consecutive measurements were obtained under basal conditions followed by the addition of 1 μ M oligomycin, which inhibits the mitochondrial ATP synthase; 1.5 μ M FCCP, which uncouples ATP synthesis from oxygen consumption; 200 μ M etomoxir, to inhibit CPT1a and block FAO; and a combination of 100 nM rotenone plus 1 μ M antimycin A, which inhibit the electron transport chain by blocking complex I and III, respectively. All chemicals used for these assays were obtained from Sigma-Aldrich. SRC is calculated as the difference between basal OCR and the maximal OCR after the addition of FCCP.

Statistical analysis. A two-tailed Student's *t* test was used for all statistical analysis: *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$.

Online supplemental material. Fig. S1 shows that etomoxir directly impairs *T. muris* survival. Online supplemental material is available at <http://www.jem.org/cgi/content/full/jem.20151448/DC1>.

ACKNOWLEDGMENTS

We thank Drs. Richard Grencis, Allison Bancroft, and John Grainger for experimental advice; the National Institute of Allergy and Infectious Diseases (NIAID) animal facility staff for animal care; and Kevin Holmes and the NIAID sorting facility, in particular Cal Eigsti for cell sorting and Kim Beach for technical assistance. We thank the Belkaid laboratory for critical discussions regarding the manuscript.

This work was supported by the Division of Intramural Research of the NIAID, the Ministry for Science and Education of North-Rhine-Westfalia (C. Wilhelm), the Deutsche Forschungsgemeinschaft Excellence Cluster ImmunoSensation (C. Wilhelm), and the Human Frontier Science Program and by funds from the U.S. Department of Agriculture/Agricultural Research Service Project Plan #8040-51000-058-00D (J.F. Urban Jr.). The NIAID Division of Intramural Research Animal Care and Use Program, as part of the National Institutes of Health Intramural Research Program, approved all of the experimental procedures (protocol LPD-7E).

The authors declare no competing financial interests.

Submitted: 8 September 2015

Accepted: 27 May 2016

REFERENCES

- Berod, L., C. Friedrich, A. Nandan, J. Freitag, S. Hagemann, K. Harmrolfs, A. Sandouk, C. Hesse, C.N. Castro, H. Bähre, et al. 2014. De novo fatty acid synthesis controls the fate between regulatory T and T helper 17 cells. *Nat. Med.* 20:1327–1333. <http://dx.doi.org/10.1038/nm.3704>
- Budischak, S.A., K. Sakamoto, L.C. Megow, K.R. Cummings, J.F. Urban Jr., and V.O. Ezenwa. 2015. Resource limitation alters the consequences of co-infection for both hosts and parasites. *Int. J. Parasitol.* 45:455–463. <http://dx.doi.org/10.1016/j.ijpara.2015.02.005>
- Chang, C.H., J.D. Curtis, L.B. Maggi Jr., B. Faubert, A.V. Villarino, D. O'Sullivan, S.C. Huang, G.J. van der Windt, J. Blagih, J. Qiu, et al. 2013. Posttranscriptional control of T cell effector function by aerobic glycolysis. *Cell.* 153:1239–1251. <http://dx.doi.org/10.1016/j.cell.2013.05.016>
- Cliffe, L.J., N.E. Humphreys, T.E. Lane, C.S. Potten, C. Booth, and R.K. Grensis. 2005. Accelerated intestinal epithelial cell turnover: A new mechanism of parasite expulsion. *Science.* 308:1463–1465. <http://dx.doi.org/10.1126/science.1108661>
- Contreras, A.V., N. Torres, and A.R. Tovar. 2013. PPAR- α as a key nutritional and environmental sensor for metabolic adaptation. *Adv. Nutr.* 4:439–452. <http://dx.doi.org/10.3945/an.113.003798>
- Cui, G., M.M. Staron, S.M. Gray, P.C. Ho, R.A. Amezcua, J. Wu, and S.M. Kaech. 2015. IL-7-induced glycerol transport and TAG synthesis promotes memory CD8⁺ T cell longevity. *Cell.* 161:750–761. <http://dx.doi.org/10.1016/j.cell.2015.03.021>
- Eberl, G., M. Colonna, J.P. Di Santo, and A.N. McKenzie. 2015. Innate lymphoid cells: A new paradigm in immunology. *Science.* 348:aaa6566. <http://dx.doi.org/10.1126/science.aaa6566>
- Fuchs, A., W. Vermi, J.S. Lee, S. Lonardi, S. Gilfillan, R.D. Newberry, M. Cella, and M. Colonna. 2013. Intraepithelial type 1 innate lymphoid cells are a unique subset of IL-12- and IL-15-responsive IFN- γ -producing cells. *Immunity.* 38:769–781. <http://dx.doi.org/10.1016/j.immuni.2013.02.010>
- Hall, J.A., J.R. Grainger, S.P. Spencer, and Y. Belkaid. 2011. The role of retinoic acid in tolerance and immunity. *Immunity.* 35:13–22. <http://dx.doi.org/10.1016/j.immuni.2011.07.002>
- Heck, A.M., J.A. Yanovski, and K.A. Calis. 2000. Orlistat, a new lipase inhibitor for the management of obesity. *Pharmacotherapy.* 20:270–279. <http://dx.doi.org/10.1592/phco.20.4.270.34882>
- Huang, S.C., B. Everts, Y. Ivanova, D. O'Sullivan, M. Nascimento, A.M. Smith, W. Beatty, L. Love-Gregory, W.Y. Lam, C.M. O'Neill, et al. 2014. Cell-intrinsic lysosomal lipolysis is essential for alternative activation of macrophages. *Nat. Immunol.* 15:846–855. <http://dx.doi.org/10.1038/ni.2956>
- Karo-Atar, D., A. Bordowitz, O. Wand, M. Pasmanik-Chor, I.E. Fernandez, M. Itan, R. Frenkel, D.R. Herbert, E.D. Finkelman, O. Eickelberg, and A. Munitz. 2016. A protective role for IL-13 receptor α 1 in bleomycin-induced pulmonary injury and repair. *Mucosal Immunol.* 9:240–253. <http://dx.doi.org/10.1038/mi.2015.56>
- Kastner, P., H.J. Lawrence, C. Waltzinger, N.B. Ghyselinck, P. Chambon, and S. Chan. 2001. Positive and negative regulation of granulopoiesis by endogenous RAR α . *Blood.* 97:1314–1320. <http://dx.doi.org/10.1182/blood.V97.5.1314>
- Klose, C.S., M. Flach, L. Möhle, L. Rogell, T. Hoyler, K. Ebert, C. Fabiunke, D. Pfeifer, V. Sexl, D. Fonseca-Pereira, et al. 2014. Differentiation of type 1 ILCs from a common progenitor to all helper-like innate lymphoid cell lineages. *Cell.* 157:340–356. <http://dx.doi.org/10.1016/j.cell.2014.03.030>
- MacIver, N.J., R.D. Michalek, and J.C. Rathmell. 2013. Metabolic regulation of T lymphocytes. *Annu. Rev. Immunol.* 31:259–283. <http://dx.doi.org/10.1146/annurev-immunol-032712-095956>
- Michalek, R.D., V.A. Gerriets, S.R. Jacobs, A.N. Macintyre, N.J. MacIver, E.F. Mason, S.A. Sullivan, A.G. Nichols, and J.C. Rathmell. 2011. Cutting edge: Distinct glycolytic and lipid oxidative metabolic programs are essential for effector and regulatory CD4⁺ T cell subsets. *J. Immunol.* 186:3299–3303. <http://dx.doi.org/10.4049/jimmunol.1003613>
- Monticelli, L.A., M.D. Buck, A.L. Flamar, S.A. Saenz, E.D. Tait Wojno, N.A. Yudanin, L.C. Osborne, M.R. Hepworth, S.V. Tran, H.R. Rodewald, et al. 2016. Arginase 1 is an innate lymphoid-cell-intrinsic metabolic checkpoint controlling type 2 inflammation. *Nat. Immunol.* 17:656–665. <http://dx.doi.org/10.1038/ni.3421>
- O'Sullivan, D., G.J. van der Windt, S.C. Huang, J.D. Curtis, C.H. Chang, M.D. Buck, J. Qiu, A.M. Smith, W.Y. Lam, L.M. DiPlato, et al. 2014. Memory CD8⁺ T cells use cell-intrinsic lipolysis to support the metabolic programming necessary for development. *Immunity.* 41:75–88. <http://dx.doi.org/10.1016/j.immuni.2014.06.005>
- Pearce, E.L., and E.J. Pearce. 2013. Metabolic pathways in immune cell activation and quiescence. *Immunity.* 38:633–643. <http://dx.doi.org/10.1016/j.immuni.2013.04.005>
- Pearce, E.L., M.C. Poffenberger, C.H. Chang, and R.G. Jones. 2013. Fueling immunity: Insights into metabolism and lymphocyte function. *Science.* 342:1242454. <http://dx.doi.org/10.1126/science.1242454>
- Robinette, M.L., A. Fuchs, V.S. Cortez, J.S. Lee, Y. Wang, S.K. Durum, S. Gilfillan, M. Colonna, L. Shaw, B. Yu, et al. Immunological Genome Consortium. 2015. Transcriptional programs define molecular characteristics of innate lymphoid cell classes and subsets. *Nat. Immunol.* 16:306–317. <http://dx.doi.org/10.1038/ni.3094>
- Spencer, S.P., C. Wilhelm, Q. Yang, J.A. Hall, N. Bouladoux, A. Boyd, T.B. Nutman, J.F. Urban Jr., J. Wang, T.R. Ramalingam, et al. 2014. Adaptation of innate lymphoid cells to a micronutrient deficiency promotes type 2 barrier immunity. *Science.* 343:432–437. <http://dx.doi.org/10.1126/science.1247606>
- Spits, H., and J.P. Di Santo. 2011. The expanding family of innate lymphoid cells: Regulators and effectors of immunity and tissue remodeling. *Nat. Immunol.* 12:21–27. <http://dx.doi.org/10.1038/ni.1962>
- Spits, H., D. Artis, M. Colonna, A. Diefenbach, J.P. Di Santo, G. Eberl, S. Koyasu, R.M. Locksley, A.N. McKenzie, R.E. Mebius, et al. 2013. Innate lymphoid cells—a proposal for uniform nomenclature. *Nat. Rev. Immunol.* 13:145–149. <http://dx.doi.org/10.1038/nri3365>
- Sun, C.M., J.A. Hall, R.B. Blank, N. Bouladoux, M. Oukka, J.R. Mora, and Y. Belkaid. 2007. Small intestine lamina propria dendritic cells promote de novo generation of Foxp3 T reg cells via retinoic acid. *J. Exp. Med.* 204:1775–1785. <http://dx.doi.org/10.1084/jem.20070602>
- Taylor, C.M., Q. Wang, B.A. Rosa, S.C. Huang, K. Powell, T. Schedl, E.J. Pearce, S. Abubucker, and M. Mitreva. 2013. Discovery of anthelmintic drug targets and drugs using chokepoints in nematode metabolic pathways. *PLoS Pathog.* 9:e1003505. <http://dx.doi.org/10.1371/journal.ppat.1003505>
- van der Windt, G.J., B. Everts, C.H. Chang, J.D. Curtis, T.C. Freitas, E. Amiel, E.J. Pearce, and E.L. Pearce. 2012. Mitochondrial respiratory capacity is a critical regulator of CD8⁺ T cell memory development. *Immunity.* 36:68–78. <http://dx.doi.org/10.1016/j.immuni.2011.12.007>

- van der Windt, G.J., D. O'Sullivan, B. Everts, S.C. Huang, M.D. Buck, J.D. Curtis, C.H. Chang, A.M. Smith, T. Ai, B. Faubert, et al. 2013. CD8 memory T cells have a bioenergetic advantage that underlies their rapid recall ability. *Proc. Natl. Acad. Sci. USA*. 110:14336–14341. <http://dx.doi.org/10.1073/pnas.1221740110>
- Vats, D., L. Mukundan, J.I. Odegaard, L. Zhang, K.L. Smith, C.R. Morel, R.A. Wagner, D.R. Greaves, P.J. Murray, and A. Chawla. 2006. Oxidative metabolism and PGC-1 β attenuate macrophage-mediated inflammation. *Cell Metab.* 4:13–24. <http://dx.doi.org/10.1016/j.cmet.2006.05.011>
- Veldhoen, M., and V. Brucklacher-Waldert. 2012. Dietary influences on intestinal immunity. *Nat. Rev. Immunol.* 12:696–708. <http://dx.doi.org/10.1038/nri3299>
- Viscarra, J.A., and R.M. Ortiz. 2013. Cellular mechanisms regulating fuel metabolism in mammals: Role of adipose tissue and lipids during prolonged food deprivation. *Metabolism*. 62:889–897. <http://dx.doi.org/10.1016/j.metabol.2012.12.014>
- WHO. 2009. Global prevalence of vitamin A deficiency in populations at risk 1995–2005. WHO Global Database on Vitamin A Deficiency. World Health Organization, Geneva. 55 pp.