Molecular Immunology 115 (2019) 3–11

Contents lists available at ScienceDirect

Molecular Immunology

journal homepage: www.elsevier.com/locate/molimm

Review

Natural killer cell metabolism

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ARTICLE INFO

Keywords:
Natural killer cells
Cellular metabolism
Metabolic regulators
Effector function
Nutrients
Hormones
Metabolites
Cancer
Obesity

ABSTRACT

Natural killer (NK) cells are a critical component in the innate immune response against disease. NK cell function is tightly regulated by specific cytokine and activation/inhibitory receptor signalling, leading to diverse effector responses. Like all living cells, energy metabolism is a fundamental requirement for NK cell activation and survival. There is growing evidence that distinct functional profiles of NK cells are determined by alterations to cellular metabolic pathways. In this review, we summarise current literature that has explored NK cell metabolism to provide insight into how metabolic regulation controls NK cell function. We focus on metabolism pathways induced by different NK cell stimuli, metabolic regulatory proteins, and nutrient and hormonal levels in health and disease which impact on NK cell metabolic and functional activity.

1. Introduction

Natural killer cells are innate lymphocytes that play a key role in controlling malignancy and infection. They primarily develop in bone marrow from haematopoietic stem cells (HSC) and mature NK cells are found in a number of peripheral sites including lymphoid organs (spleen, lymph nodes, blood) and non-lymphoid tissues (liver, lungs, adipose). NK cell activity is increased when infected or malignant cells upregulate stress-induced activating ligands or alternatively downregulate MHC class I molecules. Upon activation, NK cells rapidly produce cytotoxic molecules, and secrete pro-inflammatory cytokines and chemokines to kill targets or modulate immune responses. Although their significance in protection against various diseases is well appreciated, the mechanisms controlling their function in these settings is still to be fully elucidated.

The importance of energy metabolism in controlling immune cell function has been one of the major findings in immunology over the past few years, and we now appreciate that immune cells undergo distinct metabolic shifts to support their activity. The interplay between cellular metabolism and functional properties of immune cells is complex, particularly in highly variable disease environments where energy supply and demands are in constant change. In this review, we will summarise currently available evidence for different metabolic processes that are shown to be implicated in NK cell function – including metabolic pathways, molecular metabolic regulators, and external nutrient and hormone levels. Finally, we will raise outstanding questions in NK cell metabolism and how answering these will advance understanding of innate immunity.

2. Metabolic activation of NK cells in response to different stimuli

NK cells resemble cytotoxic CD8 T lymphocytes in their ability to exert direct cytotoxic responses and produce pro-inflammatory cytokines. CD8 T cell metabolism has been leading the field of immunometabolism research, and therefore the metabolic processes required for specific function of CD8 T cells are well characterised. CD8 T cells experience dramatic metabolic shifts in different phases of immune responses associated with differentiation into effector and memory T cells (reviewed in Buck et al. (2015), Pearce (2010)). Naive T cells have a lower metabolic rate and produce energy through the TCA cycle and electron transport chain (ETC), and oxidative phosphorylation (OXPHOS) in mitochondria. Upon encounter to cognate antigens, T cells rapidly increase the rate of glycolysis and lactate fermentation even under ample oxygen (O_2) levels to fuel OXPHOS. Once antigens are cleared, CD8 T cells undergo contraction, where effector cells are removed by apoptosis. CD8 T cells that survive this process increase mitochondrial mass and restructure mitochondrial cristae into a denser structure, which in combination allows more efficient fatty acid β-oxidation (FAO) to fuel OXPHOS supporting prolonged survival and memory T cell differentiation (van der Windt et al., 2013).

NK cells experience metabolic shifts upon activation in a stimulation-specific fashion (Fig. 1). Unlike T cells, which primarily receive signals through T cell receptors (TCR), NK cells receive a wide array of signals through NK cell activating and inhibitory receptors, stress-

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https://doi.org/10.1016/j.molimm.2017.11.021
Received 1 September 2017; Received in revised form 16 November 2017; Accepted 20 November 2017
Available online 24 November 2017

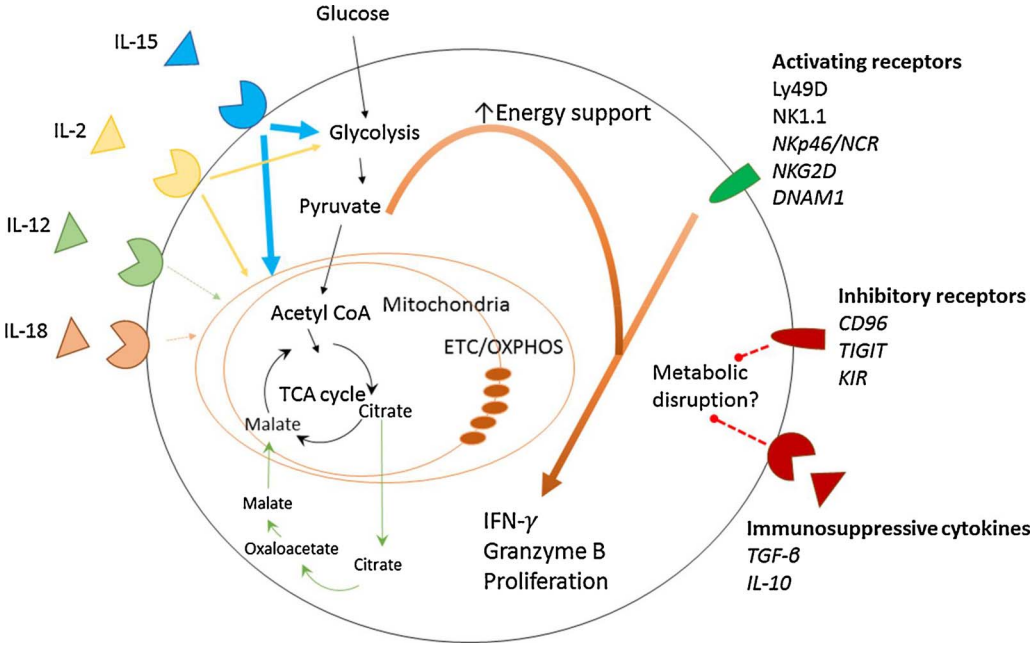

Fig. 1. Cytokine stimulation drives metabolic activation, supporting increased energetic demands. Cytokines, in particular IL-15 and IL-2, increase glycolysis and oxidative phosphorylation (OXPHOS) in NK cells that support increased demands for growth and synthesis of biomass, including effector molecules. Activation receptors (e.g. NK1.1) do not trigger metabolism alone but appear to be dependent on energy generated by cytokine signalling. Activating receptors and inhibitory receptors whose effects on cell metabolism have not yet been studied are italicized in the diagram.

induced ligand receptors, and cytokine receptors (Chan et al., 2014a; Guillerey et al., 2016; Vivier et al., 2008; Marcais et al., 2013). Rapid production of IFN-γ in response to cytokine stimuli is one of the key effector functions of NK cells. The requirement for energy metabolism in this early activation phase (up to 6 h) is dependent on the stimulus. For example, IFN-γ production induced by IL-12 and IL-15 combination, or activating receptor stimulation (NK1.1 and Ly49D) requires functional OXPHOS (Keppel et al., 2015). In contrast, activation with IL-18 is independent of changes to metabolism (Keppel et al., 2015). IL-15 is an essential survival and growth factor for NK cells, and its effect on NK cell metabolism is dose- and time-dependent. IL-15 at a low dose (10 ng/mL) for short-term stimulation supports OXPHOS to activate IFN-γ production, whilst a long-term stimulation at a higher dose increases both glycolysis and OXPHOS, with more bias towards glycolysis (Keppel et al., 2015; Marcais et al., 2014). IL-2 is another common gamma chain cytokine that shares functional similarity with IL-15 in NK cell activation. Similar to IL-15, IL-2 stimulation of NK cells increases the rate of both glycolysis and OXPHOS. This is further increased when IL-2 is provided together with IL-12. IL-12 potently stimulates IFN-γ production in NK cells. Current available evidence suggests that IL-12 does not alter rates of glycolysis or OXPHOS, indicating IL-12 receptor signalling is independent of metabolic regulation (Donnelly et al., 2014). In humans, both OXPHOS and glycolysis were required for IL-12 and IL-15 combination to induce optimal production of IFN-γ, whilst granzyme B production or degranulation were metabolism-independent (Keating et al., 2016). However, glycolysis was necessary and sufficient to support activation and IFN-γ production to multiple activation signals (IL-12 + IL-18, phorbol 1,2-myristate 13-acetate (PMA) and calcimycin (Ca), α-NK1.1 and α-Ly49D mAbs) in the presence of high levels of IL-15.

3. Metabolic regulators and NK cell function

A number of enzymes or proteins that regulate cell metabolism support fundamental biological processes. In immune cells, the activities of such regulators are associated with specific immune responses that require different metabolic demands in different circumstances. Here we will highlight a few that have been implicated in NK cell metabolism.

3.1. mTOR

Mechanistic target of rapamycin (mTOR) is a ubiquitous serine/threonine kinase that initiates protein translation by phosphorylation of ribosomal protein S6 kinase (RS6 K) and eukaryotic initiation factor 4E binding protein 1 (4E-BP1), leading to activation and inhibition of respective targets (Sengupta et al., 2010). Active mTOR consists of multiple proteins, and two regulatory proteins raptor and rictor are required to form functionally distinct mTOR complexes 1 and 2 (mTORC1 and 2), respectively. mTOR activity is also regulated by external amino acid levels (Goberdhan et al., 2016) or growth factors (Efeyan and Sabatini, 2013), and they have been shown to regulate function of T cells (Pollizzi et al., 2016; Chi, 2012; Pollizzi et al., 2015).

In NK cells, increased mTOR activity is required for cytokine signalling, to synthesize effector molecules or to support growth of cells. mTOR participates in IL-15-dependent metabolic activation of NK cells. IL-15 instructs NK cell development, from NK precursors (NKP) through immature NK (iNK) to mature NK (mNK) cells (Marcais et al., 2013; Nandagopal et al., 2014; Geiger and Sun, 2016). IL-15 stimulates mTORC1 through JAK1 and 3/PI3 K/AKT signalling axis, and the activity of the signalling proteins increases from NKP to iNK cells (Nandagopal et al., 2014; Wang et al., 2016). In mice, mNK cells are further classified into different maturation stages based on CD11b and CD27 expression; CD11b being expressed higher in the most mature (CD11b + CD27-: 'CD11b + '), CD27 being expressed in least mature (CD11b-CD27 + : 'CD27 + '), and both being expressed in intermediate (CD11b + CD27 + : 'DP') NK cell subsets (Chiossone et al., 2009). Phosphorylation status of mTOR and associated signalling molecules are consistently higher in CD27 + NK cells than DP or CD11b + NK cells, indicating NK cells progressively lose activity of mTOR as they differentiate into more mature NK cells (Marcais et al., 2014). Decreased mTOR activity of mature NK cell subsets is associated with reduced basal metabolism, as expression of nutrient transporters CD71 (transferrin receptor) and CD98, (L-amino acid transporter) and glucose uptake capacity decrease (Marcais et al., 2014). The association of higher mTOR activity with more immature CD27 + NK cells is consistent with better survival and proliferative potential of this subset (Chiossone et al., 2009). Indeed, genetic deletion of mTOR leads to defective differentiation of CD27 + NK cells into more mature subsets, suggesting that CD27 + NK cells require functional mTOR signalling for maturation into DP and CD11b + NK cells (Marcais et al., 2014).

mTOR activation is also required to support functional activation of mature NK cells in certain settings. Cytokine-dependent activation of NK cells mediated by IL-2, IL-15, IL-2 and IL-12, or IL-12 and IL-18 combinations, enhances activation of ribosomal protein S6, suggesting these signals increase mTOR activity (Marcais et al., 2014; Donnelly et al., 2014). Inhibition of mTORC1 by rapamycin specifically reduced IL-2 and IL-12 stimulation-dependent upregulation of glycolysis, leading to reduced cell growth and impairment of granzyme B and IFN-γ production (Donnelly et al., 2014). Rapamycin also reduced IL-15- or poly I:C-dependent granzyme B production, suggesting these stimuli initiate protein translation through mTOR (Marcais et al., 2014; Donnelly et al., 2014; Mao et al., 2016). However, despite increased activation of mTOR in response to cytokine stimulation, mTOR does not seem to be universally required for NK cell effector function. For example, mTOR was required for granzyme B production and proliferation, but not IFN-γ production, in response to Ly49H activation receptor stimulation (Marcais et al., 2014). Moreover, in human NK cells, IL-2-induced glycolysis upregulation was abrogated upon rapamycin treatment, whereas combined IL-12 and IL-15 stimulation activated glycolysis independent of mTOR. Correspondingly, cytokine induced activation of OXPHOS is independent of mTOR, indicating that mTOR activation is essential for response to some, but not all stimulations, and it plays a significant role in functional activation associated with activation of glycolysis. Overall, mTOR has a major role in the development of NK cells, and is also required to support effector molecule production in response to cytokine stimuli.

3.2. AMPK

Nutrient deprivation leads to a decrease in substrate levels available to produce adenosine trisphosphate (ATP), which results in a relative accumulation of intracellular adenosine monophosphate (AMP) or diphosphate. An increase in AMP to ATP, or ADP to ATP ratios activate 5′ AMP-activated protein kinase (AMPK) that promotes catabolic reactions to top up energy within the cell (Mihaylova and Shaw, 2011; Hardie et al., 2012). AMPK is also phosphorylated and positively regulated by liver kinase B1 (LKB1) and TGF-β −activated kinase 1 (TAK1), and is dephosphorylated and negatively regulated by protein phosphatases (PPs) (Andris and Leo, 2015). Active AMPK increases fatty acid oxidation (FAO) through inhibition of acetyl CoA carboxylase (ACC) and activating carnitine palmitoyl transferase 1 (CPT-1), rate-limiting step enzymes for de novo lipogenesis or FAO, respectively (Yeh et al., 1980; Saha and Ruderman, 2003; Dobrzyn et al., 2004; Zhou et al., 2015). AMPK is also known to be negatively regulated by AKT (Hahn-Windgassen et al., 2005), implying that increased AKT activity in the presence of IL-15 may be associated with decreased AMPK activity, skewing metabolism towards cellular growth. Indeed, as opposed to mTOR function, an increase in AMPK activity is associated with decreased activation of NK cells. It has been previously shown that both NKP and iNK subsets have similar levels of AMPK phosphorylation status indicating AMPK does not change catabolic status of NK cells in premature stages (Wang et al., 2016). However, in humans, AMPK expression levels are increased in terminally differentiated mature CD56dim NK cells that express KLRG1 (Muller-Durovic et al., 2016). In fact, KLRG1 ligation leads to increased AMPK activity in NK cells by preventing dephosphorylation mediated by PP2C. AMPK activation by pharmacological agents suppresses NK cell cytotoxicity, granzyme B and IFN-γ production. Furthermore, NK cells with an active KLRG1–AMPK axis displayed reduced proliferative potential, decreased telomerase reverse transcriptase (TERT) expression, and increased DNA damage, all of which are associated with reduced growth and terminally differentiated NK cell phenotype (Muller-Durovic et al., 2016). AMPK therefore negatively regulates NK cell function, and has suppressive roles primarily in mature, terminally differentiated NK cells.

3.3. GSK3β

Glycogen synthase kinase 3 β (GSK3β) is a serine threonine kinase that regulates glucose homeostasis by phosphorylating and deactivating glycogen synthase (GS), inhibiting glycogen synthesis (Zois and Harris, 2016). In addition to GS, GSK3β has a number of substrates that are involved in a broad range of biological processes, including transcription factors NFAT and Jun, and anti-apoptotic protein MCL1, each of which supports functional activation or survival of NK cells (Aramburu et al., 1995; Neilson et al., 2001; Huntington et al., 2007; Beurel et al., 2010; Wang et al., 2013; Kang et al., 2017; Lougaris et al., 2017). NK cells derived from acute myeloid leukaemia patients displayed increased expression of GSK3β associated with defective cytolytic ability (Parameswaran et al., 2016). Inhibition of GSK3β enhances NK cell killing against AML tumors by upregulation of lymphocyte function associated antigen 1 (LFA1), and an increased production of IFN-γ and TNF-α (Parameswaran et al., 2016). Although the mechanism by which increased GSK3β leads to NK cell suppression has not been investigated, this may occur through regulation of glucose homeostasis.

3.4. DGKζ

Lipids are essential constituents in membrane structures, and also signal to activate immune cell function. Diacylglycerol (DAG) is a lipid signal transducer that is important in receptor-dependent activation of T cells and NK (Chen et al., 2016; Gumbleton and Kerr, 2013). Therefore, external nutrient levels or intracellular events that affect DAG levels or localisation within the cell may alter function of NK cells. Cell membrane-associated DAG is produced through the reaction catalysed by phospholipase Cγ through hydrolysis of phophatidylinositol (4,5) bisphosphate PI(4,5,)P2 into 1,4,5-inositol trisphosphate (IP3), which also acts as Ca^2+-dependent receptor signalling (Gumbleton and Kerr, 2013). Diacylglycerol kinases (DGKs) phosphorylates DAG into phosphatidic acid (PA), and subsequently controls intracellular DAG levels. NK cells with DGKζ-deficiency, but not with DGKα-deficiency, have specifically increased sensitivity to NK activating receptor-dependent induction of IFN-γ and degranulation, which is associated with increased tumor-killing and protection (Yang et al., 2016). This enhanced activity of NK cells is mediated by increased ERK1 activity without affecting AKT or NF-kB, or changes in activation and inhibitory receptors, indicating altered signalling leads to the NK cell functional phenotype (Yang et al., 2016). It is yet to be shown that DGKζ-deficiency increases intracellular DAG levels, and that elevation in DAG, and not other secondary metabolites, is the absolute requirement for the enhancement of DGKζ-deficient NK cells. However, if DAG is the key signal transducer for this process, it is also likely that altered rates of lipolysis or lipogenesis in NK cells potentially change intracellular DAG, influencing receptor-dependent activation of NK cells (Kase et al., 2015; Serup et al., 2016; Dobrzyn et al., 2013).

3.5. FOXOs

Forkheadbox O (FOXO) transcription factors are highly conserved transcription factors across eukaryotic species and participate in transcriptional activation of catabolic process-related genes. There are 4 family members of FOXO proteins: FOXO1, FOXO3, FOXO4, and FOXO6 (Martins et al., 2015). Amongst all the FOXO proteins, FOXO1 is the most abundant, FOXO3 is expressed to a lesser extent, and FOXO4 is not detected at mRNA levels in NK cells (Wang et al., 2016). FOXO1 can be phosphorylated by AKT and glucocorticoid kinase (SGK), which leads to cytoplasmic localisation of FOXO, and subsequent senescence in its transcriptional regulation (Singh et al., 2011). FOXO1 more significantly contributes to development and function of NK cells (Wang et al., 2016; Deng et al., 2015). Total FOXO1 protein levels are high in NKP and iNK subsets, and the expression levels progressively decrease as NK cells mature into mNK cells (Wang et al., 2016). FOXO1 in NKP

cells is not phosphorylated and is exclusively found in nucleus, whilst it is primarily localised in the cytoplasm in more mature iNK and mNK cells regardless of its phosphorylation status. Consistently, FOXO1-activated genes, including Bcl2, Klf2, and IL7Ra are upregulated in NKP cells, whereas FOXO1-repressed genes, such as Tbx21, Ccr7, and Atg7, are more highly expressed in iNK cells than in NKP cells. Within mature NK cell subsets, genetic deletion of FOXO1 leads to reduction in terminally differentiated KLRG1 + CD11b + NK cells, suggesting FOXO1 sustains CD27 + NK cells or terminates NK cell maturation at this stage. FOXO1 also promotes CD62L expression and lymph node-homing of mature NK cells, which is associated with inactive and resting NK cell phenotype (Deng et al., 2015).

The role of FOXO1 in NK cell activation and survival is unclear. One study shows that genetic deletion or knock down of FOXO1 leads to increased activation of NK cells, whilst another study demonstrates that NK cells with FOXO1 lacking functional AKT-dependent phosphorylation sites cannot be optimally activated (Wang et al., 2016; Deng et al., 2015). One well-established role for FOXO proteins is the induction of autophagy during starvation, and it has been shown that NK cells also activate autophagy upon MCMV infection (O'Sullivan et al., 2015). Although FOXO1 promotes autophagy through transcriptional regulation in different cell types, phosphorylation of FOXO1 by AKT also activates Atg7-dependent autophagic response, leading to activation and improved survival of NK cells that confers protection against MCMV infection (Wang et al., 2016). In summary, FOXO1controls NK cell development into CD27+ NK cells, and is likely to promote survival of NK cells through induction of autophagy.

3.6. SREBPs

Sterol regulatory element binding proteins (SREBPs) are transcription factors that have central roles in lipid metabolism, controlling expression of lipid synthesis genes. There are two SREBP proteins: SREBP 1 and 2. SREBP1 consists of two isoforms − SREBP 1a and 1c. Sensing nutrient status or insulin signals, SREBP1c specifically enhances the lipid synthesis pathway, whereas SREBP1a more broadly regulates both lipid and cholesterol synthesis pathways. SREBP2 is regulated by sterol levels and controls cholesterol metabolism (reviewed in detail in Shimano and Sato (2017)).

Recently, it has been revealed that SREBPs have an essential role in NK cell responses to combinatory stimulation with IL-2 and IL-12, through activation of glucose metabolism (Assmann et al., 2017). Cytokine stimulation leads to upregulated gene expression of SREBPs, and activated classical SREBP targets genes that are critical for *de novo* synthesis of fatty acids (i.e. Fasn and Scd1) and mevalonate required for cholesterol biosynthesis (i.e. Hmgcs1 and Acat2). Pharmacological inhibition of SREBPs or deficiency of Scap gene, which processes inactive SREBP into its transcriptionally active form, significantly impaired both glycolysis and OXPHOS, growth and proliferation, GzmB and IFN-γ production in NK cells. This also suppressed NK cell cytotoxicity of Yac-1 tumor targets and protection against B16F10 melanoma lung metastasis. In cytokine-activated NK cells, SREBPs upregulated key metabolic genes, Slc25A1 and Acly that drive citrate-malate shuttle (CMS), which produce NADH through continuous exchanges of mitochondrial citrate with cytosolic malate (Assmann et al., 2017). Moreover, inhibition of Acly led to similar functional and metabolic impairment of NK cells observed with SREBP inhibition. These findings were recapitulated in human NK cells, and the effects of SREBP and Acly inhibition were more prominent in CD56 bright NK cells. Thus, SREBPs regulate cytokine-induced metabolic and functional activation of NK cells by causing glucose-dependent induction of CMS.

4. Effects of nutrients, metabolites, hormones on NK cell function

Immune cells are exposed to fluctuating levels of nutrients, metabolites and hormones that vary significantly as a consequence of dietary intake, circadian rhythm and stress responses. Accumulating evidence suggests that NK cells are also under the control of the endocrine system, implying NK cell activity can be tightly linked to hormone secretion. In this section, we will cover known effects of nutrient or metabolite levels and the endocrine system on function of NK cells in health and disease.

4.1. Hormones and NK cell function

4.1.1. Adiponectin

The endocrine system regulates systemic nutrient levels by promoting catabolic or anabolic reactions in various tissues, and some hormones were identified to directly regulate NK cell function. Adiponectin, a highly abundant hormone exclusively secreted by adipose tissues, participates in glucose and FA metabolism, and acts on various types of cells through adiponectin receptors 1 and 2 (AdipoR 1 and 2), and T-cadherin (Yamauchi et al., 2003; Takeuchi et al., 2007). In humans, NK cells express much higher levels of the two AdipoRs than T cells. CD56dim NK cells constitutively express a high level of surface AdipoR 1 and 2, and CD56bright NK cells have high abundance of intracellular AdipoRs, implying that more mature CD56dim NK cells have greater sensitivity to adiponectin signalling. In mice, AdipoRs are primarily stored intracellularly, and surface expression levels are minimal (Wilk et al., 2013). Adiponectin appears to be required for healthy development of NK cells. For example, IL-15-induced maturation of NK cells was reduced in adiponectin-deficient conditions, suggesting adiponectin directly promotes NK cell development or stimulates other cell types to promote functional NK cell maturation (Han et al., 2013). Functionally, adiponectin more commonly suppresses NK cell function although the effects are stimulation and signalling specific. Adiponectin treatment of human NK cells reduces IFN-γ production in response to R848 (TLR7 and 8 ligand) stimulation, and adiponectin deficiency leads to increased production of IFN-γ in mouse NK cells (Wilk et al., 2013). Adiponectin stimulation blocks IL-2-induced NF-kB activation by preventing phosphorylation of inhibitor of NF-kB (IkB), protecting it from proteasome-dependent degradation, which subsequently inhibits IFN-γ production (Kim et al., 2006). In contrast, upon stimulation with IL-12 and IL-18, or LPS, IFN-γ production was unaffected (Wilk et al., 2013). Cytotoxic activity of NK cells against YAC-1 or EL4 cells was also reduced upon adiponectin administration. However, IL-2-induced degranulation and cytotoxicity against K562 cells were both unaffected when NK cells were treated with adiponectin (Wilk et al., 2013). In a separate study, adiponectin deficiency led to reduction in degranulation, and NKG2D and CD94 expression on NK cells (Wilk et al., 2013). Taken together, effects of adiponectin on NK cell function varies dependent on species and stimulatory conditions, but is mostly associated with suppression of pro-inflammatory activities.

4.1.2. Leptin

Leptin is a hormone derived from adipose tissues, and controls energy balance through suppression of hunger. There are six isoforms of leptin receptors (ObR), amongst which two of them have been well characterised and known to signal through JAK2. Unlike adiponectin receptors that are abundantly expressed on human NK cells, only about 5% of human blood NK cells express Ob-Rs (Wrann et al., 2012).Leptin has both activation and regulatory roles in NK cells. Db/db mouse NK cells that lack ObR and resultant dysfunctional leptin signalling, displayed increased killing against YAC-1 targets and higher mRNA and protein levels of IL-10, suggesting leptin signalling promotes pro-inflammatory or cytotoxic response of NK cells (Lo et al., 2009) whilst suppressing its anti-inflammatory roles. Similarly, NK-92 cells or primary human NK cells stimulated with leptin exhibited reduced killing against colon adenocarcinoma, or possessed reduced expression of NKG2D and NKp46, as well as less IFN-γ production (Bahr et al., 2017; Jahn et al., 2015). In contrast, NK cells stimulated with leptin displayed enhanced granzyme B expression and cytotoxic response against other

tumors, indicating the effects of leptin, like adiponectin, may be context specific (Lamas et al., 2013). Leptin is also involved in regulation of NK cell apoptosis. Db/db mouse-derived NK cells exhibited reduced anti-apoptotic protein BCL2 expression and increased pro-apoptotic Bax expression (Lo et al., 2009).

4.1.3. Stress hormones: catecholamines and glucocorticoids

Psychological or physiological stressors trigger hypothalamus-pituitary-adrenal gland (HPA) axis or sympathetic nervous system activity to release stress hormones. Stress hormones suppress glucose consumption and promote gluconeogenesis by liver cells to provide essential glucose to organs, such as brain, whilst activating lipolysis by adipose tissues to increase circulating fatty acid levels as a supply of energy to other organs. There are two major groups of stress hormones; steroid-derived glucocorticoids and tyrosine-derived catecholamines, which include norepinephrine (noradrenaline), epinephrine (adrenaline) and dopamine as the main constituents (Glaser and Kiecolt-Glaser, 2005). NK cells express both adrenergic receptor B2 (AdRB2) and glucocorticoid receptor (GR). AdRB2 is highly expressed on CD56dim NK cells whereas GR is expressed higher on CD56bright NK cells (Bigler et al., 2015). An acute increase in catecholamines and glucocorticoids induced by administration of 3,4-methylenedioxymethamphetamine (MDMA) and methylphenidate (MPH) stimulated NK cell mobilisation into the blood, which was positively correlated with the proportion of CD56 dim NK cell subsets (Bigler et al., 2015). During this response, NK cells downregulated NKG2D expression, although degranulation and IFN-γ production was unaltered (Bigler et al., 2015). Similarly, an increase in catecholamines upon exercise training induced IL-6-dependent blood mobilisation of NK cells (Pedersen et al., 2016). NKCC cells treated *in vitro* with physiological levels of cortisol showed reduced cytotoxicity against K562, whilst adrenaline-treated NK cells displayed reduced cytotoxicity only when they were treated with a dose above physiological levels (Gotlieb et al., 2015). Furthermore, the adrenaline-induced suppression of NKCC cytotoxicity was reversible upon withdrawal, whilst cortisol-dependent suppression was not. Similarly, sleep deprivation or depression decreases NK cell cytotoxic ability by elevated glucocorticoid levels and adrenergic receptor signalling (Duggal et al., 2015; De Lorenzo et al., 2015). Overall, stress hormones increase mobilisation of NK cells into the blood circulation, but appear to suppress cytotoxic activity.

4.2. Alterations of nutrient and metabolite levels in disease settings

4.2.1. Cancer

In some cancers, NK cells become dysfunctional, and their suppression can be attributable to the accumulation of tumor-derived metabolites or nutrient deprivation in the tumor environment. Tumor cells have elevated energy metabolism to support their growth and development. To satisfy increased metabolic demands, tumor cells upregulate glycolysis and lactate fermentation that supply a number of substrates for the synthesis of cellular macromolecules. As a result, tumor cells decrease availability of glucose whilst increasing lactate levels (Fischer et al., 2007; Corrado et al., 2016). It has been demonstrated that glucose deprivation impedes glycolysis of CD8 T cells, dampening IFN-γ production (Chang et al., 2015; Ho et al., 2015). NK cell activation which relies on glycolytic flux may also be suppressed in a glucose-deficient tumor environment. Increased lactate levels increase the acidity of the environment, which has been demonstrated to affect the cytotoxic potential of NK cells (Potzl et al., 2017; Brand et al., 2016). In accordance, serum lactate levels are elevated in advanced stage lymphoma patients, and is strongly correlated with NK cell dysfunction (Konjevic et al., 1999; Jurisic et al., 2000). Similarly, adenosine, which is derived from increased breakdown of extracellular ATP by tumors, also negatively affects NK cell function (Williams et al., 1997; Raskovalova et al., 2006; Hoskin et al., 2008). Tumors may impact the nutrient environment indirectly via effects on other cells. For example, a number of studies demonstrate that tumors create a supportive niche for immune suppressive myeloid cells which often have increased activities of indoleamine 2,3-deoxygenase (IDO) or arginase. IDO and arginase catalyse reactions that consume tryptophan and arginine, respectively, resulting in decreased levels of the amino acids required for T cell activation (Rodriguez et al., 2003; Lee et al., 2002). Despite scant evidence available for the importance of these amino acids for NK cell activity, similar mechanisms of immune suppression via a number of other nutrients or metabolites may take place in the tumor environment. Immune suppression by tumors can be caused by elevated levels of anti-inflammatory cytokines, including TGF-β. TGF-β has been shown to counteract IL-15-induced activation of mTOR, decreasing NK cell function (Viel et al., 2016). In summary, altered nutrient, metabolite, and immunomodulatory cytokine levels in tumor environments may contribute to metabolism-dependent suppression of NK cells.

4.2.2. Obesity

Under normal conditions, nutrient levels are tightly regulated by the endocrine system, but in some circumstances, such as obesity, this nutrient regulation fails, leading to chronically increased nutrient levels, particularly glucose and lipids. One of the best characterised complications of obesity is insulin resistance. This insulin unresponsiveness is a result of defective AKT/mTOR signalling. Importantly AKT/mTOR signalling axis plays essential roles in activation of immune cells downstream of TCR or IL-15 stimulation for T cells or NK cells, respectively (Marcais et al., 2014; Chi, 2012; Pollizzi et al., 2015; Nandagopal et al., 2014; Delgoffe et al., 2011). mTOR suppression is caused by excessive systemic FA levels which ultimately leads to accumulation of toxic metabolites, such as ceramides (Teixeira and Costa, 2016). In fact, it has been demonstrated that exposure to elevated levels of FAs dampens TCR signalling (Stulnig et al., 2000). Similarly, increased cholesterol levels influence TCR membrane-clustering and subsequent activation of CD8 T cells, which leads to reduced protection against tumor. Although the interplay between increased lipid species and immune cell functions in obesity have not been fully dissected, incidence of obesity is associated with increased susceptibilities to certain cancers and infectious diseases (Bahr et al., 2017; Mori et al., 2006; Smith et al., 2007; O'Shea et al., 2010), suggesting the obese environment may negatively impact on immune surveillance. Indeed, it has been observed that NK cells in obese patients are lower in abundance in the blood (O'Shea et al., 2010; Lynch et al., 2009), although this may be due to directed migration to different organs (Lautenbach et al., 2009). Furthermore, NK cells derived from obese conditions exhibit reduced tumor killing ability (Bahr et al., 2017; Smith et al., 2007; O'Shea et al., 2010; Nave et al., 2008; Laue et al., 2015). However, obesity is also associated with increased NK cell activation. NK cells in the visceral adipose tissues (VAT) of obese mice exhibit activated phenotype. VAT NK cells skew macrophage differentiation into a pro-inflammatory M1 phenotype which further promotes inflammation (Lee et al., 2016; Wensveen et al., 2015). The resultant chronic inflammation causes insulin resistance in adipose tissues, leading to systemically dysregulated nutrient levels.

Hormone imbalance in obesity may also affect NK cell function differently from healthy conditions. As alluded to earlier, leptin transiently upregulates oxidative metabolism of NK cells. However, leptin-induced phosphorylation of JAK2, ERK1 and 2, AKT, AMPK, and GSK3b is blunted in NK cells derived from obese conditions indicating that chronic stimulation decreases NK cell responsiveness (Nave et al., 2008; Laue et al., 2015). Likewise, short-term activation of NK cells with leptin (up to 24 h) enhances both cytotoxicity and IFN-γ, but long-term stimulation (more than 3 days) reduces the expression of effector molecules (Wrann et al., 2012). Altogether, nutrient excess and hormone imbalance in obese environments perturb immune homeostasis, which potentially contributes to NK cell dysfunction (Fig. 2).

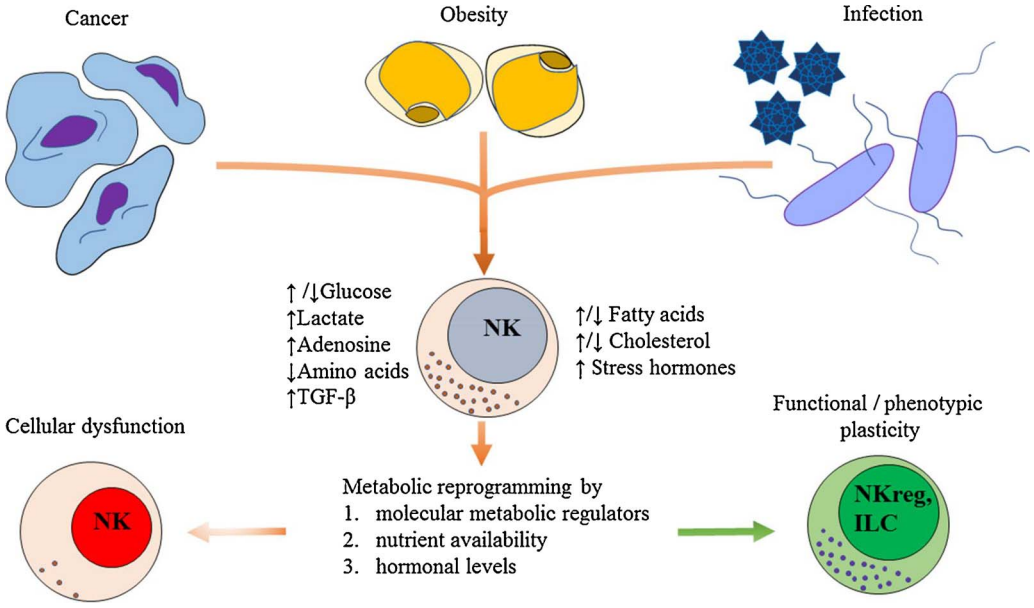

Fig. 2. Disease conditions are associated with perturbed metabolic environments, potentially affecting NK cell metabolism and function. In disease settings, nutrient, metabolite and hormonal balance are often dysregulated. In tumor microenvironments, nutrient levels (e.g. glucose, amino acids) may be decreased whilst metabolites (e.g. lactate) may be increased. In obese patients, both chronically elevated nutrient levels, such as glucose and fatty acids, and hormonal imbalance cause changes in physiological conditions. Exposure to such environments can disrupt NK cell metabolism and cause cellular dysfunction or differentiation into other cell phenotypes (e.g. regulatory NK cells [NKreg] or innate lymphoid cells [ILCs]). Such metabolic perturbations may also occur in infectious diseases, leading to similar metabolic reprogramming of NK cells.

5. Outstanding questions and conclusions

To date, studies on NK cell metabolism have largely been limited to specific *in vitro* cytokine and activating receptor stimulation (Keppel et al., 2015; Marcais et al., 2014; Donnelly et al., 2014; Keating et al., 2016), and have revealed specific metabolic requirements for different stimuli (Keppel et al., 2015). The differences in the dependency on energy metabolism between the two major stimulatory pathways may stem from distinct kinetics involved in activation. IL-12 and IL-18 signal through JAK/STAT and NF-kB pathways which boost IFN-γ production via stabilisation and enhancement of IFN-γ transcripts. In contrast, activation receptor signals (e.g. through NKG2D) are transmitted through DAG and calcium-dependent pathways, which also induce gene transcription, but in addition mediate cytoskeleton mobilization, promoting the release of cytokine or cytotoxic granules (Caraux et al., 2006; Lagrue et al., 2013; Li et al., 2008). As cytoskeleton remodelling consumes ATP (Netter et al., 2017; Sanborn et al., 2009), activation receptor-dependent pathways may require constant energy supply for optimal production of IFN-γ. Energy requirements for inhibitory receptor signals are yet to be identified and therefore raise questions – do inhibitory signals require specific metabolic pathways or induce metabolic reprogramming? If so, what metabolic pathway(s) need to be activated or suppressed to maintain inactive/inhibited states of NK cells? If there are common metabolic pathways/regulators for NK cell function, they will be important targets for immunotherapies, as blocking killer inhibitory receptors (KIR) or co-inhibitory receptors (TIGIT, CD96) has been effective in tumor control (Blake et al., 2016; Li et al., 2014; Liu et al., 2013; Stanietsky et al., 2013; Romagne et al., 2009; Kohrt et al., 2014; Vey et al., 2012; Benson et al., 2011; Chan et al., 2014b).

It has been demonstrated that NK cells increase glycolysis upon blockade of OXPHOS, indicating NK cells are capable of shifting metabolism according to their needs or pressures from the environment. Since NK cells demonstrate metabolic plasticity it raises questions whether functional differentiation or lineage plasticity of NK cells are metabolism-dependent. In various diseases settings (i.e. obesity, infections, and cancers), NK cells have been shown to acquire myeloid, regulatory, or innate lymphoid cell 1-like phenotype (Clark et al., 2016; Gao et al., 2017; Perona-Wright et al., 2009; Theurich et al., 2017) and hence it will be important to investigate metabolic processes underlying these changes.

Nutrient and metabolite levels vary according to dietary intakes and hormonal controls in various disease settings including cancers, infections, and obesity. The challenges in this field therefore are to define metabolic factors which have functional significance in specific conditions. Currently, our knowledge on effects of nutrient levels on NK cells is limited only to glucose, but it is likely that other major nutrient sources, such as glutamine and fatty acids also influence NK cell function. This possibility also extends to other amino acids and different types of lipid species.

Recent progress in immunometabolic studies has shed light on functional links between immunity and cellular metabolism. This review provided an overview of metabolic factors and regulators that can affect NK cell function in health and disease. However, our understanding of metabolic pathways in NK cell biology is still in its infancy. NK cells receive signals from a wide array of stimuli for activation and suppression, leading to a fundamental question – is activation or inhibition of NK cells controlled by one multiple metabolic pathway? Or does each stimulus cause different metabolic events resulting in distinct functional outcomes? Answers to these would help our understanding of how NK cells are (dys)regulated in different disease states and potentially allow us to target NK cell metabolism to modulate their activity.

Conflict of interest statement

The authors declare no potential conflicts of interest.

Acknowledgements

T.K. was supported by an Australian Government Research Training Program (RTP) Scholarship. S.R.M. was supported by a National Health and Medical Research Council Career Development Fellowship (APP1061429).

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