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Metabolic Regulation of Natural Killer Cell IFN-γ Production

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Abstract

Metabolism is critical for a host of cellular functions and provides a source of intracellular energy. It has been recognized recently that metabolism also regulates differentiation and effector functions of immune cells. Although initial work in this field has focused largely on T lymphocytes, recent studies have demonstrated metabolic control of innate immune cells, including natural killer (NK) cells. Here, we review what is known regarding the metabolic requirements for NK cell activation, focusing on NK cell production of interferon-gamma (IFN-γ). NK cells are innate immune lymphocytes that are poised for rapid activation during the early immune response. Although their basal metabolic rates do not change with short-term activation, they exhibit specific metabolic requirements for activation depending upon the stimulus received. These metabolic requirements for NK cell activation are altered by culturing NK cells with interleukin-15, which increases NK cell metabolic rates at baseline and shifts them toward aerobic glycolysis. We discuss the metabolic pathways important for NK cell production of IFN-γ protein and potential mechanisms whereby metabolism regulates NK cell function.

Keywords

NK cell; interferon-gamma; glycolysis; oxidative phosphorylation; mTOR; signaling; immunometabolism

I. INTRODUCTION

Natural killer (NK) cells are lymphocytes that comprise approximately 5–15% of human peripheral blood mononuclear cells and 2–3% of splenocytes in mice. They share their role as cytotoxic and interferon-gamma (IFN-γ)-producing cells with $CD8^+$ T cells. However, unlike T cells, which mount an antigen-specific response days after their initial activation, NK cells are largely antigen independent, recognize infected and transformed cells through germline-encoded NK receptors (NKRs), and carry out their cytotoxic function within minutes to hours.[1–4] NK cells produce several cytokines and chemokines that coordinate innate and adaptive immune responses and are the major source of IFN-γ in the first hours of infection before antigen-specific T-cell production of IFN-γ.[2]

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IFN-γ plays an important role in modulating the immune response, particularly host defense against intracellular pathogens and cancers. In addition to NK cells, IFN-γ is produced by NK T cells, $CD8^+$ T cells, and T-helper 1 (Th1) $CD4^+$ T cells.[5] Functions of IFN-γ include supporting Th1 differentiation, boosting macrophage function, encouraging leukocyte migration to the site of infection, and increasing major histocompatibility complex expression for better T-cell recognition of infected or malignant cells.[6–8] Mice and humans deficient in IFN-γ signaling are susceptible to tumors and infections with intracellular pathogens such as mycobacteria.[7,9] However, uncontrolled IFN-γ protein production is also detrimental to the host and overexpression of IFN-γ in mice promotes autoimmunity.[10,11] Therefore, tight regulation of IFN-γ expression is essential for balancing inflammation and immune tolerance.

Recently, it has been found that cellular metabolism regulates IFN-γ expression by NK and T cells.[12–17] In this review, we discuss the mechanisms of IFN-γ regulation, metabolic regulation of cytokine production by other immune cells, and the specific metabolic requirements for NK cell IFN-γ production in mice and humans. We conclude with speculation about the mechanisms by which metabolism may regulate IFN-γ production.

II. REGULATION OF IFN-γ PROTEIN PRODUCTION

IFN-γ protein expression is regulated at multiple levels, including epigenetically, transcriptionally, and post-transcriptionally. Therefore, a thorough understanding of the pathways leading to IFN-γ production is important when considering potential mechanisms of metabolic regulation of this critical cytokine in NK cells. Much of the work examining IFN- regulation in NK cells has been done in mice, but studies with human NK cells suggest similarities between mice and humans.[18,19] Regulation of IFN-γ production in NK cells shares many aspects with T cells, including the signaling pathways and transcription factors (TFs) required for efficient transcription. Acute transcription of *Ifng* requires nuclear factor kappa light-chain enhancer of activated B cells (NF-κB) activation, which is induced by the activating receptors NKR or T-cell receptor (TCR) and/or the cytokines interleukin-1 (IL-1) or IL-18, and signal transducer and activator of transcription 4 (STAT4), which is induced by IL-12.[20,21] However, a crucial difference between NK and T cells is that mature NK cells have an epigenetically accessible *Ifng* locus and constitutively express IFN-γ transcript, whereas T cells do not.[21–24] Here, we review known mechanisms regulating IFN-γ production in NK cells, including induction of transcription through activating signaling, effects of noncoding RNA, and post-transcriptional regulation through mRNA stability.

A. Transcriptional Regulation

Expression at the *Ifng* locus is controlled by its promoter and several upstream enhancer regions. In resting murine NK cells, the *Ifng* locus is epigenetically “primed” and available to the TFs T-bet and Eomes, which are expressed in mature NK cells.[22–24] Human NK cells have a similarly accessible *IFNG* locus.[19] In both mice and humans, IFN-γ transcript is produced constitutively at low levels, although it is unclear what is driving this transcription and why NK cells do not constitutively produce IFN-γ protein.[23] It is possible that retention of pre-formed transcripts is one of the mechanisms allowing NK cells to respond rapidly to

activation because resting murine NK cells also constitutively express low levels of granzyme B and perforin transcript but not protein.[25] In contrast, the T-cell *Ifng* locus is "closed" and relatively inaccessible, requiring epigenetic changes and up-regulation of several TFs, including T-bet and Eomes, before transcription of *Ifng*.[21,22]

Interestingly, one proximal enhancer regulating both mouse and human IFN-γ transcription, CNS1, is methylated and "closed" in naive NK cells, but is de-methylated in two types of "memory" NK cells: human cytomegalovirus-adapted NKG2C^{hi} human NK cells and murine cytokine-induced memory-like NK cells that were treated with cytokines (IL-12/15/18) and transferred adoptively into lymphopenic hosts.[26,27] This finding provides a potential molecular basis as to why some types of memory NK cells have an enhanced capacity for IFN-γ production.

NK cells can be activated by three complementary and often overlapping routes: (1) engagement of germline-encoded activating NKRs that recognize stress- and virally induced ligands on target cells in the absence of a strong inhibitory NKR signal, (2) recognition of antibody-coated target cells through the activating Fc receptor CD16 (antibody-dependent cellular cytotoxicity), and/or (3) activation by innate and adaptive immune cytokines including IL-1, IL-2, IL-12, IL-15, and IL-18.[3,28] Experimentally, NK cell IFN-γ production can be induced by brief incubation (4–24 hours) with purified cytokines or cytokine-producing antigen presenting cells, co-culture with tumor cells expressing activating ligands, or culture with plate- or bead-bound antibodies against activating NKRs.

Most activating NKRs signal through adapters containing immunoreceptor tyrosine-based activation motifs (ITAMs), which activate the Ras/mitogen-activated protein kinase (MAPK), phosphoinositide 3-kinase (PI3K)/AKT, and phospholipase C-gamma (PLC-γ) phosphorylation cascades.[29–32] Cytokine receptors for IL-2, IL-12, and IL-15 activate a variety of STATs (Fig. 1). Combined with constitutive expression of T-bet, and likely Eomes, in NK cells, these TFs bind the *Ifng* promoter, intronic enhancers, and both upstream and distal conserved noncoding sequences to induce transcription (Fig. 1.)[5,33] In particular, IL-12 co-stimulation and STAT4 activation are required for optimal cytokine-induced IFN-γ transcription. In murine T cells, this appears to be due to STAT4 stabilization of the RelA subunit of NF-κB when binding to the *Ifng* locus.[34] In T cells, AP-1, Ets-1, Runx3, NFAT, and other STATs are also recruited, but their role in NK cell IFN-γ production is unclear.[21]

B. Regulation by Noncoding RNA

Several microRNAs (miRNAs) have been shown to regulate IFN-γ production in NK and T cells.[35,36] These ~22 nucleotide RNA molecules recognize specific sequences on messenger RNAs (mRNAs) and classically lead to their degradation or inhibit their translation. In NK and T cells, miR-29 binds directly to the 3′ untranslated region (UTR) of IFN-γ and represses translation.[37] MiRNAs might also inhibit IFN-γ production by interfering with upstream activating signaling; for example, miR-146a decreases IRAK/TRAF6 activity in T cells.[38] However, some miRNAs play a more complex role, as is the case of miR-155. Both overexpression and deletion of miR-155 in NK cells leads to increased IFN-γ production.[39–41] Acute deletion of the miRNA-processing enzyme *Dicer* in all cells, including mature NK cells, using a drug-inducible Cre model caused decreased NK cell

IFN-γ production in response to NKRs.[42] However, in a more specific model of *Dicer* deletion in lymphocytes only, NK cell IFN-γ production was enhanced in response to multiple activating stimuli including NKRs.[43] These results suggest that miRNAs may play different roles in developing versus mature NK cells, affecting how NK cells respond to activation and controlling IFN-γ translation directly.

Long noncoding RNAs (lncRNAs) are >200 nucleotide RNAs that can regulate gene transcription and affect recruitment of TFs. The lncRNA *Tmevpg1*, also known as NeST, has been shown to regulate IFN-γ production in T cells. Knock-down of NeST in Th1 cells decreased IFN-γ production, whereas induced expression increased IFN-γ production.[44,45] Transgenic expression of this lncRNA was associated with increased H3K4 methylation, a mark of active chromatin, at the *Ifng* locus.[45] Although NeST is the only lncRNA known to affect IFN-γ production thus far, its regulation of this cytokine suggests that there might be other lncRNAs regulating IFN-γ production and effector function. The role of lnRNAs, including NeST, in the production of IFN-γ by NK cells has not been studied.

C. Post-Transcriptional Regulation

Post-transcriptional regulation is another important mechanism for controlling IFN-γ protein production. The secondary structure of the 5′ UTR of human, but not mouse, IFN-γ mRNA activates PKR, which inhibits translation of IFN-γ transcript.[46,47] The 3′ UTR of murine IFN-γ has several AU-rich elements that signal for rapid degradation and deletion of this region in a mouse model led to high circulating levels of IFN-γ.[11] Several cytokines, including IL-12, stabilize IFN-γ transcript in T cells through a p38 MAPK-dependent pathway involving AU-binding proteins such as tristetraprolin.[48–50] Knockout of tristetraprolin in T cells led to a doubling of the half-life of the IFN-γ transcript and increased expression upon activation.[50] In NK cells, there is also evidence that the stability of IFN-γ mRNA is increased upon activation because stimulation with IL-2/12 increases IFN-γ mRNA processing efficiency, export from the nucleus, and half-life compared with IL-12 alone or no stimulation.[51,52] Therefore, IL-12 signaling stabilizes IFN-γ in both T and NK cells, athough the mechanisms may not be shared between cell types. It is unclear what post-transcriptional mechanism NK cells use to control the translation of pre-formed transcript, but this process is likely unique to NK cells because T cells do not appear to express IFN-γ transcript without translation of protein.

In summary, IFN-γ is transcribed constitutively at low levels in NK cells and increased expression and translation upon activation is tightly regulated by TF signaling and stabilization of mRNA. In addition to these regulatory strategies, recent studies have suggested that metabolism also regulates NK cell IFN-γ production.

III. METABOLIC REGULATION IN T CELLS, MACROPHAGES, AND DENDRITIC CELLS

The study of immunometabolism examines how changes in metabolic pathway usage regulate and are regulated by immune cell activation and function. Significant progress has been made in elucidating the importance of metabolism for development, differentiation,

activation, effector function, and/or memory formation in murine T cells, macrophages, and dendritic cells (DCs).[53–56] Several recent studies have demonstrated that NK cell functions, in particular IFN-γ production, are also metabolically regulated.[12–15] Here, we review briefly the major metabolic pathways and how metabolism regulates activation and cytokine production in these immune cell types.

A. Cellular Metabolic Pathways

Major cellular metabolic pathways active in immune cells are shown in Figure 2. The main fuel used by most cells is glucose, which is processed by glycolysis into pyruvate, high-energy electrons carried by NADH and two molecules of ATP. The rate of glycolysis is regulated by diverse signals including the cell's energy balance, the abundance of downstream intermediates, and cell signaling events.[57] Several metabolic regulators, including mTOR, HIF-1α, ERRα, and c-myc, increase the rate of glycolysis through up-regulation of glycolytic enzymes and/or glucose transporters.[53] Aerobic glycolysis, or fermentation of pyruvate into lactate in the presence of oxygen, is a distinguishing feature of proliferating cells first described in cancer cells and T lymphocytes.[58,59] Glucose can be diverted from glycolysis to fuel nucleotide synthesis, regeneration of the reducing agent NADPH,[60] and glycosylation of proteins for their correct folding, function, and expression.[61]

Pyruvate from glycolysis is converted into acetyl-CoA to enter the tricarboxylic acid (TCA) cycle. The TCA cycle occurs in the mitochondria and processes acetyl-CoA into CO_2 and high-energy electrons. It contains multiple entry and exit points into other metabolic pathways, and can be fueled by glycolysis, glutamine, or fatty acid oxidation (FAO). TCA intermediates such as citrate can be used for several biosynthetic reactions, including fatty acid (FA) and amino acid synthesis.[62] Acetyl-CoA is required for acetylation, which can regulate metabolism directly by promoting degradation of metabolic enzymes and affect the transcription of many genes indirectly through acetylation of histones, which allows greater chromatin accessibility.[61]

Conversion of the high-energy electrons produced by the TCA cycle and glycolysis into energy in the form of ATP occurs during oxidative phosphorylation (OXPHOS), which takes place in the mitochondrial membrane. A series of enzymes accepts electrons from carriers such as NADH and create a proton gradient that fuels ATP synthesis.[63] OXPHOS processing of one molecule of glucose yields approximately 30 ATP molecules, making it much more efficient than anaerobic glycolysis. Reactive oxygen species (ROS) are also generated and can serve as intracellular signals or, in excess, can induce damage and an oxidative stress response.[64–66]

B. T-Cell Activation Induces Aerobic Glycolysis and Releases Enzymatic Repression of IFN-γ

Naive murine T cells are relatively metabolically quiescent and rely primarily upon glucose for energy production through OXPHOS (Fig. 2, left panel).[67] T cells up-regulate aerobic glycolysis following activation with a variety of mitogens, including TCR activation, which requires CD28 co-stimulation to enhance T-cell metabolism (Fig. 2, middle panel).[67–71]

Increased glycolytic flux encourages differentiation into inflammatory effector cells (i.e. Th1, Th2, or Th17 cells), whereas blockade of glycolysis or mTOR, which regulates glycolysis positively, promotes regulatory T-cell (Treg) differentiation.[53] Although not essential for all aspects of T-cell activation (e.g. IL-2 production), increased glycolytic activity is necessary for maximal IFN-γ production.[16,17] Although NK cells and T cells use similar ITAM signaling pathways downstream of their activating receptors, NK cells do not exhibit increased glycolysis with short-term receptor stimulation.[12]

Aerobic glycolysis assists in the rapid proliferation of T cells by shunting glucose toward production of biosynthetic precursors needed for proliferation, including nucleotide precursors, amino acids, and FAs for lipid membranes, although it is not strictly required (Fig. 2).[17,72,73] It was shown recently that the glycolytic enzyme GAPDH affects IFN-γ expression directly in $CD8^+$ T cells by binding to and preventing translation of IFN-γ transcript.[17] Increased glycolysis after activation sequesters GAPDH, allowing for efficient translation of IFN-γ. Therefore, glycolysis not only enhances the ability of activated T cells to proliferate, but also controls production of the IFN-γ protein directly. Whether glycolytic enzymes control IFN-γ translation in NK cells is unknown.

C. Macrophages and DCs Also Induce Aerobic Glycolysis with Activation

Similar to T cells, murine macrophages and DCs switch to aerobic glycolysis after activation, although the role of increased glycolytic flux appears to be different than in T cells. Pro-inflammatory (M1) macrophages switch to aerobic glycolysis upon activation,[54,56] shunting TCA intermediates and OXPHOS enzymes toward the production of ROS and nitric oxide (Fig. 2, middle panel).[74,75] A high glycolytic rate also assists in macrophage production of IL-1β in two ways: (1) buildup of the TCA intermediate succinate stabilizes HIF-1α, which enhances IL-1β transcription,[76] and (2) glycolysis increases activity of the NLRP3 inflammasome.[77] Therefore, glycolysis affects production of cytokines in macrophages as well as T cells.

Metabolic changes during dendritic cell activation are more complex. Murine bone marrow-derived DCs (BMDCs) have increased glucose consumption and glycolytic rates within minutes of activation through TLRs.[78–80] Increased glycolytic flux is thought to aid rapid cytokine synthesis by increasing citrate levels for FA synthesis, which fuels massive expansion of the endoplasmic reticulum (ER) and Golgi complex membranes.[55,80] After prolonged activation (12 hours), BMDCs rely exclusively on aerobic glycolysis, possibly because their production of nitric oxide inhibits OXPHOS.[81] Although primary murine DCs do not produce nitric oxide, they also up-regulate glycolysis and decrease OXPHOS upon activation.[80] It is interesting to speculate whether there are similar mechanisms for ER/Golgi expansion in NK cells to combat the protein folding stress of rapidly producing cytokines or cytotoxic granules.

D. Roles of OXPHOS in Immune Cell Activation and Cytokine Production

The ability to up-regulate OXPHOS is important for the generation of Tregs, memory T cells, and anti-inflammatory/tolerogenic (M2) macrophages (Fig. 2, right panel).[53,75] Memory $CD4^+$ and $CD8^+$ T cells have greater mitochondrial mass and are highly oxidative,

which is thought to support longevity and an increased ability to reactivate.[82–84] Disruption of FA processing in T cells decreases the number of memory $CD8^+$ T cells and their ability to produce IFN-γ upon re-stimulation.[85] Because both NK cells and memory T cells produce IFN-γ efficiently and quickly upon activation, there may be similarities in metabolic regulation between these two cell types.

OXPHOS is also important for T-cell activation via generation of ROS. TCR signaling induces mitochondrial ROS,[64,65] which can activate several signaling pathways, in particular MAPK.[66] In murine $CD8^+$ T cells, the lymphocyte expansion molecule assists in the formation of the mitochondrial electron transport chain, increases ROS, improves memory formation, and enhances effector functions including IFN-γ production.[86] However, direct regulation of IFN-γ production by ROS has not been shown.

In conclusion, metabolic signals can alter cytokine production directly by several means. Increased glycolytic flux can draw glycolytic enzymes away from their roles repressing mRNA translation. Metabolites can be shunted to fuel cell growth, to prepare the cell for cytokine production, or to generate effector molecules. Different cell types also use different strategies to fuel specific functions and, although various mechanisms of metabolic control have been found in other immune cell types, their relevance to NK cells has not been fully defined.

IV. METABOLIC REGULATION OF IFN-γ PRODUCTION BY MURINE NK CELLS

Although T and NK cells share many signaling pathways for activation, constitutive transcription of IFN-γ in NK cells suggests that transcription and translation, and therefore potential mechanisms of metabolic regulation, are quite different in these two cell types. Here, we review primary studies on the metabolism of murine NK cells, specifically their metabolic rates during activation (Fig. 3) and metabolic signals required for IFN-γ production (summarized in Table 1). We compare short versus extended stimulation and *in vitro* versus *in vivo* models of metabolic manipulation.

A. Resting NK Cells Use Low Levels of Glucose-Driven OXPHOS and Up-Regulate Their Metabolic Pathways after Extended Stimulation

Studies with freshly isolated murine splenic NK cells have demonstrated that naive NK cells rely on glucose-driven OXPHOS at baseline, similar to resting T cells.[12] Production of IFN-γ protein after short-term (4–6 h) stimulation with either cytokines or NKR stimulation was not accompanied by significant changes in flux through glycolysis (measured as extracellular acidification rate, ECAR) or OXPHOS (measured as oxygen consumption rate, OCR) within 6 hours of activation (Fig. 3).[12,87]

However, we and others have observed significant up-regulation of glycolysis and OXPHOS after culture in IL-15.[12,14] IL-15 is produced by multiple cell types and supports NK cell differentiation, survival, proliferation, and cytotoxicity.[88,89] In cell culture, “low” doses of IL-15 (1–10 ng/mL) promote survival but not proliferation of NK cells, whereas “high” doses (50–100 ng/mL) induce proliferation and increased cytotoxicity, but not IFN-γ

production, in the absence of a second signal.[12] One caveat to *in vitro* studies is that it is not clear how these dosages correspond to IL-15 produced *in vivo* during inflammation because IL-15 is presented to NK cells in *trans* by DCs and other antigen-presenting cells expressing the IL-15Rα chain.[90] However, the fact that NK cells proliferate rapidly after viral infection, for example, during murine cytomegalovirus (MCMV) infection,[91] suggests that IL-15 signals *in vivo* in the context of an inflammatory challenge are similar to the "high" dose levels required to induce proliferation *in vitro*. After 18 hours of culture with high-dose IL-15, NK cells increased their maximal respiratory capacity and basal OCR and ECAR significantly.[14] Longer (72–120 hours) culture in high-dose IL-15 led to increases in both ECAR and OCR, with a decreased OCR:ECAR ratio, suggesting a greater reliance on glycolysis (Fig. 3).[12,14] This parallels findings in T cells, in which several days of activation induces a transition to aerobic glycolysis.

Up-regulation of glycolysis after activation in T cells has been attributed to the activity of master metabolic regulators such as c-myc, HIF-1α, ERRα, and mTOR.[53] Marçais et al. demonstrated that high-dose IL-15 up-regulates mTOR signaling preferentially in NK cells, suggesting a mechanism by which glycolysis is enhanced.[14] NK cells also require mTOR for development and activation through IL-15 signaling, as demonstrated in mice with NK-specific deletion of *Mtor*.[14] Nandagopal et al. showed that blockade of PI3K signaling upstream of mTOR also blocks IL-15-induced enhancement of NK cell IFN-γ production.[92] These data link mTOR to NK cell development and IL-15-induced IFN-γ production, although it is challenging to differentiate the relative contributions of glycolysis versus other downstream targets of mTOR.[93–95]

Together, these findings demonstrate that short-term activation of NK cells is not accompanied by changes in metabolic flux, but extended (3 days) stimulation with high-dose IL-15 leads to up-regulation of metabolism, enhancing glycolysis preferentially. Although it is clear that extended activation of NK cells can alter cellular metabolism, further studies investigating different routes of activation will be important to gain insight into how and why NK cells increase glycolysis and OXPHOS. The observation that prolonged activation alters NK cell metabolism significantly is particularly relevant to the many experiments in the literature using IL-2 or IL-15 *in vitro*-expanded NK cells. As the role of metabolism in NK cell function is further investigated, caution should be used in interpreting findings from *in vitro*-expanded NK cells and extension of those findings to naive NK cells *in vivo* that have not been highly activated.

B. IFN-γ Production by Naive NK Cells Requires Glycolysis and OXPHOS if Activated by Receptors but Not Cytokines

Although short-term (≤6 hours) activation was not accompanied by changes in the rate of glycolysis or OXPHOS, metabolism is required for NK cell IFN-γ production within this time period. Interestingly, there was a difference in the metabolic requirements when NK cells were stimulated through NKRs versus cytokines.[12] IFN-γ production stimulated through the NK1.1 or Ly49D receptors was almost completely blocked by glucose deprivation or OXPHOS inhibition. However, IFN-γ produced downstream of IL-12/18 signaling was not susceptible to inhibition of OXPHOS, glycolysis, or FAO (OXPHOS

inhibition is shown in Fig. 4). IFN-γ was even produced in IL-12/18-stimulated NK cells with blockade of multiple metabolic fuels (glucose, glutamine, FA).[12] Therefore, IFN-γ production induced by IL-12/18 appears to be relatively metabolism independent and these findings suggest that there might be significant flexibility in the fuels that NK cells use to assist with the production of IFN-γ. This is in contrast to T cells, in which glycolysis is required for IFN-γ production in response to both receptor stimulation and IL-12/IL-18.[16,17] It is not clear why stimulation by receptors versus cytokines leads to dramatically differential metabolic requirements in NK cells. The requirement for OXPHOS in NKR-activated NK cells is unlikely to be due to a lack of ROS because supplementation with H_2O_2 does not rescue IFN-γ production under OXPHOS inhibition.[12] This and several other hypotheses are discussed in section VI.

C. Prolonged IL-15 Exposure Changes the Metabolic Requirements of NK Cell IFN-γ Production

Culture with IL-15 alters, not only the baseline metabolism of NK cells, but also their metabolic requirements for IFN-γ production. Although fresh murine NK cells require both OXPHOS and glycolysis for receptor-stimulated IFN-γ production, we found that cells primed for at least 3 days in high-dose IL-15 produce IFN-γ in response to receptor stimulation even when OXPHOS was inhibited (Fig. 4).[12] These IL-15-activated NK cells were also significantly less susceptible to glucose deprivation. This “metabolic priming” was not apparent at shorter time periods or with low-dose IL-15, suggesting both time- and dose-dependent effects of IL-15. Although high-dose IL-15 causes robust NK cell proliferation, metabolic changes were not dependent on proliferation because IFN-γ production in both highly and poorly proliferated NK cells was no longer susceptible to OXPHOS inhibition.[12]

IL-15-activated NK cells make an interesting comparison with activated T cells, which require several days of activation and undergo metabolic reprogramming during that time.[53] Both increasingly rely on glycolysis; however, whereas glycolytic inhibition limits TCR- and IL-12/18-stimulated IFN-γ production severely, it has no effect on IL-12/18 stimulation of NK cells that have been cultured in IL-15 and a modest effect with NKR stimulation.[12,16,17] These findings suggest fundamental differences in metabolic regulation of IFN-γ production between T cells and NK cells.

Donnelly et al. investigated the metabolic requirements of NK cells that had been expanded in intermediate-dose IL-15 for 7 days.[13] IFN-γ production in these IL-15-expanded NK cells in response to IL-2/12 was impaired significantly in the presence of glycolytic or mTOR inhibitors and was accompanied by increased rates in glycolysis and OXPHOS. Whether the requirement for glycolysis and mTOR is an intrinsic property of IL-2/12 stimulation or is the result of IL-15 expansion is unknown.

The mechanism by which IL-15 makes NK cells more or less dependent on specific metabolic signals is unknown, but may be related to how IL-15 primes other NK cell functions, including cytotoxicity. Priming by IL-15 causes translation of pre-formed mRNA for cytotoxic proteins,[25] so it is possible that priming also enhances translation of IFN-γ mRNA.

D. NK Cell Metabolism *in Vivo*

It has been technically challenging to investigate NK cell metabolism *in vivo* and there is less evidence regarding the NK-intrinsic pathways important for *in vivo* NK cell function. Donnelly et al. examined the effects of inhibiting glycolysis or mTOR on NK cell responses to *in vivo* stimulation with the TLR3 ligand poly(I:C).[13] Treatment of mice with inhibitors to either pathway decreased the percentage of IFN-γ^+ NK cells significantly 24 hours after injection of poly(I:C). These findings suggest that TLR3-induced NK cell IFN-γ is dependent upon glycolysis and mTOR, although the mechanism and whether it is NK specific or due to an alteration in IL-15 or type I IFN signaling in other cell types that drive NK cell IFN-γ production in this model is unknown.

In the NK-specific model of *Mtor* deletion developed by Marçais et al., there was a significant decrease in mature, peripheral NK cells despite normal bone marrow numbers.[14] Mature cells that were present did produce IFN-γ normally when stimulated by NKR or IL-12/18. Interestingly, NK cells genetically lacking mTOR had no significant differences in their ability to produce IFN-γ in response to MCMV infection at day 2 after infection.[14] This is in contrast to a study by Nandagopal et al., who administered the mTOR inhibitor rapamycin to MCMV-infected mice and observed decreased NK cell IFN-γ production at 1.5 days after infection.[92] However, it is difficult to compare these two studies because *Mtor*-deficient NK cells are immature and present at a much lower frequency. It would be interesting to investigate whether the effects of rapamycin on MCMV-induced NK cell IFN-γ production are limited to mature, rather than immature, NK cells.

V. METABOLIC REQUIREMENTS FOR IFN-γ IN HUMAN NK CELLS

A recent study demonstrated that human peripheral blood NK cells have metabolic requirements similar to murine NK cells. Human NK cells have several differences compared with murine cells, one key distinction being that, in human cells, the expression of CD56 is linked to maturation and distinguishes less mature and cytokine-producing $CD56^{bright}$ NK cells from more mature and cytotoxic $CD56^{dim}$ NK cells.[96]

Keating et al. found recently that, similar to murine NK cells, human NK cells have relatively low metabolism at rest, but increase glycolysis and OXPHOS significantly after 18 hours of stimulation with IL-2 or IL-12/15.[15] Human NK cells also have specific metabolic requirements for IFN-γ production. Inhibition of OXPHOS decreased IFN-γ production in response to IL-12/15,[15] but had no effect on the small quantity of IFN-γ produced in response to IL-2 alone.[12] Limiting the rate of glycolysis by culturing cells in the presence of galactose rather than glucose led to decreased IFN-γ production by $CD56^{bright}$ NK cells in response to IL-12/15,[15] although the effect of direct glycolytic inhibition is unknown. $CD56^{bright}$ NK cells were more glycolytic and had increased metabolism after stimulation compared with $CD56^{dim}$ cells, suggesting either developmentally regulated differences in metabolism or that cytokine synthesis requires increased metabolic capacity.

Inhibition of mTOR in human NK cells inhibited significantly the ability of IL-2 alone to up-regulate glycolysis, but glycolytic up-regulation with IL-12/15 was unchanged.[15] The fact that mTOR was required for IL-2-driven but not IL-12/15-driven glycolysis in human

NK cells is intriguing because IL-2 and IL-15 use the same signaling receptors. These results suggest that IL-12 signaling might drive increased glycolysis independently from IL-15 and mTOR signaling.

VI. POSSIBLE MECHANISMS OF METABOLIC CONTROL

A. Metabolic Regulation during Signaling and Transcription of IFN-γ

Metabolic requirements for murine NK cell IFN-γ production depend on the type and length of stimulus (i.e., NKR vs. cytokine activated) and the state of the NK cells (i.e., naive vs. IL-15-activated). There are several possible reasons for these findings. First, signaling downstream of different receptors might have distinct metabolic requirements due to the signaling components involved. For example, if signaling proteins characteristic of NKR activation such as PLC-γ or PKC-θ require a metabolic signal, this could explain why IFN-γ downstream of activating receptors required OXPHOS and glycolysis, whereas stimulation from IL-12/18 did not have the same requirement.[12] How metabolism might alter signal transduction is an interesting question. ROS and other metabolites have been shown to regulate signaling pathways, although we did not find a role for ROS in production of IFN-γ through NKR stimulation.[12] Another possibility is that phosphorylation might be altered due to decreased ATP availability with glycolytic or OXPHOS inhibition. Production or stability of IFN-γ transcript might also be regulated by metabolism in NK cells, as is found in T cells. This could be due to a metabolic cue required for efficient mRNA splicing, similar to regulation of *FOXP3* splicing by the glycolytic enzyme enolase-1 in human T cells.[97]

Differences in signaling pathways, activation of downstream TFs, or the stability of IFN-γ mRNA would be expected to result in different levels of transcript. In response to stimulation with IL-12/18, NK cell IFN-γ transcript increases >300 fold within 6 hours. However, stimulation through the activating NKR NK1.1 did not lead to significant *Ifng* transcription above baseline during the same timeframe despite the emergence of abundant IFN-γ protein.[12] Neither the high levels of IFN-g mRNA in IL-12/18-stimulated NK cells nor the low levels of transcript present in NK cells were altered with OXPHOS inhibition.[12] These findings suggest fundamental differences in the regulation of IFN-γ protein production between cytokine and NKR pathways (i.e., transcriptional control of IL-12/18 stimulation and post-transcriptional control of NK1.1 IFN-γ production), potentially providing the best clue as to the reason for distinct metabolic requirements for NK cell activation.

B. Metabolic Regulation during Translation or Post-Translational Processing

Another way in which metabolism could regulate IFN-γ protein production is through translational regulation. One possible explanation for the different metabolic regulation of NKR versus cytokine stimulation is that a metabolically regulated factor inhibits translation of pre-formed transcript during NKR activation but is overwhelmed by increased transcription during cytokine activation. This could be a direct effect of metabolic enzymes or via actions on other factors such as miRNAs. One candidate metabolic enzyme is GAPDH, which in $CD8^+$ T cells binds to and inhibits translation of IFN-γ transcript unless

recruited for glycolysis.[17] However, unlike NK cells, glycolytic inhibition in T cells affects both receptor- (TCR) and IL-12/18-driven IFN-γ production.[16,17] NK cell IFN-γ production in response to IL-12/18 is preserved with glycolytic inhibition and it is unclear whether GAPDH is important for translational control of IFN-γ in NK cells.

Metabolism could also regulate translation of IFN-γ by increasing the cell's capacity for cytokine production because rapid translation of protein is energetically costly. Activation of mTOR might assist in IFN-γ translation by increasing ribosomal synthesis and translational rates.[98] NK cells increase their mitochondrial mass 7 days after MCMV infection,[99] which could help them to meet the increased demand for ATP. It is not known whether NK cells use aerobic glycolysis to increase ER/Golgi synthesis, as has been described in DCs,[55] but, theoretically, this strategy could decrease ER stress during translation of cytokines and cytotoxic granule proteins. Therefore, metabolic changes could increase cytokine synthesis capacity immediately, as with mTOR activation, or gradually, as with organelle synthesis.

Finally, metabolism could affect protein folding or post-translational modification of IFN-γ and thus its stability and rate of production. Glucose metabolism is required for protein glycosylation, a crucial step in the folding and stability of many proteins, including IFN-γ, which in humans can be glycosylated at two residues, increasing secretion and stability.[100,101]

VII. CONCLUSION

In conclusion, several recent studies have demonstrated for the first time that the two primary metabolic pathways, glycolysis and OXPHOS, are important for mouse and human NK cell IFN-γ production under certain circumstances. The master metabolic regulator mTOR is essential for NK cell maturation and is also necessary for maximal IFN-γ production after IL-15 stimulation. The interactions between metabolic pathways and IFN-γ production are complex and dependent on the specific stimuli used, the length of stimulation, and the activation status of the NK cell. Application of these findings has the potential to inform the development of new protocols for NK cell activation in the context of immunotherapy that take into account metabolic requirements for NK cell activation. For example, NK cells cultured with IL-15 have distinct metabolism compared with naive NK cells and have enhanced function when metabolically stressed. This suggests that treatment of patients with IL-2, which is used clinically for a number of diseases and shares the same signaling pathways as IL-15, might have similar metabolic effects on NK cells. In addition, investigation of the metabolic requirements for NK cell cytotoxicity, a key property for anti-tumor effects of NK cells, warrants further investigation. Continued investigation of the metabolic regulation of NK cells will provide valuable insight into the regulation of NK cell effector functions and the metabolic programs essential for the activation of different immune cell types.

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ABBREVIATIONS

BMDC	bone marrow-derived dendritic cell
DC	dendritic cell
ECAR	extracellular acidification rate
ER	endoplasmic reticulum
FA	fatty acid
FAO	fatty acid oxidation
IFN-γ	interferon-gamma
IL	interleukin
ITAM	immunoreceptor tyrosine-based activation motif
lncRNA	long noncoding RNA
MAPK	mitogen-activated protein kinase
MCMV	murine cytomegalovirus
miRNA	microRNA
mRNA	messenger ribonucleic acid
mTor	mammalian target of rapamycin
NF-κB	nuclear factor kappa light-chain enhancer of activated B cells
NK	natural killer
NKR	natural killer receptor
OCR	oxygen consumption rate
OXPHOS	oxidative phosphorylation
PI3K	phosphoinositide 3-kinase
PLC-γ	phospholipase C-gamma
ROS	reactive oxygen species
STAT	signal transducer and activator of transcription
TCA	tricarboxylic acid
TCR	T-cell receptor

TF	transcription factor
TLR	Toll-like receptor
Treg	regulatory T cell
UTR	untranslated region

References

1. Biron CA, Brossay L. NK cells and NKT cells in innate defense against viral infections. Curr Opin Immunol. 2001; 13:458–64. [PubMed: 11498302]
2. French AR, Yokoyama WM. Natural killer cells and viral infections. Curr Opin Immunol. 2003; 15:45–51. [PubMed: 12495732]
3. Vivier E, Tomasello E, Baratin M, Walzer T, Ugolini S. Functions of natural killer cells. Nat Immunol. 2008; 9:503–10. [PubMed: 18425107]
4. Morvan MG, Lanier LL. NK cells and cancer: you can teach innate cells new tricks. Nat Rev Cancer. 2016; 16:7–19. [PubMed: 26694935]
5. Schoenborn JR, Wilson CB. Regulation of interferon-γ during innate and adaptive immune responses. Adv Immunol. 2007; 96:41–101. [PubMed: 17981204]
6. Farrar MA, Schreiber RD. The molecular cell biology of interferon-gamma and its receptor. Annu Rev Immunol. 1993; 11:571–611. [PubMed: 8476573]
7. Ikeda H, Old LJ, Schreiber RD. The roles of IFNγ in protection against tumor development and cancer immunoediting. Cytokine Growth Factor Rev. 2002; 13:95–109. [PubMed: 11900986]
8. Schroder K, Hertzog PJ, Ravasi T, Hume DA. Interferon-γ: an overview of signals, mechanisms and functions. J Leukoc Biol. 2004; 75:163–89. [PubMed: 14525967]
9. Bustamante J, Boisson-Dupuis S, Abel L, Casanova JL. Mendelian susceptibility to mycobacterial disease: genetic, immunological, and clinical features of inborn errors of IFN-gamma immunity. Semin Immunol. 2014; 26:454–70. [PubMed: 25453225]
10. Lees JR. Interferon gamma in autoimmunity: A complicated player on a complex stage. Cytokine. 2015; 74:18–26. [PubMed: 25464925]
11. Hodge DL, Berthet C, Coppola V, Kastenmuller W, Buschman MD, Schaughency PM, Shirota H, Scarzello AJ, Subleski JJ, Anver MR, Ortaldo JR, Lin F, Reynolds DA, Sanford ME, Kaldis P, Tessarollo L, Klinman DM, Young HA. IFN-gamma AU-rich element removal promotes chronic IFN-gamma expression and autoimmunity in mice. J Autoimmun. 2014; 53:33–45. [PubMed: 24583068]
12. Keppel MP, Saucier N, Mah AY, Vogel TP, Cooper MA. Activation-specific metabolic requirements for NK Cell IFN-gamma production. J Immunol. 2015; 194:1954–62. [PubMed: 25595780]
13. Donnelly RP, Loftus RM, Keating SE, Liou KT, Biron CA, Gardiner CM, Finlay DK. mTORC1-dependent metabolic reprogramming is a prerequisite for NK cell effector function. J Immunol. 2014; 193:4477–84. [PubMed: 25261477]
14. Marcais A, Cherfils-Vicini J, Viant C, Degouve S, Viel S, Fenis A, Rabilloud J, Mayol K, Tavares A, Bienvenu J, Gangloff YG, Gilson E, Vivier E, Walzer T. The metabolic checkpoint kinase mTOR is essential for IL-15 signaling during the development and activation of NK cells. Nat Immunol. 2014; 15:749–57. [PubMed: 24973821]
15. Keating SE, Zaiatz-Bittencourt V, Loftus RM, Keane C, Brennan K, Finlay DK, Gardiner CM. Metabolic reprogramming supports IFN-γ production by CD56bright NK cells. J Immunol. 2016; 196:2552–60. [PubMed: 26873994]
16. Cham CM, Gajewski TF. Glucose availability regulates IFN-gamma production and p70S6 kinase activation in CD8+ effector T cells. J Immunol. 2005; 174:4670–77. [PubMed: 15814691]
17. Chang CH, Curtis JD, Maggi LB Jr, Faubert B, Villarino AV, O'Sullivan D, Huang SC, van der Windt GJ, Blagih J, Qiu J, Weber JD, Pearce EJ, Jones RG, Pearce EL. Posttranscriptional control of T cell effector function by aerobic glycolysis. Cell. 2013; 153:1239–51. [PubMed: 23746840]

18. Savan R, Ravichandran S, Collins JR, Sakai M, Young HA. Structural conservation of interferon gamma among vertebrates. Cytokine Growth Factor Rev. 2009; 20:115–24. [PubMed: 19268624]
19. Luetke-Eversloh M, Cicek BB, Siracusa F, Thom JT, Hamann A, Frischbutter S, Baumgrass R, Chang HD, Thiel A, Dong J, Romagnani C. NK cells gain higher IFN-γ competence during terminal differentiation. Eur J Immunol. 2014; 44:2074–84. [PubMed: 24752800]
20. Lieberman LA, Hunter CA. Regulatory pathways involved in the infection-induced production of IFN-γ by NK cells. Microbes Infect. 2002; 4:1531–38. [PubMed: 12505525]
21. Balasubramani A, Mukasa R, Hatton RD, Weaver CT. Regulation of the Ifng locus in the context of T-lineage specification and plasticity. Immunol Rev. 2010; 238:216–32. [PubMed: 20969595]
22. Chang S, Aune TM. Histone hyperacetylated domains across the Ifng gene region in natural killer cells and T cells. Proc Natl Acad Sci U S A. 2005; 102:17095–100. [PubMed: 16286661]
23. Stetson DB, Mohrs M, Reinhardt RL, Baron JL, Wang ZE, Gapin L, Kronenberg M, Locksley RM. Constitutive cytokine mRNAs mark natural killer (NK) and NK T cells poised for rapid effector function. J Exp Med. 2003; 198:1069–76. [PubMed: 14530376]
24. Tato CM, Martins GA, High FA, DiCioccio CB, Reiner SL, Hunter CA. Cutting edge: innate production of IFN-γ by NK cells is independent of epigenetic modification of the IFN-γ promoter. J Immunol. 2004; 173:1514–17. [PubMed: 15265878]
25. Fehniger TA, Cai SF, Cao X, Bredemeyer AJ, Presti RM, French AR, Ley TJ. Acquisition of murine NK cell cytotoxicity requires the translation of a pre-existing pool of granzyme B and perforin mRNAs. Immunity. 2007; 26:798–811. [PubMed: 17540585]
26. Luetke-Eversloh M, Hammer Q, Durek P, Nordstrom K, Gasparoni G, Pink M, Hamann A, Walter J, Chang HD, Dong J, Romagnani C. Human cytomegalovirus drives epigenetic imprinting of the IFNG locus in NKG2Chi natural killer cells. PLoS Pathog. 2014; 10:e1004441. [PubMed: 25329659]
27. Ni, Jing, Hölsken, Oliver, Miller, Matthias, Hammer, Quirin, Luetke-Eversloh, Merlin, Romagnani, Chiara, Cerwenka, Adelheid. Adoptively transferred natural killer cells maintain long-term antitumor activity by epigenetic imprinting and CD4+ T cell help. OncoImmunology. 2016:e1219009. [PubMed: 27757318]
28. Marcais A, Viel S, Grau M, Henry T, Marvel J, Walzer T. Regulation of mouse NK cell development and function by cytokines. Front Immunol. 2013; 4:450. [PubMed: 24376448]
29. Vivier E, Nunès JA, Vély F. Natural killer cell signaling pathways. Science. 2004; 306:1517–9. [PubMed: 15567854]
30. Chiesa S, Tomasello E, Vivier E, Vely F. Coordination of activating and inhibitory signals in natural killer cells. Mol Immunol. 2005; 42:477–84. [PubMed: 15607802]
31. Lanier LL. Up on the tightrope: natural killer cell activation and inhibition. Nat Immunol. 2008; 9:495–502. [PubMed: 18425106]
32. Long EO, Kim HS, Liu D, Peterson ME, Rajagopalan S. Controlling NK cell responses: integration of signals for activation and inhibition. Annu Rev Immunol. 2013; 31:227–58. [PubMed: 23516982]
33. Murphy KM, Ouyang W, Farrar JD, Yang J, Ranganath S, Asnagli H, Afkarian M, Murphy TL. Signaling and transcription in T helper development. Annu Rev Immunol. 2000; 18:451–94. [PubMed: 10837066]
34. Balasubramani A, Shibata Y, Crawford GE, Baldwin AS, Hatton RD, Weaver CT. Modular utilization of distal cis-regulatory elements controls Ifng gene expression in T cells activated by distinct stimuli. Immunity. 2010; 33:35–47. [PubMed: 20643337]
35. Baumjohann D, Ansel KM. MicroRNA-mediated regulation of T helper cell differentiation and plasticity. Nat Rev Immunol. 2013; 13:666–78. [PubMed: 23907446]
36. Leong JW, Sullivan RP, Fehniger TA. microRNA management of NK cell developmental and functional programs. Eur J Immunol. 2014; 44:2862–8. [PubMed: 25142111]
37. Ma F, Xu S, Liu X, Zhang Q, Xu X, Liu M, Hua M, Li N, Yao H, Cao X. The microRNA miR-29 controls innate and adaptive immune responses to intracellular bacterial infection by targeting interferon-γ. Nat Immunol. 2011; 12:861–9. [PubMed: 21785411]

38. Yang L, Boldin MP, Yu Y, Liu CS, Ea CK, Ramakrishnan P, Taganov KD, Zhao JL, Baltimore D. miR-146a controls the resolution of T cell responses in mice. J Exp Med. 2012; 209:1655–70. [PubMed: 22891274]
39. Sullivan RP, Fogel LA, Leong JW, Schneider SE, Wong R, Romee R, Thai TH, Sexl V, Matkovich SJ, Dom GW 2nd, French AR, Fehniger TA. MicroRNA-155 tunes both the threshold and extent of NK cell activation via targeting of multiple signaling pathways. J Immunol. 2013; 191:5904–13. [PubMed: 24227772]
40. Zawislak CL, Beaulieu AM, Loeb GB, Karo J, Canner D, Bezman NA, Lanier LL, Rudensky AY, Sun JC. Stage-specific regulation of natural killer cell homeostasis and response against viral infection by microRNA-155. Proc Natl Acad Sci U S A. 2013; 110:6967–72. [PubMed: 23572582]
41. Trotta R, Chen L, Ciarlariello D, Josyula S, Mao C, Costinean S, Yu L, Butchar JP, Tridandapani S, Croce CM, Caligiuri MA. miR-155 regulates IFN-γ production in natural killer cells. Blood. 2012; 119:3478–85. [PubMed: 22378844]
42. Bezman NA, Cedars E, Steiner DF, Blelloch R, Hesslein DG, Lanier LL. Distinct requirements of microRNAs in NK cell activation, survival, and function. J Immunol. 2010; 185:3835–46. [PubMed: 20805417]
43. Sullivan RP, Leong JW, Schneider SE, Keppel CR, Germino E, French AR, Fehniger TA. MicroRNA-deficient NK cells exhibit decreased survival but enhanced function. J Immunol. 2012; 188:3019–30. [PubMed: 22379033]
44. Collier SP, Collins PL, Williams CL, Boothby MR, Aune TM. Influence of Tmevpg1, a long intergenic noncoding RNA, on the expression of Ifng by Th1 cells. J Immunol. 2012; 189:2084–8. [PubMed: 22851706]
45. Gomez JA, Wapinski OL, Yang YW, Bureau JF, Gopinath S, Monack DM, Chang HY, Brahic M, Kirkegaard K. The NeST long ncRNA controls microbial susceptibility and epigenetic activation of the interferon-γ locus. Cell. 2013; 152:743–54. [PubMed: 23415224]
46. Ben-Asouli Y, Banai Y, Pel-Or Y, Shir A, Kaempfer R. Human interferon-γ mRNA autoregulates its translation through a pseudoknot that activates the interferon-inducible protein kinase PKR. Cell. 2002; 108:221–32. [PubMed: 11832212]
47. Cohen-Chalamish S, Hasson A, Weinberg D, Namer LS, Banai Y, Osman F, Kaempfer R. Dynamic refolding of IFN-[gamma] mRNA enables it to function as PKR activator and translation template. Nat Chem Biol. 2009; 5:896–903. [PubMed: 19801993]
48. Nagy E, Buhlmann JE, Henics T, Waugh M, Rigby WFC. Selective modulation of IFN-γ mRNA stability by IL-12/NKSF. Cell Immunol. 1994; 159:140–51. [PubMed: 7994750]
49. Mavropoulos A, Sully G, Cope AP, Clark AR. Stabilization of IFN-γ mRNA by MAPK p38 in IL-12- and IL-18-stimulated human NK cells. Blood. 2005; 105:282–8. [PubMed: 15345584]
50. Ogilvie RL, SternJohn JR, Rattenbacher B, Vlasova IA, Williams DA, Hau HH, Blackshear PJ, Bohjanen PR. Tristetraprolin mediates interferon-γ mRNA decay. J Biol Chem. 2009; 284:11216–23. [PubMed: 19258311]
51. Ye J, Ortaldo JR, Conlon K, Winkler-Pickett R, Young HA. Cellular and molecular mechanisms of IFN-gamma production induced by IL-2 and IL-12 in a human NK cell line. J Leukoc Biol. 1995; 58:225–33. [PubMed: 7643015]
52. Hodge DL, Martinez A, Julias JG, Taylor LS, Young HA. Regulation of nuclear gamma interferon gene expression by interleukin 12 (IL-12) and IL-2 represents a novel form of posttranscriptional control. Mol Cell Biol. 2002; 22:1742–53. [PubMed: 11865054]
53. Buck MD, O'Sullivan D, Pearce EL. T cell metabolism drives immunity. J Exp Med. 2015; 212:1345–60. [PubMed: 26261266]
54. Galván-Peña S, O'Neill LA. Metabolic reprogramming in macrophage polarization. Front Immunol. 2014; 5:420. [PubMed: 25228902]
55. Pearce EJ, Everts B. Dendritic cell metabolism. Nat Rev Immunol. 2015; 15:18–29. [PubMed: 25534620]
56. O'Neill LA, Pearce EJ. Immunometabolism governs dendritic cell and macrophage function. J Exp Med. 2016; 213:15–23. [PubMed: 26694970]

57. Berg, J., Tymoczko, J., Stryer, L. Biochemistry: Glycolysis and gluconeogenesis [monograph on the Internet]. New York: W H Freeman; 2002. [cited 2016 Jun 2]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK21154/
58. Warburg O. On the Origin of Cancer Cells. Science. 1956; 123:309–14. [PubMed: 13298683]
59. Warburg O, Gawehn K, Geissler AW. Metabolism of leukocytes. Zeitschrift fur Naturforschung Teil B: Chemie, Biochemie, Biophysik, Biologie. 1958; 13b:515–6.
60. Berg, J., Tymoczko, J., Stryer, L. Biochemistry: The Calvin cycle and the pentose phosphate pathway [monograph on the Internet]. New York: W H Freeman; 2002. [cited 2016 Jun 2]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK21154/
61. Wellen KE, Thompson CB. A two-way street: reciprocal regulation of metabolism and signalling. Nat Rev Mol Cell Biol. 2012; 13:270–6. [PubMed: 22395772]
62. Berg, J., Tymoczko, J., Stryer, L. Biochemistry: The citric acid cycle [monograph on the Internet]. New York: W H Freeman; 2002. [cited 2016 Jun 2]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK21154/
63. Berg, J., Tymoczko, J., Stryer, L. Biochemistry: Oxidative phosphorylation [monograph on the Internet]. New York: W H Freeman; 2002. [cited 2016 Jun 2]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK21154/
64. Sena LA, Li S, Jairaman A, Prakriya M, Ezponda T, Hildeman DA, Wang CR, Schumacker PT, Licht JD, Perlman H, Bryce PJ, Chandel NS. Mitochondria are required for antigen-specific T cell activation through reactive oxygen species signaling. Immunity. 2013; 38:225–36. [PubMed: 23415911]
65. Belikov AV, Schraven B, Simeoni L. T cells and reactive oxygen species. J Biomed Sci. 2015; 22:85. [PubMed: 26471060]
66. Simeoni L, Bogeski I. Redox regulation of T-cell receptor signaling. Biol Chem. 2015; 396:555–68. [PubMed: 25781677]
67. Frauwirth KA, Thompson CB. Regulation of T lymphocyte metabolism. J Immunol. 2004; 172:4661–5. [PubMed: 15067038]
68. Culvenor JG, Weidemann MJ. Phytohaemagglutinin stimulation of rat thymus lymphocyte glycolysis. Biochim Biophys Acta. 1976; 437:354–63. [PubMed: 1085166]
69. Roos D, Loos JA. Changes in the carbohydrate metabolism of mitogenically stimulated human peripheral lymphocytes. Biochim Biophys Acta. 1970; 222:565–82. [PubMed: 5496487]
70. Frauwirth KA, Riley JL, Harris MH, Parry RV, Rathmell JC, Plas DR, Elstrom RL, June CH, Thompson CB. The CD28 signaling pathway regulates glucose metabolism. Immunity. 2002; 16:769–77. [PubMed: 12121659]
71. Pearce EL, Poffenberger MC, Chang CH, Jones RG. Fueling immunity: insights into metabolism and lymphocyte function. Science. 2013; 342:1242454. [PubMed: 24115444]
72. Lunt SY, Vander Heiden MG. Aerobic glycolysis: meeting the metabolic requirements of cell proliferation. Annu Rev Cell Dev Biol. 2011; 27:441–64. [PubMed: 21985671]
73. Vander Heiden MG, Cantley LC, Thompson CB. Understanding the Warburg effect: the metabolic requirements of cell proliferation. Science. 2009; 324:1029–33. [PubMed: 19460998]
74. Infantino V, Convertini P, Cucci L, Panaro Maria A, Di Noia Maria A, Calvello R, Palmieri F, Iacobazzi V. The mitochondrial citrate carrier: a new player in inflammation. Biochem J. 2011; 438:433–6. [PubMed: 21787310]
75. Galván-Peña S, O'Neill LAJ. Metabolic reprograming in macrophage polarization. Front Immunol. 2014; 5:420. [PubMed: 25228902]
76. Tannahill GM, Curtis AM, Adamik J, Palsson-McDermott EM, McGettrick AF, Goel G, Frezza C, Bernard NJ, Kelly B, Foley NH, Zheng L, Gardet A, Tong Z, Jany SS, Corr SC, Haneklaus M, Caffrey BE, Pierce K, Walmsley S, Beasley FC, Cummins E, Nizet V, Whyte M, Taylor CT, Lin H, Masters SL, Gottlieb E, Kelly VP, Clish C, Auron PE, Xavier RJ, O'Neill LA. Succinate is an inflammatory signal that induces IL-1beta through HIF-1alpha. Nature. 2013; 496:238–42. [PubMed: 23535595]
77. Moon JS, Hisata S, Park MA, DeNicola GM, Ryter SW, Nakahira K, Choi AM. mTORC1-induced HK1-dependent glycolysis regulates NLRP3 inflammasome activation. Cell Rep. 2015; 12:102–15. [PubMed: 26119735]

78. Krawczyk CM, Holowka T, Sun J, Blagih J, Amiel E, DeBerardinis RJ, Cross JR, Jung E, Thompson CB, Jones RG, Pearce EJ. Toll-like receptor-induced changes in glycolytic metabolism regulate dendritic cell activation. Blood. 2010; 115:4742–9. [PubMed: 20351312]

79. Jantsch J, Chakravortty D, Turza N, Prechtel AT, Buchholz B, Gerlach RG, Volke M, Glasner J, Warnecke C, Wiesener MS, Eckardt KU, Steinkasserer A, Hensel M, William C. Hypoxia and hypoxia-inducible factor-1 alpha modulate lipopolysaccharide-induced dendritic cell activation and function. J Immunol. 2008; 180:4697–705. [PubMed: 18354193]

80. Everts B, Amiel E, Huang SC, Smith AM, Chang CH, Lam WY, Redmann V, Freitas TC, Blagih J, van der Windt GJ, Artyomov MN, Jones RG, Pearce EL, Pearce EJ. TLR-driven early glycolytic reprogramming via the kinases TBK1-IKKε supports the anabolic demands of dendritic cell activation. Nat Immunol. 2014; 15:323–32. [PubMed: 24562310]

81. Everts B, Amiel E, van der Windt GJ, Freitas TC, Chott R, Yarasheski KE, Pearce EL, Pearce EJ. Commitment to glycolysis sustains survival of NO-producing inflammatory dendritic cells. Blood. 2012; 120:1422–31. [PubMed: 22786879]

82. van der Windt GJ, Everts B, Chang CH, Curtis JD, Freitas TC, Amiel E, Pearce EJ, Pearce EL. Mitochondrial respiratory capacity is a critical regulator of CD8+ T cell memory development. Immunity. 2012; 36:68–78. [PubMed: 22206904]

83. van der Windt GJW, O'Sullivan D, Everts B, Huang SC, Buck MD, Curtis JD, Chang CH, Smith AM, Ai T, Faubert B, Jones RG, Pearce EJ, Pearce EL. CD8 memory T cells have a bioenergetic advantage that underlies their rapid recall ability. Proc Natl Acad Sci U S A. 2013; 110:14336–41. [PubMed: 23940348]

84. Dimeloe S, Mehling M, Frick C, Loeliger J, Bantug GR, Sauder U, Fischer M, Elle R, Develioglu L, Tay S, Langenkamp A, Hess C. The immune-metabolic basis of effector memory CD4+ T cell function under hypoxic conditions. J Immunol. 2016; 196:106–14. [PubMed: 26621861]

85. Pearce EL, Walsh MC, Cejas PJ, Harms GM, Shen H, Wang LS, Jones RG, Choi Y. Enhancing CD8 T-cell memory by modulating fatty acid metabolism. Nature. 2009; 460:103–7. [PubMed: 19494812]

86. Okoye I, Wang L, Pallmer K, Richter K, Ichimura T, Haas R, Crouse J, Choi O, Heathcote D, Lovo E, Mauro C, Abdi R, Oxenius A, Rutschmann S, Ashton-Rickardt PG. The protein LEM promotes CD8+ T cell immunity through effects on mitochondrial respiration. Science. 2015; 348:995–1001. [PubMed: 25883318]

87. Keppel MP, Cooper MA. Assessment of NK cell metabolism. Methods Mol Biol. 2016; 1441:27–42. [PubMed: 27177654]

88. Carson WE, Giri JG, Lindemann MJ, Linett ML, Ahdieh M, Paxton R, Anderson D, Eisenmann J, Grabstein K, Caligiuri MA. Interleukin (IL) 15 is a novel cytokine that activates human natural killer cells via components of the IL-2 receptor. J Exp Med. 1994; 180:1395–403. [PubMed: 7523571]

89. Becknell B, Caligiuri MA. Interleukin-2, Interleukin-15, and their roles in human natural killer cells. Adv Immunol. 2005; 86:209–39. [PubMed: 15705423]

90. Koka R, Burkett P, Chien M, Chai S, Boone DL, Ma A. Cutting edge: murine dendritic cells require IL-15Rα to prime NK cells. J Immunol. 2004; 173:3594–8. [PubMed: 15356102]

91. Dokun AO, Kim S, Smith HR, Kang HS, Chu DT, Yokoyama WM. Specific and nonspecific NK cell activation during virus infection. Nat Immunol. 2001; 2:951–6. [PubMed: 11550009]

92. Nandagopal N, Ali AK, Komal AK, Lee SH. The critical role of IL-15-PI3K-mTOR pathway in natural killer cell effector functions. Front Immunol. 2014; 5:187. [PubMed: 24795729]

93. Chi H. Regulation and function of mTOR signalling in T cell fate decisions. Nat Rev Immunol. 2012; 12:325–38. [PubMed: 22517423]

94. Yang K, Chi H. mTOR and metabolic pathways in T cell quiescence and functional activation. Semin Immunol. 2012; 24:421–8. [PubMed: 23375549]

95. Weichhart T, Hengstschlager M, Linke M. Regulation of innate immune cell function by mTOR. Nat Rev Immunol. 2015; 15:599–614. [PubMed: 26403194]

96. Freud AG, Caligiuri MA. Human natural killer cell development. Immunol Rev. 2006; 214:56–72. [PubMed: 17100876]

97. De Rosa V, Galgani M, Porcellini A, Colamatteo A, Santopaolo M, Zuchegna C, Romano A, De Simone S, Procaccini C, La Rocca C, Carrieri PB, Maniscalco GT, Salvetti M, Buscarinu MC, Pranzese A, Mozzilo E, La Cava A, Matarese G. Glycolysis controls the induction of human regulatory T cells by modulating the expression of FOXP3 exon 2 splicing variants. Nat Immunol. 2015; 16:1174–84. [PubMed: 26414764]

98. Wang X, Proud CG. The mTOR pathway in the control of protein synthesis. Physiology. 2006; 21:362–9. [PubMed: 16990457]

99. O'Sullivan TE, Johnson LR, Kang HH, Sun JC. BNIP3- and BNIP3L-mediated mitophagy promotes the generation of natural killer cell memory. Immunity. 2015; 43:331–42. [PubMed: 26253785]

100. Sareneva T, Pirhonen J, Cantell K, Kalkkinen N, Julkunen I. Role of N-glycosylation in the synthesis, dimerization and secretion of human interferon-gamma. Biochem J. 1994; 303:831–40. [PubMed: 7980452]

101. Sareneva T, Pirhonen J, Cantell K, Julkunen I. N-glycosylation of human interferon-gamma: glycans at Asn-25 are critical for protease resistance. Biochem J. 1995; 308:9–14. [PubMed: 7755594]

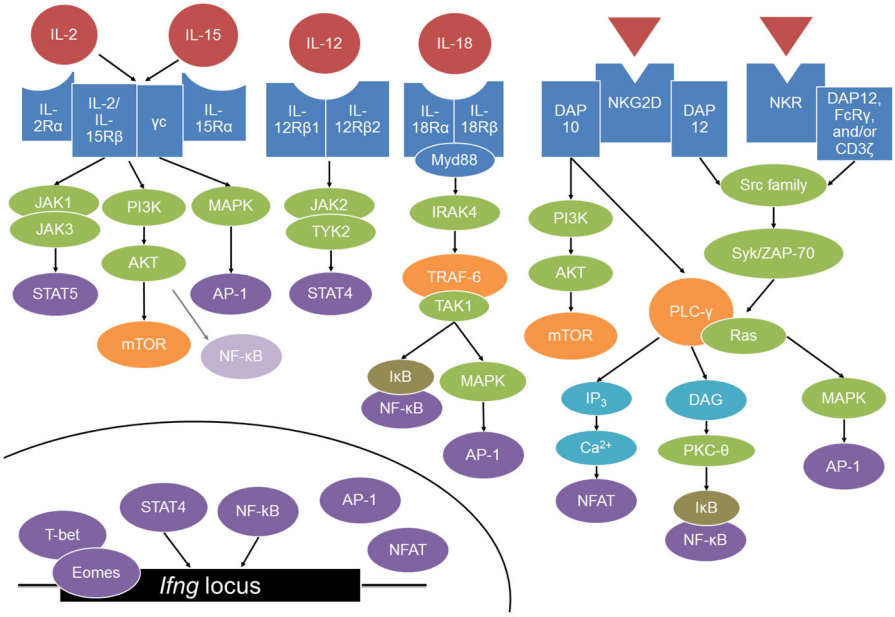

FIG. 1.

Signaling pathways leading to IFN-γ transcription in NK cells. NK cells up-regulate the transcription of *Ifng* in response to several signaling pathways, most of which converge on the TFs STAT4 and NF-κB to cause acute transcription. In NK cells, the *Ifng* locus is bound by constitutively active T-bet and Eomes. Shown here are the primary signaling pathways downstream of cytokines and receptors leading to IFN-γ transcription. IL-12-induced STAT4 is essential for optimal cytokine co-stimulation of IFN-γ. IL-2 and IL-15 share common signaling receptors and downstream Janus kinase (JAK)/STAT, PI3K, and MAPK signaling. There is evidence that NF-κB and STAT4 can also be activated downstream of IL-2 in NK cells, although this signaling is poorly described (lightened). Activation receptors can trigger IFN-γ production independently of cytokine signaling and associate with ITAM-containing adapters, leading to multiple downstream signaling cascades including PI3K, MAPK, and PLC-γ, which cause cytokine production and cytotoxicity. Red indicates ligand; blue, receptor; green, kinase; purple, transcription factor; and teal, second messenger.

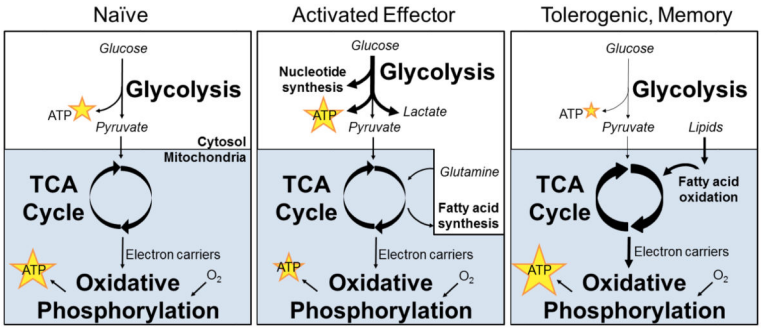

FIG. 2.

Major metabolic pathways and programs in immune cells. Left: Naive immune cells use glucose to fuel glycolysis, producing ATP and pyruvate. Pyruvate is converted into acetyl-CoA, enters into the mitochondria, and is processed by the TCA cycle into high-energy electrons. Carriers bring these electrons to the electron transport chain for OXPHOS, which requires oxygen to synthesize large amounts of ATP. Center: In activated effector T cells, DCs, and M1 macrophages, glycolysis is highly up-regulated, leading to the production of lactate. Metabolites are shunted toward nucleotide synthesis in T cells, ROS/nitric oxide in macrophages, and fatty acid synthesis in DCs. Depleted TCA intermediates can be replaced by glutamine. OXPHOS functions at a low level in activated effector cells. Right: Alternatively activated M2 macrophages, Tregs, and memory T cells down-regulate glycolysis, instead oxidizing lipids to fuel the TCA cycle. Increased mitochondrial mass leads to greater respiratory capacity and increased OXPHOS.

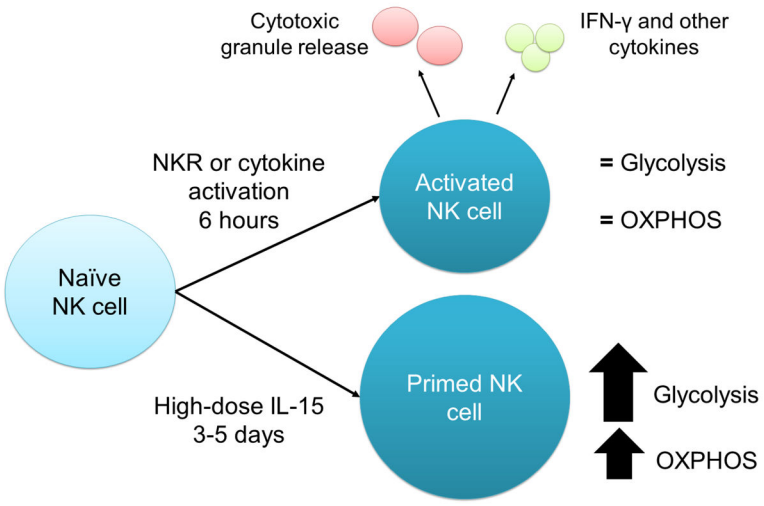

FIG. 3.

Increased rates of metabolism with prolonged IL-15 exposure, but not short-term activation. Short-term activation (6 hours) by NKRs or cytokines does not change the rates of glycolysis or OXPHOS despite the production of IFN-γ and degranulation. Culture with IL-15 for 3+ days increases both glycolysis and OXPHOS, with a greater enhancement of glycolysis but, by itself, does not lead to significant IFN-γ production. These IL-15-"primed" NK cells also have different metabolic requirements for IFN-γ production compared with naive NK cells.

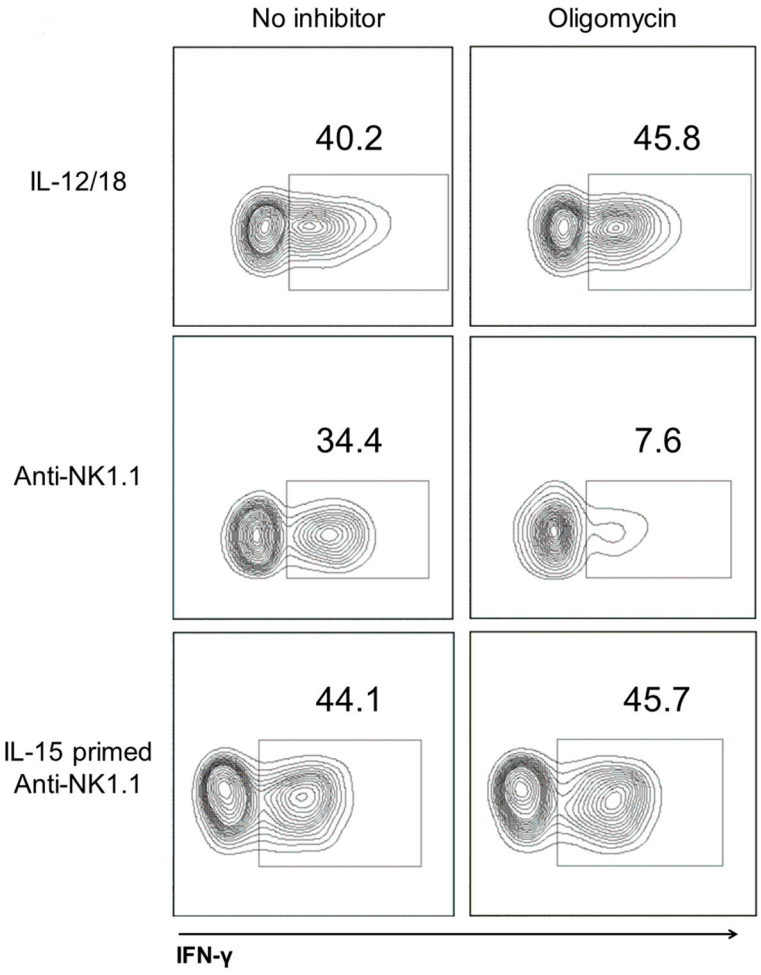

FIG. 4.

Naive NK cells require OXPHOS for receptor-stimulated IFN-γ production, but activation with IL-15 eliminates this requirement. Fresh murine splenic NK cells (top and middle) or IL-15-activated "primed" NK cells (bottom, 72-hour stimulation with 100 ng/mL IL-15) were activated with IL-12/18 (top) or plate-bound anti-NK1.1 (center and bottom) in the presence or absence of the OXPHOS inhibitor oligomycin (100 nM). NK cell production of IFN-γ protein was measured after 6 hours.

TABLE 1

Metabolic Regulation of NK Cell IFN-γ Production

	Stimulus	Glycolytic Inhibition	OXPHOS Inhibition	mTOR Inhibition
Primary Murine NK Cells	Cytokines	+++	+ (IL-12/15) +++(IL-12/18)	n/a
	NKR	+	X	n/a
	PMA/Ca	++	+	n/a
IL-15-Primed Murine NK Cells	Cytokines	+ to ++	+++	X to ++
	NKR	++	+++	+
	PMA/Ca	n/a	+++	n/a
In Vivo Activated Murine NK Cells	Poly(I:C)	++	n/a	++
	MCMV	n/a	n/a	++
Human NK Cells	IL-2	+++*	+++	+ to ++
	IL-12/15	++*	X to +	+++

For each stimulus, the effect of inhibition of glycolysis, OXPHOS, or mTOR on the percentage of NK cells producing IFN-γ is shown. +++, similar or <20% change with inhibitor; ++, 20–50% decrease with inhibitor; +, 50–80% decrease with inhibitor; X, >80% decrease with inhibitor; n/a, not available/not tested.[12,13,15,85]

*Culture of human NK cells in the presence of galactose, which stimulates OXPHOS preferentially compared with glycolysis, caused slightly decreased IFN-γ production in NK cells in response to IL-12/15; direct glycolytic inhibition has not been tested.