

# Interleukin-18 up-regulates amino acid transporters and facilitates amino acid-induced mTORC1 activation in natural killer cells

Received for publication, September 17, 2018, and in revised form, January 16, 2019. Published, Papers in Press, January 29, 2019, DOI 10.1074/jbc.RA118.005892

Saeedah Mused Almutairi<sup>‡§1,2</sup>, Alaa Kassim Ali<sup>‡1,3</sup>, William He<sup>‡</sup>,  Doo-Seok Yang<sup>‡</sup>, Peyman Ghorbani<sup>‡</sup>, Lisheng Wang<sup>‡</sup>, Morgan D. Fullerton<sup>‡</sup>, and  Seung-Hwan Lee<sup>‡4</sup>

From the <sup>‡</sup>Department of Biochemistry, Microbiology and Immunology, Faculty of Medicine, University of Ottawa, Ottawa, Ontario K1H 8M5, Canada and <sup>§</sup>Botany and Microbiology Department, College of Sciences, King Saud University, Riyadh 11451, Saudi Arabia

Edited by Luke O'Neill

Upon inflammation, natural killer (NK) cells undergo metabolic changes to support their high energy demand for effector function and proliferation. The metabolic changes are usually accompanied by an increase in the expression of nutrient transporters, leading to increased nutrient uptake. Among various cytokines inducing NK cell proliferation, the mechanisms underlying the effect of interleukin (IL)-18 in promoting NK cell proliferation are not completely understood. Here, we demonstrate that IL-18 is a potent cytokine that can enhance the expression of the nutrient transporter CD98/LAT1 for amino acids independently of the mTORC1 pathway and thereby induce a dramatic metabolic change associated with increased proliferation of NK cells. Notably, treatment of IL-18-stimulated NK cells with leucine activates the metabolic sensor mTORC1, indicating that the high expression of amino acid transporters induces amino acid-driven mTORC1 activation. Inhibition of the amino acid transporter CD98/LAT1 abrogated the leucine-driven mTORC1 activation and reduced NK cell effector function. Taken together, our study identified a novel role of IL-18 in up-regulating nutrient transporters on NK cells and thereby inducing metabolic changes, including the mTORC1 activation by amino acids.

Inflammatory signals induce a metabolic change in immune cells as they shift from a quiescent state to an activated state, resulting in increased proliferation, cytokine production, and effector functions. Natural killer (NK)<sup>5</sup> cells are innate lympho-

cytes that play a critical role in antiviral immunity. NK cells are the first proliferating lymphocytes and serve as the principal cytotoxic cells during the early stages of infection (1–3). During virus infection, naïve NK cells undergo rapid metabolic reprogramming to support their expansion and differentiation into potent effector NK cells (4, 5). Interleukin (IL)-2 and IL-15 activate the mammalian target of rapamycin (mTOR) complex 1 (mTORC1), a key molecule required for glycolytic reprogramming that induces the up-regulation of several glycolytic enzymes and GLUT1 in NK cells (4–9). In immune cells, including NK cells, the mTORC1 pathway is linked to the acquisition of effector functions, which is exemplified by defective effector functions in mTORC1-deficient or rapamycin-treated mice (4–6, 8).

Several cytokines, including IL-2, -10, -12, -15, -18, and -21 and type I IFNs, are known to modulate NK cell proliferation and effector functions during infection and inflammation. Among them, IL-18 is a member of the IL-1 family of cytokines and was originally identified as IFN- $\gamma$ -inducing factor because NK cells and T cells secrete IFN- $\gamma$  upon stimulation with IL-18 (10). It is mainly produced by macrophages and dendritic cells and is processed from an inactive precursor by caspase 1/11-mediated cleavage into the active form upon inflammasome activation in mice (11, 12). The cytosolic DNA sensor AIM2 inflammasome is known to induce systemic biologically active IL-18 during infection *in vivo* (13). So far, most studies on IL-18 have shown synergistic functions with IL-12, including IFN- $\gamma$  induction (11, 12). The critical role of IL-12 and IL-18 in IFN- $\gamma$  production, which is important in directly or indirectly controlling virus replication, was previously demonstrated during murine cytomegalovirus infection (14, 15). In addition, we have identified that IL-12 and IL-18 can up-regulate IL-2R $\alpha$  chain, which renders NK cells highly sensitive to IL-2 stimulation (16). This IL-12/18 pathway enhanced our understanding of NK cell proliferation and is currently being employed for the adoptive transfer of *ex vivo* expanded NK cells that can be sustained

This work was supported by Canadian Institutes of Health Research Grants MOP-130385 and PJT-156106 and Canadian Cancer Society Research Institute Grant 704316 (to S.-H. L.). The authors declare that they have no conflicts of interest with the contents of this article.

This article contains Figs. S1–S3 and Table S1.

<sup>1</sup> Both authors contributed equally to this work.

<sup>2</sup> Recipient of a scholarship from King Saud University and the Saudi Ministry of High Education, Saudi Arabia.

<sup>3</sup> Recipient of an Ontario graduate scholarship.

<sup>4</sup> To whom correspondence should be addressed. Tel.: 613-562-5800 (ext. 8868); Fax: 613-562-5452; E-mail: [seunglee@uottawa.ca](mailto:seunglee@uottawa.ca).

<sup>5</sup> The abbreviations used are: NK, natural killer; IL, interleukin; IFN, interferon; LAT1, L-type amino acid transporter 1; LNAA, large neutral amino acid; CD71, transferrin receptor; SLC, solute carrier; mTOR, mammalian target of rapamycin; mTORC1, mammalian target of rapamycin complex 1; pS6, phosphorylated ribosomal protein S6; LPS, lipopolysaccharide; ECAR, extracel-

lular acidification rate; 2-NBDG, 2-deoxy-2-[(7-nitro-2,1,3-benzoxadiazol-4-yl) amino]-D-glucose; BCH, 2-aminobicyclo-(2,2,1)-heptanecarboxylic acid; BrdU, 5-bromo-2'-deoxyuridine; IL-R, IL-receptor; TNF, tumor necrosis factor; S6K, S6 kinase; MeAIB,  $\alpha$ -(methylamino)isobutyric acid; GPNA, L- $\gamma$ -glutamyl-*p*-nitroanilide; NALA, *N*-acetyl-leucine amide; rh, recombinant human; TCR, T cell receptor; MFI, mean fluorescence intensity.

longer *in vivo* (17, 18). Another mechanism by which IL-18 exhibits its synergistic effect with IL-12 was described in previous work, where IL-18 was shown to prime NK cells *in vivo* to produce IFN- $\gamma$  upon subsequent stimulation with IL-12 (19).

Interestingly, several reports presented the supportive role of IL-18 in NK cell proliferation during IL-2 stimulation *in vitro* (20, 21). Because IL-18 is not a proliferative cytokine that induces the STAT5 pathway, the effect of IL-18 in inducing proliferation might be indirect and influenced by other unidentified factors. We have shown that IL-18 induces the expression of CD25, the IL-2R $\alpha$  chain, on NK cells (16), and thus the enhanced proliferation could be mediated by IL-18-induced CD25 up-regulation on NK cells. However, a similar synergistic role between IL-18 and IL-15 was also demonstrated during NK cell proliferation (22), indicating that IL-18 utilizes an alternative pathway to promote NK cell proliferation. In addition, IL-18 was shown to support the selective expansion of the Ly49H<sup>+</sup> NK cells during murine cytomegalovirus infection (23). Taken together, IL-18 is suggested to support the proliferation of NK cells; however, the mechanisms of IL-18 in promoting NK cell proliferation have not been clearly established.

In multicellular organisms, glucose and amino acids are plentiful in the extracellular milieu, but these molecules have to cross the cell membrane through transporters to be used as building blocks or for generating ATP (24). The nutrient transporters comprise the numerous solute carrier (SLC) groups of membrane transport proteins (>400 members) and show redundancy and promiscuity in their specificity (25, 26). For example, there are 11 SLC families dedicated to the transport of all 20 amino acids (27, 28). One well-studied amino acid transporter is CD98, which is encoded by *Slc3a2*. CD98 is not in itself an amino acid transporter but forms disulfide-linked heterodimers with a variety of multiple membrane-spanning light chains that are responsible for the amino acid transport properties of the complex (27). One of the light chains is L-type amino acid transporter 1 (LAT1), which is encoded by *Slc7a5*. CD98 and LAT1 form a complex called the System L transporter, which preferentially imports large neutral amino acids (LNAAs) such as leucine, isoleucine, and valine in exchange for the efflux of intracellular glutamine (27, 29). In addition to their roles as metabolites, accumulating evidence has demonstrated that essential amino acids such as leucine, tryptophan, phenylalanine, and arginine are known to activate mTORC1 in lysosomal compartments (30–33). A previous report demonstrated the ability of Rag GTPases to physically interact with mTORC1 and regulate its subcellular redistribution in response to leucine (34, 35).

In the present study, we characterized a mechanism by which IL-18 induces NK cell proliferation. Despite the largely overlapping functions of IL-12 and IL-18, the signaling pathways of these two cytokines are different, suggesting a unique function of IL-18. We identified a novel IL-18-specific role on NK cells in which IL-18 can up-regulate nutrient transporters and thereby modulate cellular metabolism. More importantly, the increased expression of the System L amino acid transporter CD98/LAT1 enabled the activation of mTORC1 by enhanced uptake of leucine. Altogether, our findings demonstrated that

IL-18 is the potential cytokine inducing metabolic changes in NK cells.

## Results

### **Nutrient transporters are highly expressed on proliferating NK cells upon IL-18 stimulation**

As previously demonstrated, enriched NK cells stimulated by IL-2/IL-18 showed increased proliferation upon increasing concentrations of IL-18 in a dose-dependent manner. Stimulation with 300 units/ml IL-2 and 30 ng/ml IL-18 for 3 days increased NK cells number up to 60-fold (Fig. 1A). Because we reasoned that the metabolic demands needed for the intense NK cell proliferation can be fulfilled by high expression of nutrient transporters, the expression of amino acid transporter CD98 on proliferating NK cells in regard to cell division was analyzed. Notably, higher expression of CD98 was observed in NK cells that were dividing fast, showing a strong correlation (Fig. 1B).

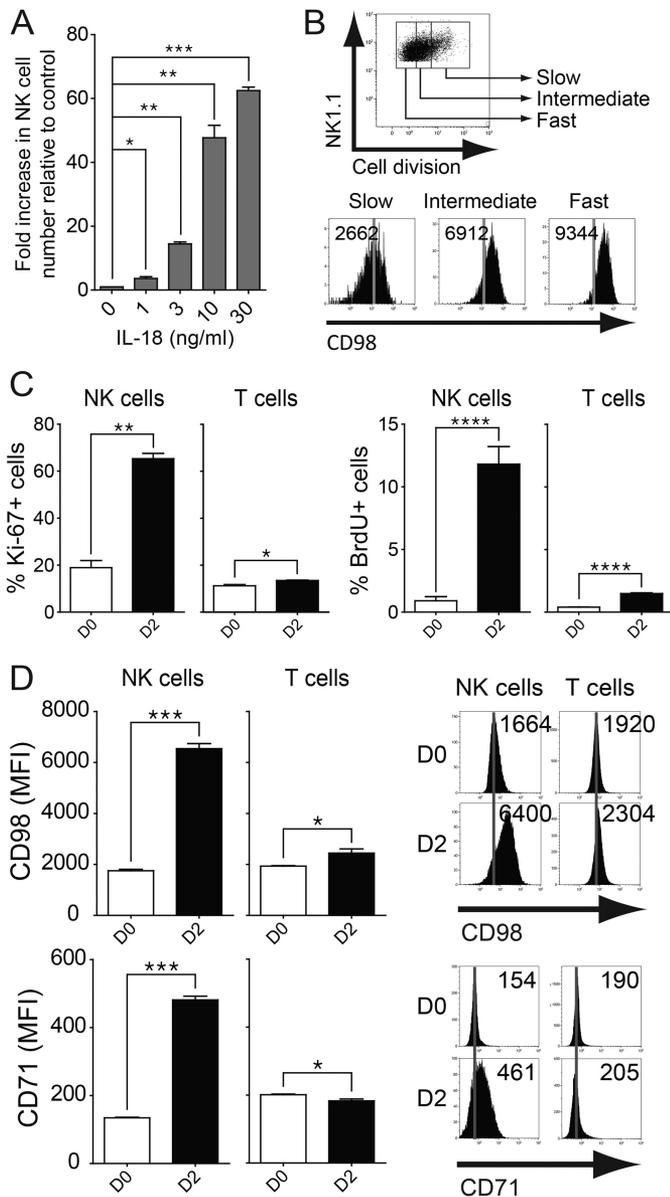
LPS treatment is known to induce IL-18 production through the inflammasome-dependent pathway (36). Notably, NK cells proliferate upon LPS treatment (37, 38), and the proliferation antigen Ki-67 and BrdU incorporation were highly increased in NK cells on day 2 post-LPS treatment (Fig. 1C). To investigate whether the expression of nutrient transporters is also up-regulated in LPS-induced inflammation, we analyzed the expression of nutrient transporters on NK and T cells from mice injected with LPS for 2 days. The LPS treatment *in vivo* resulted in the up-regulation of CD98 and transferrin receptor (CD71) expression on NK cells (Fig. 1D). There was a minor change in CD98 and CD71 expression on T cells. Thus, NK cell proliferation upon IL-18 stimulation is accompanied with a high expression of nutrient transporters, suggesting that their high expression is required to support the robust NK cell proliferation.

### **IL-18 can up-regulate nutrient transporters on NK cells**

To determine whether IL-18 can directly induce the up-regulation of the nutrient transporters CD98 and CD71, we stimulated enriched NK cells for 18 h with IL-18 or various other recombinant cytokines that are known to activate NK cells. The list of cytokines includes IL-4, -12, -15, -18, -21, IFN- $\alpha$ , - $\beta$ , - $\gamma$ , and TNF $\alpha$ . 100 units/ml IL-2 was included to maintain NK cell survival during stimulation. Even though IL-2 slightly increased the expression of CD98 and CD71, the surface expression of these nutrient transporters was exceedingly up-regulated upon treatment with IL-18 (Fig. 2A). Despite the largely synergistic functions of IL-12 and IL-18, it is noteworthy that IL-18, but not IL-12, could induce high expression of CD98 and CD71 on NK cells upon stimulation with increasing concentrations of IL-12 or IL-18 (Fig. 2B), indicating a function of IL-18 that is unique from that of IL-12 in regard to the up-regulation of nutrient transporters. Similarly, IL-18, but not IL-12, could drastically increase glucose uptake in NK cells (Fig. 2C), suggesting that IL-18 signaling induces the uptake of various nutrients.

Because IL-12 and IL-18 induce the expression of IL-2R $\alpha$  chain, which renders NK cells highly responsive to IL-2 (16), we investigated the effect of cytokines on enriched NK cells supplemented with IL-15 to exclude the possibility that the

## IL-18 enhances amino acid transporter expression on NK cells



**Figure 1. Higher proliferation of NK cells is associated with enhanced expression of nutrient receptors upon IL-18 treatment *in vitro* and LPS treatment *in vivo*.** *A*, the number of expanded NK cells upon *ex vivo* stimulation with various concentrations of IL-18 relative to IL-2 alone was quantified. *B*, NK cells from the spleen of naïve C57BL/6 mice were *ex vivo* stimulated with IL-2 and IL-18. NK cells were prelabeled with cell proliferation dye and cultured with 300 units/ml IL-2 and 3 ng/ml IL-18 for 3 days. The dot plot depicts the dilution of cell proliferation dye on NK cells, and the histograms depict the MFI of CD98 expression on NK cells gated in regard to cell proliferation. *C* and *D*, C57BL/6 mice were either left untreated or challenged with 100  $\mu$ g of LPS intraperitoneally and sacrificed on the indicated days for further analysis. *C*, NK cell proliferation upon treatment with LPS as measured by Ki-67 expression and BrdU incorporation. *D*, representative plots depicting the MFI of CD98 and CD71 expression on NK cells and T cells in the spleens of naïve (*D0*) or LPS-treated mice at day 2 postinjection (*D2*). For *A* and *B*, data are from one experiment representative of two independent experiments, with three replicates per group. For *C* and *D*, data are from one experiment representative of three independent experiments, with two to three mice per group. Data represent mean  $\pm$  S.D. (error bars). \*,  $p < 0.05$ ; \*\*,  $p < 0.01$ ; \*\*\*,  $p < 0.001$ .

increased nutrient receptor expression was due to an increased sensitivity of NK cells to IL-2 by IL-18. Consistent with the results from IL-2/18-stimulated NK cells, although IL-15/18-stimulated NK cells greatly up-regulated the expression of nutrient transporters and showed increased glucose uptake,

IL-15/12-stimulated NK cells failed to induce any significant change (Fig. S1).

Interestingly, the up-regulation of nutrient transporters by IL-18 stimulation predominately occurs on NK cells and not on other immune cells such as T cells (Fig. 1*D* and Fig. S1*C*). To determine whether the selective effect of IL-18 on NK cells is determined by the differential expression level of its receptor, we analyzed IL-18R $\alpha$  surface expression on several cell populations among naïve splenic leukocytes. Notably, NK cells are the only cell population expressing a basally high level of IL-18R $\alpha$  among naïve splenic leukocytes (Fig. 3*A*). Real-time PCR analysis also indicated that genes encoding the IL-18 receptor  $\alpha$ - and  $\beta$ -chains, *Il18r1* and *Il18rap*, are highly expressed on sorted NK cells compared with NK cell-depleted total leukocytes (non-NK cells) (Fig. 3*B*). Similar to IL-18, IL-1 $\alpha$  and IL-1 $\beta$  are known to signal via a Toll/interleukin-1 receptor motif in the cytoplasmic domain of their receptors. Treatment with IL-1 $\alpha$ / $\beta$  failed to up-regulate nutrient transporters on NK cells (Fig. 3*C*), presumably due to the low expression level of genes for the IL-1 receptor, *Il1r1* and *Il1rap*, on NK cells (Fig. 3*B*). Taken together, IL-18 can up-regulate nutrient transporters on NK cells, and the basally high expression of IL-18 receptor presumably supports the immediate induction of nutrient transporters upon IL-18 stimulation.

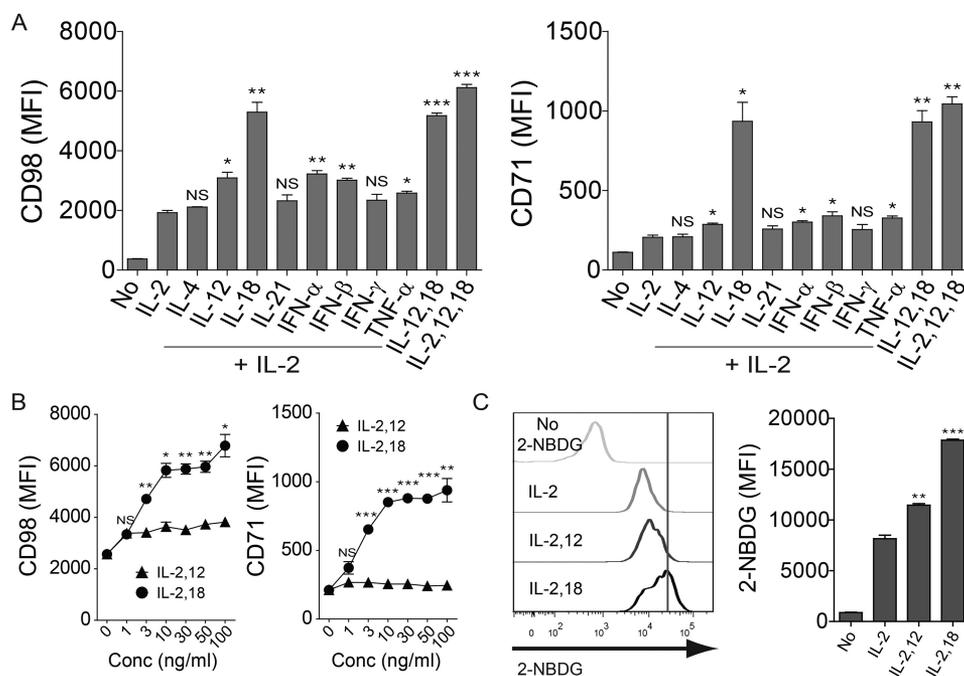
### IL-18 up-regulates nutrient transporters on NK cells independently of mTORC1 pathway

The mTORC1 pathway is known to induce the up-regulation of nutrient transporters on NK cells (4, 6). To determine whether IL-18 stimulation is a nonredundant pathway distinct from the mTORC1 pathway for inducing the expression of nutrient transporters, we stimulated NK cells with IL-18 in the presence of rapamycin, an mTORC1 inhibitor, and analyzed the expression of CD98 and CD71 on NK cells. In general, the rapamycin treatment reduced the expression of CD98 and CD71. However, it was noteworthy that IL-18, but not IL-12, can induce the up-regulation of CD98 and CD71 expression by similar -fold changes (Fig. 4, *A* and *B*), indicating that IL-18 can induce the up-regulation of nutrient transporters independently of mTORC1 pathway. The abrogated S6 phosphorylation indicated the efficiency of mTORC1 inhibition by rapamycin (Fig. 4*C*). To exclude the possibility that IL-18 signaling activates the mTORC1 pathway before the pathway is completely blocked by rapamycin, we measured the expression of CD98 and CD71 on NK cells preincubated with rapamycin before IL-18 stimulation. Consistently, we observed the up-regulation of CD98 and CD71 by similar -fold changes (Fig. S2). Therefore, our results indicated that IL-18 can induce the expression of nutrient receptors independently of the mTORC1 pathway.

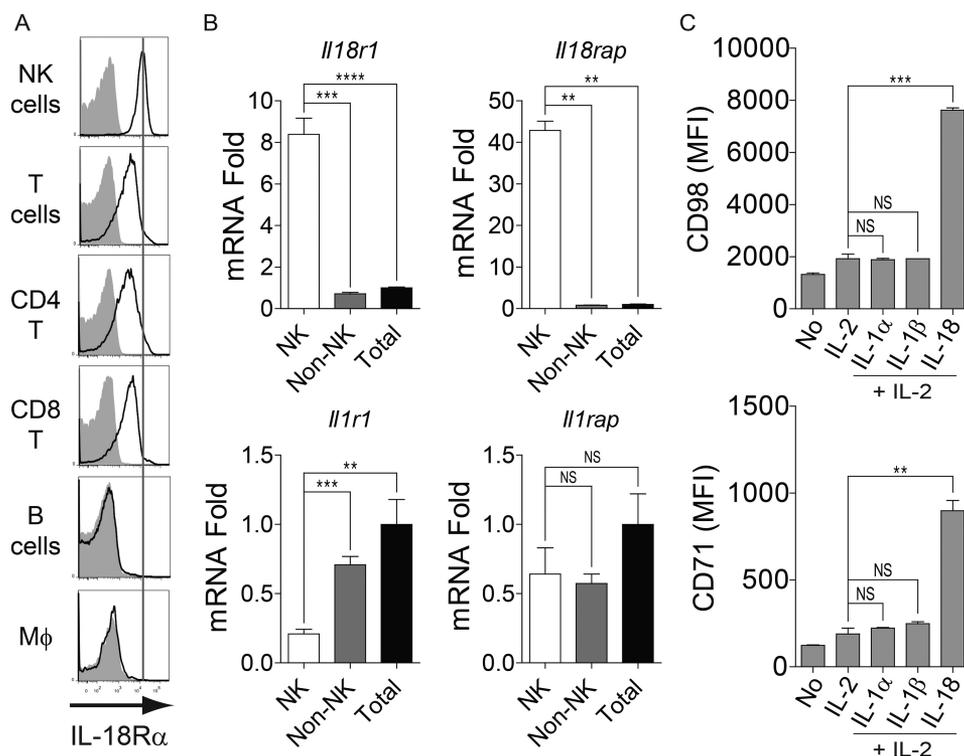
### IL-18 induces metabolic changes in NK cells

Heterodimers of CD98 (SLC3A2) and LAT1 (SLC7A5) form the bidirectional System L transporter that exchanges the simultaneous efflux of glutamine and influx of leucine (27, 29). For this exchange to occur, SLC1A5, a high-affinity transporter for glutamine, is required to preload the cells with glutamine. To investigate whether the effect of IL-18 can induce the up-

## IL-18 enhances amino acid transporter expression on NK cells

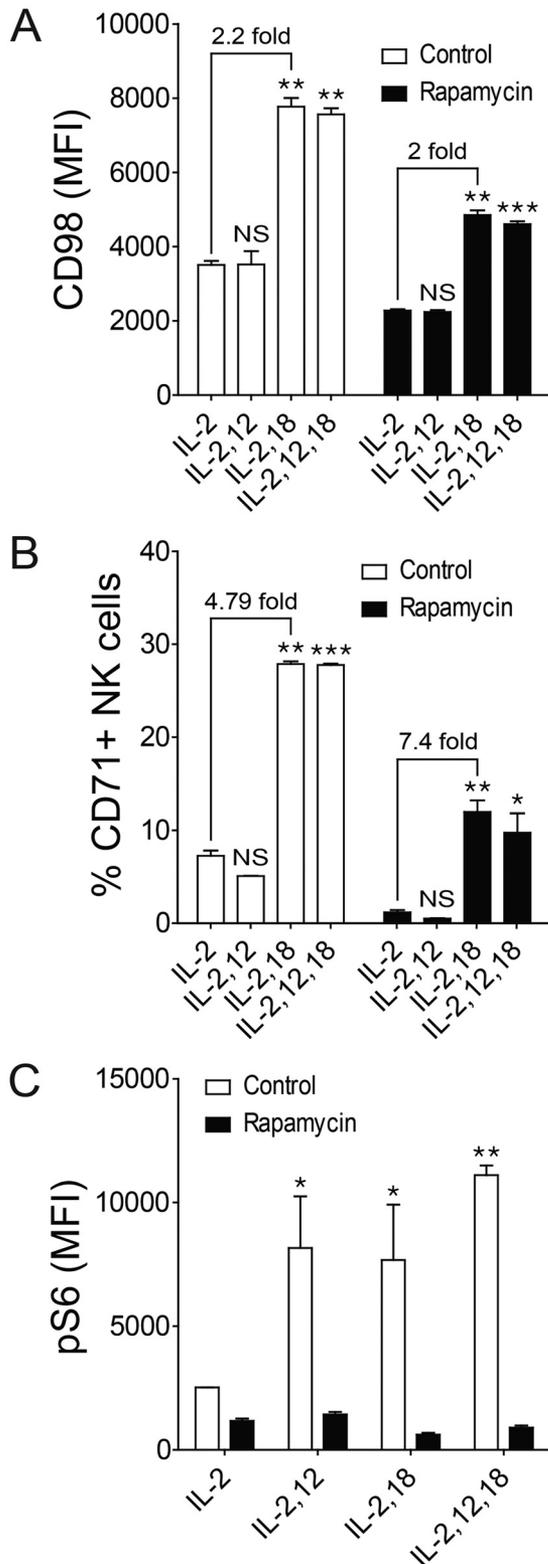


**Figure 2. IL-18 can induce the up-regulation of nutrient transporters on NK cells *in vitro*.** NK cells were enriched from the spleens of naïve C57BL/6 mice and stimulated with the indicated cytokines *in vitro* for 24 h. 100 units/ml rhIL-2 was added to maintain NK cell survival. **A**, representative plots depict the MFI of CD98 and CD71 expression on cytokine-stimulated NK cells. Statistics are comparing samples with IL-2-stimulated NK cells. **B**, the expression of CD98 and CD71 on NK cells was measured upon stimulation with different concentrations of IL-12 and IL-18. For the statistical analyses of the data, the *p* values were obtained by comparing the stimulated NK cells with the unstimulated NK cells (without IL-12 or IL-18 treatment). **C**, representative histograms of the glucose uptake by cytokine-stimulated NK cells as measured by the MFI of 2-NBDG. Statistics are comparing samples with IL-2-stimulated NK cells. Data are from one experiment representative of four independent experiments, with two replicates per group. Data represent mean  $\pm$  S.D. (error bars). NS, not significant; \*, *p* < 0.05; \*\*, *p* < 0.01; \*\*\*, *p* < 0.001.



**Figure 3. IL-18 and IL-1 receptor expression on NK cells.** **A**, surface protein expression of the IL-18 receptor  $\alpha$  subunit on various splenic leukocytes from C57BL/6 mice. **B**, NK cells were isolated by flow sorting from the spleen of C57BL/6 mice and compared with non-NK cells (total splenic leukocytes except NK cells) and total splenic leukocytes. mRNA was extracted from these three populations, and the transcript levels of IL-18 and IL-1 receptors were quantified by quantitative real-time PCR. **C**, representative plots depict the MFI of CD98 and CD71 expression on enriched NK cells from the spleen of naïve C57BL/6 mice upon stimulation with different cytokines for 24 h. Data are from one experiment representative of three independent experiments, with three replicates per group. Data represent mean  $\pm$  S.D. (error bars). NS, nonsignificant; \*\*, *p* < 0.01; \*\*\*, *p* < 0.001; \*\*\*\*, *p* < 0.0001. *Mφ*, macrophages.

## IL-18 enhances amino acid transporter expression on NK cells



**Figure 4. IL-18 induces the expression of nutrient receptors independently of mTORC1 pathway.** NK cells from the spleen of naïve C57BL/6 mice were stimulated with different cytokines for 24 h in the presence or absence of rapamycin. *A*, representative plots depict the MFI of CD98 expression on NK cells. *B*, representative plots depict the percentage of CD71-expressing NK cells. *C*, representative plots depict the MFI of pS6 in NK cells. Data are from one experiment representative of three independent experiments, with two replicates per group. Data represent mean  $\pm$  S.D. (error bars). NS, not significant; \*,  $p < 0.05$ ; \*\*,  $p < 0.01$ ; \*\*\*,  $p < 0.001$ .

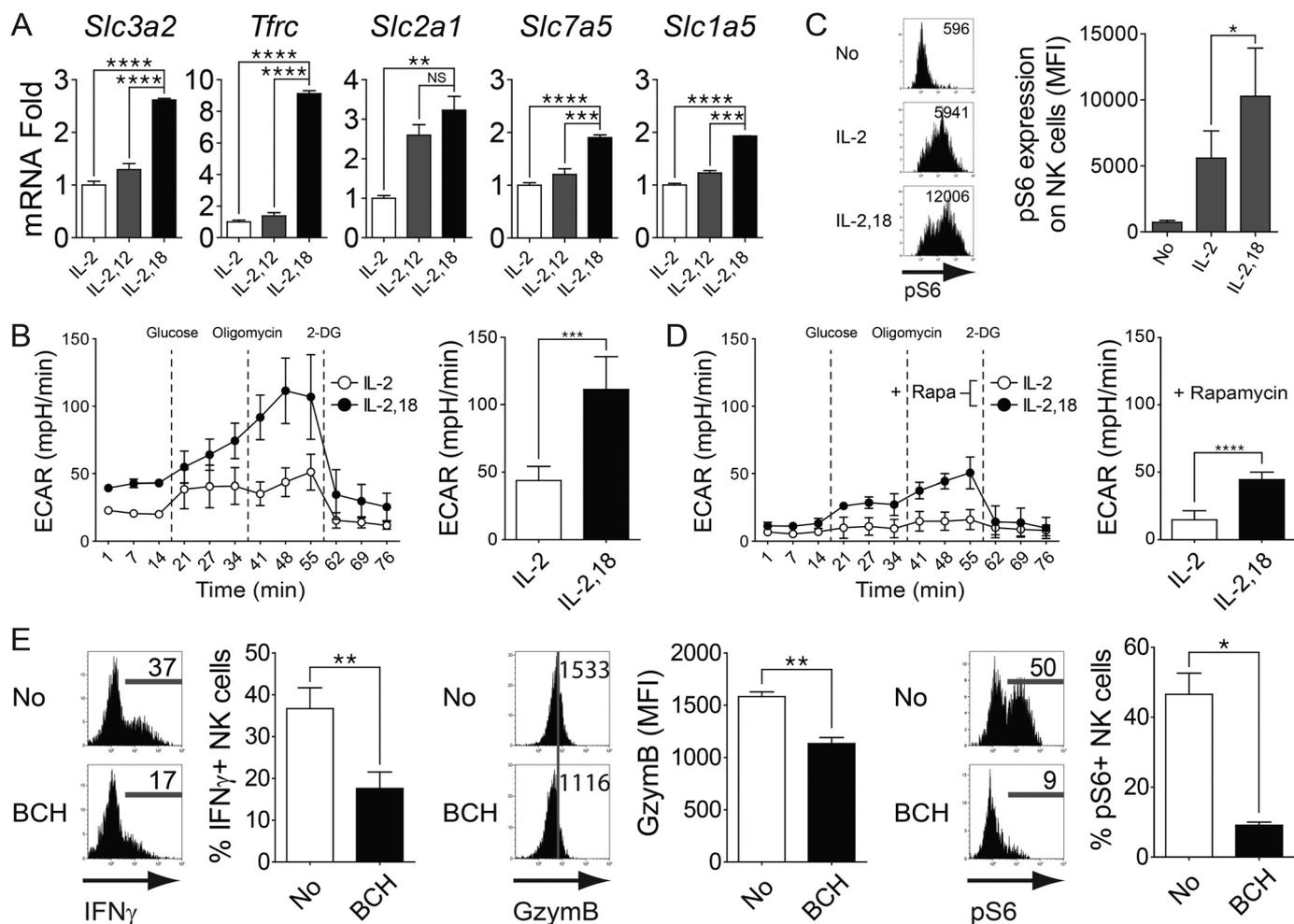
regulation of all the necessary nutrient transporters for the exchange of glutamine for leucine, we purified splenic NK cells via FACS, stimulated the NK cells with IL-2, IL-2/12, or IL-2/18 for 18 h, and analyzed the transcripts of nutrient transporters by real-time PCR. The mRNA expression of *Slc3a2* (*Cd98*), *Tfrc* (*Cd71*), and *Slc2a1* (*Glut1*) was highly up-regulated upon stimulation with IL-18, consistent with the flow cytometry data (Fig. 5A). Notably, expression of *Slc7a5* and *Slc1a5* was also up-regulated by IL-18 (Fig. 5A), indicating that all nutrient transporters necessary for the bidirectional transport of leucine/glutamine via the System L transporter are highly induced by IL-18 stimulation.

To elucidate the metabolic change of NK cells by IL-18, we analyzed the metabolic profiles of glycolysis of expanded NK cells upon IL-2/18 stimulation by measuring the extracellular acidification rate (ECAR) using Seahorse technology. Clearly, NK cells treated with IL-18 showed enhanced glycolysis, suggesting the activation of mTORC1 (Fig. 5B). Indeed, NK cells stimulated with IL-2/18 elicited higher expression of phosphorylated S6 ribosomal protein (pS6), a substrate of mTORC1 activation, compared with those stimulated with IL-2 alone (Fig. 5C). Interestingly, IL-18 was able to enhance the rate of glycolysis in the presence of rapamycin (Fig. 5D), similar to the mTORC1-independent up-regulation of nutrient transporters on NK cells by IL-18 shown in Fig. 4.

Next, to evaluate the importance of the System L transporter, consisting of the CD98/LAT1 heterodimer, for the induction of effector functions in NK cells upon stimulation with IL-2/18, we used 2-aminobicyclo-(2,2,1)-heptanecarboxylic acid (BCH), a competitive inhibitor of the System L transporter. Notably, NK cells stimulated with IL-2/18 in the presence of BCH showed reduced effector functions in regard to IFN- $\gamma$  production, granzyme B expression, and mTORC1 activation (Fig. 5E). Altogether, these results demonstrated that IL-18 can up-regulate the expression of a wide range of amino acid transporters and induce metabolic changes in NK cells. Moreover, CD98 expression on NK cells stimulated with IL-18 is required for the induction of effector functions.

### IL-18 can induce leucine-driven mTORC1 activation

Amino acids such as leucine can directly activate mTORC1 (30–33). Because NK cells stimulated with IL-2/18 showed increased transcript expression of the three necessary transporter components for the System L transporter, we investigated whether increased expression of these transporters allows leucine to activate mTORC1. NK cells expanded with IL-2 were further stimulated with either IL-2 or IL-2/18. The cells were then washed and starved for 3 h in amino acid-depleted medium. mTORC1 activity in NK cells was diminished during amino acid starvation (Fig. 6A). The NK cells showing inert mTORC1 activity were preloaded with L-glutamine and then subjected to stimulation with L-leucine. Remarkably, robust S6 phosphorylation was only observed in IL-2/18-stimulated NK cells by the addition of L-leucine in a dose-dependent manner (Fig. 6A) and was abrogated by rapamycin treatment (Fig. 6B). Signaling through the mTORC1 pathway leads to the activation of the 70-kDa ribosomal protein S6 kinase (S6K), which then phosphorylates S6. L-Leucine–



**Figure 5.** NK cells show increased glycolysis (ECAR) upon IL-18 stimulation. *A*, NK cells isolated by flow sorting were stimulated with IL-2 and either IL-12 or IL-18 for 24 h. The graph depicts the transcript levels of several nutrient receptors as quantified by quantitative real-time PCR. *B*, the ECAR levels of NK cells stimulated with IL-2 or IL-2/18 are shown. *C*, representative histograms depict the MFI of pS6 in NK cells upon stimulation with the indicated cytokines for 24 h. *D*, the ECAR levels of stimulated NK cells upon rapamycin treatment are shown. *E*, NK cell functions are reduced upon inhibition of the amino acid transporter LAT1. NK cells from the spleen of naïve C57BL/6 mice were stimulated with IL-2 and IL-18 cytokines for 18 h in the presence or absence of BCH. Representative histograms depict the proportion of NK cells expressing IFN- $\gamma$ , granzyme B (*Gzymb*), and pS6. Data are from one experiment representative of three independent experiments, with three to five replicates per group. Data represent mean  $\pm$  S.D. (error bars). NS, not significant; \*,  $p < 0.05$ ; \*\*,  $p < 0.01$ ; \*\*\*,  $p < 0.001$ ; \*\*\*\*,  $p < 0.0001$ .

induced mTORC1 activation in IL-2/18-stimulated NK cells was also confirmed by Western blotting, as these cells showed high phosphorylation of S6K upon leucine treatment (Fig. 6C). The enhanced S6 phosphorylation returned to the basal level within 60 min following stimulation with L-leucine (Fig. 6D).

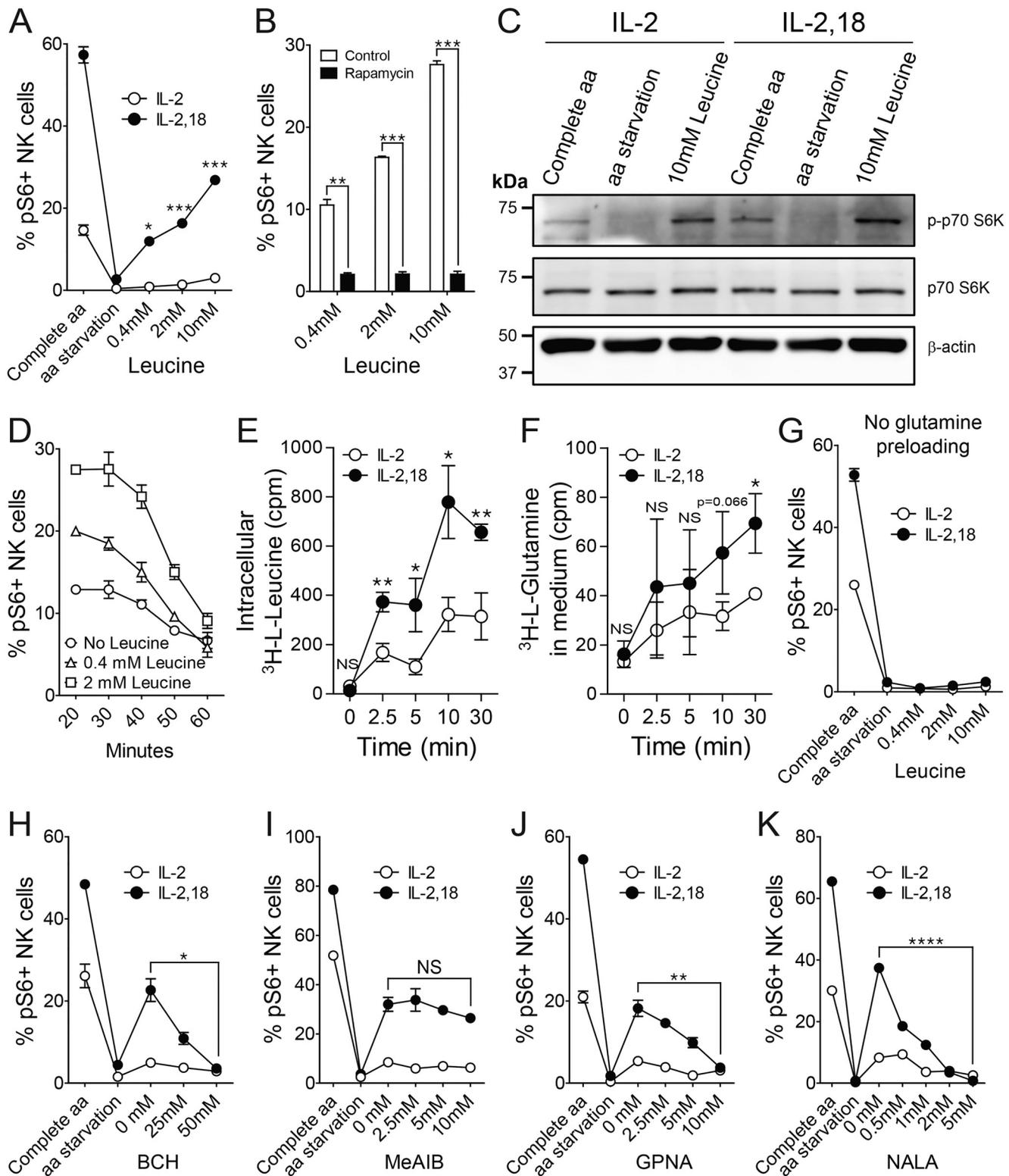
The System L transporter allows the bidirectional exchange of L-glutamine for essential amino acids such as L-leucine (27, 29). To determine whether the L-leucine-driven mTORC1 activation is mediated by the bidirectional exchange of L-glutamine for L-leucine in IL-2/18-stimulated NK cells, we analyzed L-leucine influx and L-glutamine efflux upon L-leucine treatment using  $^3\text{H}$ -labeled amino acids. Notably, increased L-leucine influx and L-glutamine efflux were observed during L-leucine treatment in IL-2/18-stimulated cells compared with IL-2-stimulated cells (Fig. 6, E and F).

The cellular uptake of L-glutamine was shown to be the rate-limiting step that activates mTORC1 (29). To determine the importance of intracellular L-glutamine for the L-leucine-induced mTORC1 activation, we performed an experiment in

which IL-18-activated cells were treated with leucine without L-glutamine preloading. Notably, L-leucine could not induce mTORC1 activation without the intracellular L-glutamine (Fig. 6G). To exclude the possibility that *de novo* protein synthesis is required for the increased mTORC1 activity, we repeated the assay in the presence of cycloheximide, an inhibitor of protein synthesis, and observed that L-leucine-induced mTORC1 activation was observed independently of protein synthesis (data not shown).

To determine the requirement of components for the System L transporter consisting of the CD98/LAT1 heterodimer, BCH, an inhibitor of LAT1, was used. Treatment with BCH abrogated S6 phosphorylation in NK cells stimulated with IL-2/18 in a dose-dependent manner (Fig. 6H). Such changes were negligible in conditioned medium of NK cells stimulated with IL-2.  $\alpha$ -(Methylamino)isobutyric acid (MeAIB), an inhibitor of System A transporters, did not show any effect on leucine-induced mTORC1 activation (Fig. 6I). In addition, L- $\gamma$ -glutamyl-*p*-nitroanilide (GPNA), an inhibitor of SLC1A5-regulated transport

## IL-18 enhances amino acid transporter expression on NK cells



**Figure 6. IL-18 can induce mTORC1 activation by leucine treatment.** *Ex vivo* expanded NK cells were stimulated with IL-2 or IL-2/IL-18 for 18 h, starved for 3 h in amino acid–depleted medium, then replenished with L-glutamine for 1 h before being treated with L-leucine for 30 min. *A*, phosphorylation of ribosomal protein S6 upon addition of leucine in NK cells was measured by flow cytometry. *B*, NK cells were treated with leucine, and the effect of rapamycin on mTORC1 activation was measured. *C*, phosphorylation of p70 S6 kinase upon addition of leucine was measured by Western blotting. *D*, IL-2/18–stimulated NK cells were treated with leucine for the indicated time before measuring pS6. *E*, starved NK cells were preloaded with unlabeled L-glutamine for 1 h and treated with <sup>3</sup>H-labeled L-leucine for the indicated time up to 30 min. The intracellular levels of <sup>3</sup>H-labeled L-leucine are shown. *F*, starved NK cells were preloaded with unlabeled glutamine and <sup>3</sup>H-labeled glutamine for 1 h and treated with unlabeled leucine for the indicated time up to 30 min. The levels of <sup>3</sup>H-labeled glutamine in the culture medium are shown. *G*, NK cells were treated with leucine without preloading with glutamine. *H–K*, NK cells stimulated with IL-2 or IL-2/IL-18 were treated with leucine, and the effects of BCH (*H*), MeAIB (*I*), GPNA (*J*), and NALA (*K*) on mTORC1 activation were measured. Data are from one experiment representative of three to four independent experiments, with two to three replicates per group. Data represent mean ± S.D. (error bars). *aa*, amino acid; NS, nonsignificant; \**p* < 0.05; \*\**p* < 0.01; \*\*\**p* < 0.001; \*\*\*\**p* < 0.0001.

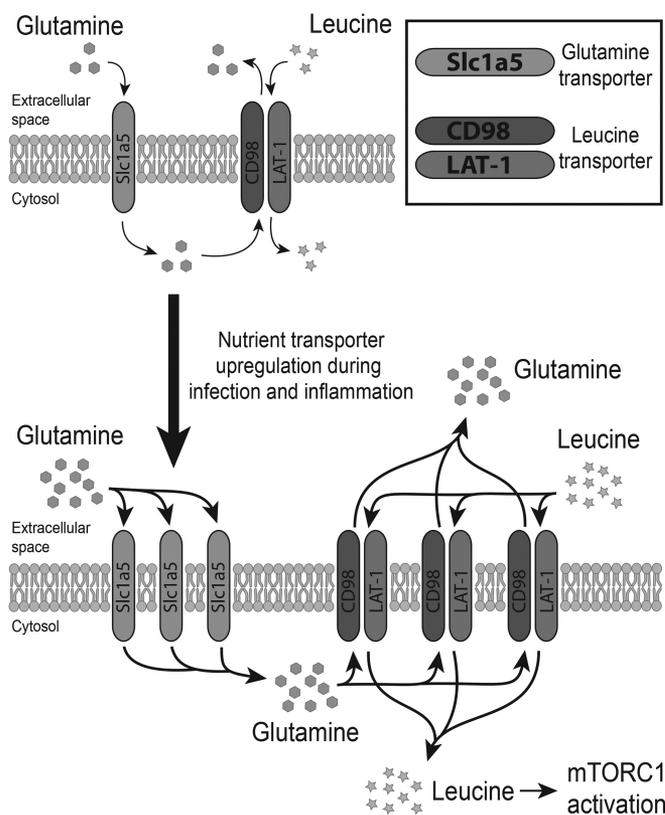
(39), suppressed S6 phosphorylation in a dose-dependent manner (Fig. 6J), indicating that intracellular L-glutamine, transported into the cell via SLC1A5, is critical as an efflux substrate for the System L transporter to activate the mTORC1 pathway. The leucine structural antagonist *N*-acetyl-leucine amide (NALA) exerted similar inhibitory effects on S6 phosphorylation (Fig. 6K). Treatments of IL-18-stimulated NK cells with the aforementioned inhibitors did not induce noticeable cell death in any of the tested concentrations of inhibitors (Fig. S3). Taken together, the data demonstrated that increased expression of the System L amino acid transporter by IL-18 stimulation can intensify mTORC1 activation by leucine.

**Discussion**

The inflammasome pathway induces inflammation in response to infectious microbes and molecules derived from host proteins by the activation of caspase-1 (40–42). Because the signaling pathway emanating from the IL-18 receptor is distinct from that of IL-12 receptor, the inflammasome-induced IL-18 was thought to play nonredundant roles on immune cells. Here, we demonstrated a previously unappreciated role of IL-18 in increasing nutrient accessibility by up-regulating nutrient transporters, including the amino acid transporters and the transferrin receptor, on NK cells.

In this report, we demonstrated that the nutrient transporter up-regulation by IL-18 stimulation occurs predominately on NK cells. Notably, the increased expression of CD98/LAT1, the System L amino acid transporter, granted dramatic activation of mTORC1 in NK cells upon the addition of leucine, one of the substrates for the System L transporter. To our knowledge, this is the first time that the activation of mTORC1 by leucine has been observed in primary immune cells (Fig. 7), highlighting the effect of IL-18 on immunometabolism during inflammation. Therefore, our data demonstrated that IL-18 is a potent cytokine for inducing high expression of nutrient transporters on NK cells and thereby intensifying mTORC1 activation through the transport of leucine.

Several discrete pathways have been described to induce CD98 expression. In addition to our results showing that IL-18 can induce high expression of CD98 on IL-18 receptor-expressing cells such as NK cells, early studies showed that conventional stimulation with phytohemagglutinin, phorbol 12-myristate-13-acetate, ionomycin, or  $\alpha$ -CD3 resulted in a dramatic increase of the CD98 mRNA level in resting human T cells (43, 44). In naïve NK cells, the proliferative cytokines IL-2 and IL-15 also induce a marginal expression of CD98 via the phosphatidylinositol 3-kinase–mTORC1 pathway (4–6, 45). Our data showing the potent effect of IL-18 on the up-regulation of CD98 in the presence of rapamycin supported the conclusion that IL-18 modulates the nutrient uptake by NK cells through a mechanism that is nonredundant of the mTORC1 pathway. The presence of multiple pathways capable of inducing CD98 might guarantee that immune cells are able to up-regulate nutrient transporters during inflammation. Interestingly, the Seahorse data showed that IL-18 is able to enhance the rate of glycolysis in the presence of rapamycin (Fig. 5D). Even though we demonstrated in this report that IL-18 can induce metabolic changes via the leucine-driven mTORC1 pathway,



**Figure 7. Enhanced expression of CD98/LAT1 amino acid transporter by IL-18 induces leucine-driven mTORC1 activation.** The System L transporter is a bidirectional transporter that imports leucine into the cell in exchange for the export of glutamine, requiring intracellular glutamine for the efficient transport of leucine into the cell. IL-18 up-regulates the glutamine transporter (SLC1A5) and leucine transporter (CD98/LAT1), which increases the uptake of leucine and the efflux of glutamine, resulting in mTORC1 activation.

the Seahorse data suggest that IL-18 can increase glycolysis independently of mTORC1. Presumably, the up-regulation of glucose transporters by IL-18 contributes to the increased glycolysis.

The mechanism by which CD98 promotes the proliferation of immune cells is an emerging area of recent research. It is conceivable that CD98 allows the robust proliferation of immune cells by boosting the transport of amino acids because the rapidly proliferating immune cells have intense metabolic demands. However, several lines of evidence have recently indicated that amino acids can have a predominate role in metabolic processes in addition to their role as protein building blocks (35). Notably, the CD98-mediated exchange of glutamine for leucine has been identified as a rate-limiting step in the activation of mTORC1 (29), suggesting the existence of a critical pathway through which CD98 could stimulate the proliferation of immune cells by sustaining mTORC1 activity. To understand the sustained mTORC1 activity during inflammation, we propose a new model with two distinct mTORC1 activation pathways: 1) IL-2/15 initiates the metabolic reprogramming and induces mTORC1 activity, and 2) CD98/LAT1 sustains mTORC1 activity by amino acid-driven mTORC1 activation. For the first time, our model indicates that IL-18 is a potent cytokine for inducing high expression of nutrient trans-

## IL-18 enhances amino acid transporter expression on NK cells

porters on metabolically active immune cells and thereby sustains mTORC1 activation by amino acid transport. Our model is supported by a recent report demonstrating that amino acid transport through CD98 is required to sustain levels of c-Myc, a central regulator of metabolism (46).

This specific role of IL-18 for up-regulating nutrient transporters on naïve NK cells might be due to the basally high level of both chains of the heterodimeric complex of IL-18 receptor as shown here and elsewhere (22, 47). IL-18 receptor expression on T cells has been known to be induced in effector T cells and maintained in memory T cells (48–50). Therefore, our model is also informative to understand the sustained mTORC1 activation during T cell proliferation (47–50). Presumably, the high expression of IL-18 receptor supports the immediate induction of the System L amino acid transporter on NK and memory T cells upon IL-18 stimulation during infections. This single transporter system seems responsible for mediating the uptake of LNAAs such as leucine in immunologically activated NK and T cells and is thus the dominant LNAAs transporter in such cells without redundancy with other System L1 or System  $\gamma$ +L transporters. For example, the roles of CD98/LAT1 in T cells have been extensively studied in the context of infection and autoimmune models. Anti-CD98 mAb completely prevented the onset of cyclophosphamide-induced diabetes in NOD mice, coincident with decreased proliferation of pathogenic CD4<sup>+</sup> T cells (51). T cell-specific deletion of LAT1 resulted in dramatically reduced T cell clonal expansion in an autoimmune model (52). Moreover, LAT1-deficient T cells were unable to reprogram their metabolism in response to antigens and did not undergo clonal expansion or effector differentiation. Interestingly, naïve mice deficient in CD98 or LAT1 in T cells do not show any developmental defects, suggesting that the transporter is exclusively required as a critical metabolic checkpoint for immune cell proliferation during inflammation (52, 53).

Preactivation of NK cells with a combination of IL-12/15/18 was shown to generate so-called cytokine-induced memory-like NK cells that can maintain sustained proliferation and effector function after adoptive transfer and reject established tumors in mice (17). Similarly, the first human clinical trial with NK cells preactivated *ex vivo* with IL-12/15/18 demonstrated that adoptively transferred memory-like NK cells proliferated and expanded in acute myeloid leukemia patients and generated robust responses against leukemia targets (54, 55). For the mechanism underlying the sustained proliferation *in vivo*, we have shown that both IL-12 and IL-18 can induce expression of CD25, the IL-2R $\alpha$  chain, on NK cells (16). Even though the enhanced survival and proliferation of adoptively transferred NK cells are mediated by their increased sensitivity to endogenous levels of low IL-2 due to the high-affinity IL-2 receptor on NK cells, the unique role of IL-18 in the cytokine combination has not been demonstrated. Notably, IL-18 was able to act synergistically with IL-15 in stimulating *in vitro* NK cell proliferation (22); however, the mechanism has not been characterized. Our results demonstrated that IL-18 can induce the metabolic changes of NK cells via the modulation of nutrient transporter expression to support NK cell proliferation and provide an insight for therapeutic applications in infection, cancer, and autoimmune diseases by modulating the metab-

olism and function of immune cells via targeting the IL-18-mediated pathways.

### Experimental procedures

#### Mice and LPS injection

WT C57BL/6 mice from Charles River were housed in a specific pathogen-free environment. All mice used for experiments were aged between 6 and 12 weeks old. All procedures were approved by and conducted in accordance with the institution's animal guidelines of the University of Ottawa. C57BL/6 mice were injected intraperitoneally with 100  $\mu$ g of LPS in PBS. To measure BrdU incorporation *in vivo*, the mice were injected intraperitoneally with 2 mg of BrdU 2 h prior to sacrifice.

#### Cell isolation, NK cell isolation, and *in vitro* stimulation

Spleens were harvested, and a single-cell suspension was generated following red blood cell lysis and filtration through a 70- $\mu$ m filter. NK cells were enriched from the spleen by negative selection using the MagniSort Mouse NK Cell Enrichment kit (eBioscience). Purities of enriched NK cells (NK1.1<sup>+</sup>TCR $\beta$ <sup>-</sup>) were >80%. Isolated NK cells were cultured for 24 h in the presence of 100 units/ml recombinant human (rh) IL-2 with the following different cytokines at the indicated concentrations: IL-1 $\alpha$  and  $\beta$  (100 ng/ml), IL-4 (100 ng/ml), IL-12 (10 ng/ml), IL-15/IL-15R $\alpha$  complex (3 ng/ml), IL-18 (50 ng/ml), IL-21 (100 ng/ml), IFN- $\alpha$  (1,000 units/ml), IFN- $\beta$  (1,000 units/ml), IFN- $\gamma$  (100 ng/ml), and TNF $\alpha$  (100 ng/ml). For expanding NK cells, NK cells were cultured in RP-10 medium (RPMI 1640 medium containing 10% fetal bovine serum, 1 $\times$  penicillin/streptomycin, 2 mM L-glutamine, 10 mmol of HEPES, 50  $\mu$ mol of 2-mercaptoethanol) for 7–10 days in the presence of 1,000 units/ml rhIL-2 (obtained from National Cancer Institute Preclinical Repository). For the NK cell proliferation assay, NK cells were enriched from the spleen as mentioned above. Enriched NK cells were labeled with Cell Proliferation Dye eFluor 450 (eBioscience). Cells were then cultured in RP-10 medium with 300 units/ml rhIL-2 and various concentrations of IL-18 for 3 days.

#### Flow cytometric analysis

The following mAbs were used: anti-CD3 (17A2 and 145-2C11), anti-TCR $\beta$  (H57-597), anti-CD8 (53-6.7), anti-CD49b (DX5), anti-granzyme B (NGZB), anti-IL-18R $\alpha$  (P3TUNYA), and anti-phospho-S6(Ser-235/Ser-236) (CUPK43K) from eBioscience; anti-CD19 (1D3), anti-CD4 (RM4-5), anti-F4/80 (T45-2342), anti-NK1.1 (PK136), anti-CD107a (1D4B), anti-IFN $\gamma$  (XMG1.2), anti-Ki-67 (B56), and anti-BrdU (3D4) from BD Biosciences; and anti-CD71 (RT7217) and anti-CD98 (RL388) from BioLegend. Live/Dead Fixable Yellow Dead Cell Stain was from Invitrogen. Intracellular staining of Ki-67 was carried out using a Foxp3 staining kit (eBioscience). Cells were acquired using a BD LSRFortessa or BD FACSCelesta and analyzed using Kaluza 1.3 Analysis software (Beckman Coulter) or FlowJo (Tree Star). The intracellular staining of anti-phospho-S6 (Ser-235/Ser-236) was performed using BD Cytofix/Cytoperm protocols (BD Biosciences).

### Quantitative real-time PCR

For the quantification of transcripts of IL-1 and IL-18 receptors, NK cells from C57BL/6 mice were isolated by cell sorting. Purities of sorted NK cells were >98%. Total RNA was extracted from the sorted NK cells using TRIzol reagent (Life Technologies) according to the manufacturer's instructions. For the quantification of transcripts of nutrient receptor genes, NK cells from C57BL/6 mice were isolated by cell sorting as mentioned above. The sorted NK cells were then stimulated with IL-12 (30 ng/ml) and/or IL-18 (30 ng/ml) for 18 h, and the total RNA was extracted as mentioned above. cDNA was reverse transcribed in a 20- $\mu$ l reaction using the iScript Reverse Transcription Supermix (Bio-Rad). For quantification of target genes by real-time PCR, cDNA was added to a 20- $\mu$ l reaction of FastStart Universal SYBR Green Master (Rox) (Roche Applied Science) and amplified using an Applied Biosystems 7500 Real-Time PCR System (Thermo Fisher Scientific). Expression of target genes was normalized to  $\beta$ -actin levels. The primer sequences for the target genes are shown in Table S1.

### ECAR measurement by Seahorse technology

Isolated NK cells were cultured for 7–10 days in the presence of 1,000 units/ml recombinant human IL-2 and treated with or without 30 ng/ml IL-18 for 24 h in the presence or absence of 1.6  $\mu$ M rapamycin. XF 24-well microplates (Seahorse Bioscience) were precoated with CellTaq (BD Biosciences) for 2 h before seeding the NK cells on the plate for real-time analysis of the ECAR.  $10^6$  NK cells were cultured per well, and various inhibitors were added (Agilent Seahorse XF Cell glycolysis Stress Test) at the following concentrations: oligomycin (2  $\mu$ M), 2-deoxyglucose (30 mM), and glucose (5 mM), which allow the accurate calculation of glycolysis (ECAR).

### Glucose uptake assay

$5 \times 10^5$ – $10^6$  spleen cells/ml were washed with PBS and incubated for 15 min in RPMI 1640 medium without glucose (Corning) supplemented with 10% dialyzed serum (Thermo Fisher Scientific), 2 mM L-glutamine, 1 mM HEPES, 1% penicillin/streptomycin, and 50  $\mu$ mol of 2-mercaptoethanol at 37 °C. Cells were incubated for 1 h in the glucose-free medium with 50  $\mu$ M 2-NBDG (Life Technologies) at 37 °C. Cells were washed twice with PBS and stained for NK1.1, TCR $\beta$ , and Live/Dead Fixable Yellow (Invitrogen) on ice for 25 min before being analyzed using flow cytometry.

### Leucine-driven mTORC1 activation

Expanded NK cells were stimulated with either 1,000 units/ml recombinant human IL-2 alone or with 30 ng/ml recombinant mouse IL-18 for 18 h. Cells were washed with PBS three times to remove the remaining amino acids and fetal bovine serum. Cells were cultured in RPMI 1640 medium without L-glutamine and amino acids (MyBioSource) for starvation for 3 h. Cells were pretreated with 15 mM L-glutamine (Sigma) for 1 h and washed three times. Cells were cultured in starvation medium supplemented with different concentrations of leucine (0.4, 2, and 10 mM) for different time points (20, 30, 40, 50, and 60 min). The mTOR inhibitor rapamycin (1.6  $\mu$ M) and protein

synthesis inhibitor cycloheximide (10  $\mu$ M) were purchased from Calbiochem. The leucine antagonist NALA was used for 30 min and was purchased from Bachem. The following inhibitors were purchased from Sigma and used for the indicated periods: BCH, and inhibitor of the System L amino acid transporter, 30 min; GPNA, a glutamine uptake inhibitor, 1 h; and MeAIB, an inhibitor of System A amino acid transporters, 1 h. Cells were stained for NK1.1, TCR $\beta$ , pS6, and Live/Dead Fixable Yellow and analyzed using flow cytometry. To study the bidirectional exchange of glutamine for leucine,  $^3$ H-labeled L-glutamine (NET551250UC) and  $^3$ H-labeled L-leucine (NET460250UC) were purchased from PerkinElmer Life Sciences. Cells were treated as mentioned above with the addition of  $^3$ H-labeled amino acids. To measure leucine influx, starved NK cells were preloaded with unlabeled L-glutamine and treated with 0.05  $\mu$ Ci/ml  $^3$ H-labeled L-leucine. The cells were then washed twice with ice-cold PBS, resuspended in 50  $\mu$ l of PBS, lysed with 50  $\mu$ l of 2% SDS, and transferred into liquid scintillation vials. To measure glutamine efflux into the culture medium, starved NK cells were preloaded with both 15 mM unlabeled L-glutamine and 0.5  $\mu$ Ci/ml  $^3$ H-labeled L-glutamine and treated with unlabeled L-leucine. The cells were then centrifuged, and 50  $\mu$ l of conditioned medium was transferred to liquid scintillation vials. 1 ml of EcoLite(+)<sup>TM</sup> liquid scintillation mixture (MP Biomedicals) was added to each vial, and the  $^3$ H-labeled amino acids were measured using the Tri-Carb 2910TR liquid scintillation analyzer (PerkinElmer Life Sciences).

### Western blotting

Samples were separated by SDS-PAGE and transferred onto nitrocellulose membranes. Membranes were probed for total p70 S6K (catalog number 9202), phospho-p70 S6K (Thr-389, clone 108D2), and  $\beta$ -actin (clone 13E5) rabbit antibodies, all from Cell Signaling Technology, in 5% BSA in Tris-buffered saline, 0.05% Tween 80. Membranes were then incubated with horseradish peroxidase-linked anti-rabbit IgG (Cell Signaling Technology), developed with Clarity enhanced chemiluminescent substrate (Bio-Rad), and chemiluminescence signals were visualized with the ImageQuant LAS 4010 system (GE Healthcare).

### Statistical analysis

The mean values in the experiment were tested by analysis of variance. If the analysis of variance rejected the null hypothesis of the same means among the conditions ( $p < 0.01$ ), multiple comparisons were performed between selected pairs of means by two-tailed unpaired *t* test (\*,  $p < 0.05$ ; \*\*,  $p < 0.01$ ; \*\*\*,  $p < 0.001$ ; \*\*\*\*,  $p < 0.0001$ ) using Prism version 5 (GraphPad Software).

*Author contributions*—S. M. A. and A. K. A. conceptualization; S. M. A. and A. K. A. resources; S. M. A., A. K. A., W. H., D.-S. Y., and P. G. data curation; S. M. A. and A. K. A. software; S. M. A., A. K. A., and M. D. F. formal analysis; S. M. A. and A. K. A. validation; S. M. A. and A. K. A. investigation; S. M. A. and A. K. A. methodology; S. M. A., A. K. A., and S.-H. L. writing-original draft; S. M. A. and A. K. A. project administration; S. M. A., A. K. A., L. W., and S.-H. L. writing-review and editing; S.-H. L. supervision.

*Acknowledgments*—We thank Georges Kanaan in Dr. Mary Ellen Harper's laboratory at the University of Ottawa for help with the Seahorse assay. We also thank the National Institutes of Health/NCI at Frederick Biological Resources Branch Preclinical Repository for providing the rhIL-2 cytokine.

### References

- Biron, C. A., Turgiss, L. R., and Welsh, R. M. (1983) Increase in NK cell number and turnover rate during acute viral infection. *J. Immunol.* **131**, 1539–1545 [Medline](#)
- Nguyen, K. B., Salazar-Mather, T. P., Dalod, M. Y., Van Deusen, J. B., Wei, X. Q., Liew, F. Y., Caligiuri, M. A., Durbin, J. E., and Biron, C. A. (2002) Coordinated and distinct roles for IFN- $\alpha$ , IL-12, and IL-15 regulation of NK cell responses to viral infection. *J. Immunol.* **169**, 4279–4287 [CrossRef Medline](#)
- Williams, H., McAulay, K., Macsween, K. F., Gallacher, N. J., Higgins, C. D., Harrison, N., Swerdlow, A. J., and Crawford, D. H. (2005) The immune response to primary EBV infection: a role for natural killer cells. *Br. J. Haematol.* **129**, 266–274 [CrossRef Medline](#)
- Marçais, A., Cherfils-Vicini, J., Viant, C., Degouve, S., Viel, S., Fenis, A., Rabilloud, J., Mayol, K., Tavares, A., Bienvenu, J., Gangloff, Y. G., Gilson, E., Vivier, E., and Walzer, T. (2014) The metabolic checkpoint kinase mTOR is essential for IL-15 signaling during the development and activation of NK cells. *Nat. Immunol.* **15**, 749–757 [CrossRef Medline](#)
- Nandagopal, N., Ali, A. K., Komal, A. K., and Lee, S. H. (2014) The critical role of IL-15-PI3K-mTOR pathway in natural killer cell effector functions. *Front. Immunol.* **5**, 187 [CrossRef Medline](#)
- Donnelly, R. P., Loftus, R. M., Keating, S. E., Liou, K. T., Biron, C. A., Gardiner, C. M., and Finlay, D. K. (2014) mTORC1-dependent metabolic reprogramming is a prerequisite for NK cell effector function. *J. Immunol.* **193**, 4477–4484 [CrossRef Medline](#)
- Huang, K., and Fingar, D. C. (2014) Growing knowledge of the mTOR signaling network. *Semin. Cell Dev. Biol.* **36**, 79–90 [CrossRef Medline](#)
- Keppel, M. P., Saucier, N., Mah, A. Y., Vogel, T. P., and Cooper, M. A. (2015) Activation-specific metabolic requirements for NK cell IFN- $\gamma$  production. *J. Immunol.* **194**, 1954–1962 [CrossRef Medline](#)
- Laplanche, M., and Sabatini, D. M. (2012) mTOR signaling in growth control and disease. *Cell* **149**, 274–293 [CrossRef Medline](#)
- Okamura, H., Tsutsi, H., Komatsu, T., Yutsudo, M., Hakura, A., Tanimoto, T., Torigoe, K., Okura, T., Nukada, Y., Hattori, K., Akita, K., Namba, M., Tanabe, F., Konishi, K., Fukuda, S., *et al.* (1995) Cloning of a new cytokine that induces IFN- $\gamma$  production by T cells. *Nature* **378**, 88–91 [CrossRef Medline](#)
- Wawrocki, S., Druszczynska, M., Kowalewicz-Kulbat, M., and Rudnicka, W. (2016) Interleukin 18 (IL-18) as a target for immune intervention. *Acta Biochim. Pol.* **63**, 59–63 [CrossRef Medline](#)
- Novick, D., Kim, S., Kaplanski, G., and Dinarello, C. A. (2013) Interleukin-18, more than a Th1 cytokine. *Semin. Immunol.* **25**, 439–448 [CrossRef Medline](#)
- Rathinam, V. A., Jiang, Z., Waggoner, S. N., Sharma, S., Cole, L. E., Waggoner, L., Vanaja, S. K., Monks, B. G., Ganesan, S., Latz, E., Hornung, V., Vogel, S. N., Szomolanyi-Tsuda, E., and Fitzgerald, K. A. (2010) The AIM2 inflammasome is essential for host defense against cytosolic bacteria and DNA viruses. *Nat. Immunol.* **11**, 395–402 [CrossRef Medline](#)
- Pien, G. C., Satoskar, A. R., Takeda, K., Akira, S., and Biron, C. A. (2000) Cutting edge: selective IL-18 requirements for induction of compartmental IFN- $\gamma$  responses during viral infection. *J. Immunol.* **165**, 4787–4791 [CrossRef Medline](#)
- Loh, J., Chu, D. T., O'Guin, A. K., Yokoyama, W. M., and Virgin, H. W., 4th (2005) Natural killer cells utilize both perforin and  $\gamma$  interferon to regulate murine cytomegalovirus infection in the spleen and liver. *J. Virol.* **79**, 661–667 [CrossRef Medline](#)
- Lee, S. H., Fragoso, M. F., and Biron, C. A. (2012) Cutting edge: a novel mechanism bridging innate and adaptive immunity: IL-12 induction of CD25 to form high-affinity IL-2 receptors on NK cells. *J. Immunol.* **189**, 2712–2716 [CrossRef Medline](#)
- Ni, J., Miller, M., Stojanovic, A., Garbi, N., and Cerwenka, A. (2012) Sustained effector function of IL-12/15/18-preactivated NK cells against established tumors. *J. Exp. Med.* **209**, 2351–2365 [CrossRef Medline](#)
- Leong, J. W., Chase, J. M., Romee, R., Schneider, S. E., Sullivan, R. P., Cooper, M. A., and Fehniger, T. A. (2014) Preactivation with IL-12, IL-15, and IL-18 induces CD25 and a functional high-affinity IL-2 receptor on human cytokine-induced memory-like natural killer cells. *Biol. Blood Marrow Transplant.* **20**, 463–473 [CrossRef Medline](#)
- Chaix, J., Tessmer, M. S., Hoebe, K., Fuséri, N., Ryffel, B., Dalod, M., Alexopoulou, L., Beutler, B., Brossay, L., Vivier, E., and Walzer, T. (2008) Cutting edge: priming of NK cells by IL-18. *J. Immunol.* **181**, 1627–1631 [CrossRef Medline](#)
- Lauwerys, B. R., Renauld, J. C., and Houssiau, F. A. (1999) Synergistic proliferation and activation of natural killer cells by interleukin 12 and interleukin 18. *Cytokine* **11**, 822–830 [CrossRef Medline](#)
- Son, Y. I., Dallal, R. M., Mailliard, R. B., Egawa, S., Jonak, Z. L., and Lotze, M. T. (2001) Interleukin-18 (IL-18) synergizes with IL-2 to enhance cytotoxicity, interferon- $\gamma$  production, and expansion of natural killer cells. *Cancer Res.* **61**, 884–888 [Medline](#)
- French, A. R., Holroyd, E. B., Yang, L., Kim, S., and Yokoyama, W. M. (2006) IL-18 acts synergistically with IL-15 in stimulating natural killer cell proliferation. *Cytokine* **35**, 229–234 [CrossRef Medline](#)
- Andrews, D. M., Scalzo, A. A., Yokoyama, W. M., Smyth, M. J., and Degli-Esposti, M. A. (2003) Functional interactions between dendritic cells and NK cells during viral infection. *Nat. Immunol.* **4**, 175–181 [CrossRef Medline](#)
- Edinger, A. L. (2007) Controlling cell growth and survival through regulated nutrient transporter expression. *Biochem. J.* **406**, 1–12 [CrossRef Medline](#)
- Lin, L., Yee, S. W., Kim, R. B., and Giacomini, K. M. (2015) SLC transporters as therapeutic targets: emerging opportunities. *Nat. Rev. Drug Discov.* **14**, 543–560 [CrossRef Medline](#)
- Verrey, F., Closs, E. I., Wagner, C. A., Palacin, M., Endou, H., and Kanai, Y. (2004) CATs and HATs: the SLC7 family of amino acid transporters. *Pflugers Arch.* **447**, 532–542 [CrossRef Medline](#)
- McCracken, A. N., and Edinger, A. L. (2013) Nutrient transporters: the Achilles' heel of anabolism. *Trends Endocrinol. Metab.* **24**, 200–208 [CrossRef Medline](#)
- Bröer, S., and Palacín, M. (2011) The role of amino acid transporters in inherited and acquired diseases. *Biochem. J.* **436**, 193–211 [CrossRef Medline](#)
- Nicklin, P., Bergman, P., Zhang, B., Triantafellow, E., Wang, H., Nyfeler, B., Yang, H., Hild, M., Kung, C., Wilson, C., Myer, V. E., MacKeigan, J. P., Porter, J. A., Wang, Y. K., Cantley, L. C., *et al.* (2009) Bidirectional transport of amino acids regulates mTOR and autophagy. *Cell* **136**, 521–534 [CrossRef Medline](#)
- Goberdhan, D. C., Wilson, C., and Harris, A. L. (2016) Amino acid sensing by mTORC1: intracellular transporters mark the spot. *Cell Metab.* **23**, 580–589 [CrossRef Medline](#)
- Weichhart, T., Hengstschläger, M., and Linke, M. (2015) Regulation of innate immune cell function by mTOR. *Nat. Rev. Immunol.* **15**, 599–614 [CrossRef Medline](#)
- Hara, K., Yonezawa, K., Weng, Q. P., Kozlowski, M. T., Belham, C., and Avruch, J. (1998) Amino acid sufficiency and mTOR regulate p70 S6 kinase and eIF-4E BP1 through a common effector mechanism. *J. Biol. Chem.* **273**, 14484–14494 [CrossRef Medline](#)
- Wang, X., Campbell, L. E., Miller, C. M., and Proud, C. G. (1998) Amino acid availability regulates p70 S6 kinase and multiple translation factors. *Biochem. J.* **334**, 261–267 [CrossRef Medline](#)
- Sancak, Y., Peterson, T. R., Shaul, Y. D., Lindquist, R. A., Thoreen, C. C., Bar-Peled, L., and Sabatini, D. M. (2008) The Rag GTPases bind raptor and mediate amino acid signaling to mTORC1. *Science* **320**, 1496–1501 [CrossRef Medline](#)
- Jewell, J. L., Russell, R. C., and Guan, K. L. (2013) Amino acid signalling upstream of mTOR. *Nat. Rev. Mol. Cell Biol.* **14**, 133–139 [CrossRef Medline](#)

36. Strowig, T., Henao-Mejia, J., Elinav, E., and Flavell, R. (2012) Inflammasomes in health and disease. *Nature* **481**, 278–286 [CrossRef Medline](#)
37. Tsujimoto, H., Uchida, T., Efron, P. A., Scumpia, P. O., Verma, A., Matsu-moto, T., Tschoeke, S. K., Ungaro, R. F., Ono, S., Seki, S., Clare-Salzler, M. J., Baker, H. V., Mochizuki, H., Ramphal, R., and Moldawer, L. L. (2005) Flagellin enhances NK cell proliferation and activation directly and through dendritic cell-NK cell interactions. *J. Leukoc. Biol.* **78**, 888–897 [CrossRef Medline](#)
38. Goodier, M. R., and Londei, M. (2000) Lipopolysaccharide stimulates the proliferation of human CD56+CD3– NK cells: a regulatory role of mono-cytes and IL-10. *J. Immunol.* **165**, 139–147 [CrossRef Medline](#)
39. Esslinger, C. S., Cybulski, K. A., and Rhoderick, J. F. (2005) N $\gamma$ -aryl glutamine analogues as probes of the ASCT2 neutral amino acid transporter binding site. *Bioorg. Med. Chem.* **13**, 1111–1118 [CrossRef Medline](#)
40. Cui, W., and Kaech, S. M. (2010) Generation of effector CD8+ T cells and their conversion to memory T cells. *Immunol. Rev.* **236**, 151–166 [CrossRef Medline](#)
41. Guo, H., Callaway, J. B., and Ting, J. P. (2015) Inflammasomes: mechanism of action, role in disease, and therapeutics. *Nat. Med.* **21**, 677–687 [CrossRef Medline](#)
42. Latz, E., Xiao, T. S., and Stutz, A. (2013) Activation and regulation of the inflammasomes. *Nat. Rev. Immunol.* **13**, 397–411 [CrossRef Medline](#)
43. Gottesdiener, K. M., Karpinski, B. A., Lindsten, T., Strominger, J. L., Jones, N. H., Thompson, C. B., and Leiden, J. M. (1988) Isolation and structural characterization of the human 4F2 heavy-chain gene, an inducible gene involved in T-lymphocyte activation. *Mol. Cell. Biol.* **8**, 3809–3819 [CrossRef Medline](#)
44. Lindsten, T., June, C. H., Thompson, C. B., and Leiden, J. M. (1988) Reg-ulation of 4F2 heavy-chain gene expression during normal human T-cell activation can be mediated by multiple distinct molecular mechanisms. *Mol. Cell. Biol.* **8**, 3820–3826 [CrossRef Medline](#)
45. Jensen, H., Potempa, M., Gotthardt, D., and Lanier, L. L. (2017) Cutting edge: IL-2-induced expression of the amino acid transporters SLC1A5 and CD98 is a prerequisite for NKG2D-mediated activation of human NK cells. *J. Immunol.* **199**, 1967–1972 [CrossRef Medline](#)
46. Loftus, R. M., Assmann, N., Kedia-Mehta, N., O'Brien, K. L., Garcia, A., Gillespie, C., Hukelmann, J. L., Oefner, P. J., Lamond, A. I., Gardiner, C. M., Dettmer, K., Cantrell, D. A., Sinclair, L. V., and Finlay, D. K. (2018) Amino acid-dependent cMyc expression is essential for NK cell metabolic and functional responses in mice. *Nat. Commun.* **9**, 2341 [CrossRef Medline](#)
47. Hyodo, Y., Matsui, K., Hayashi, N., Tsutsui, H., Kashiwamura, S., Yamauchi, H., Hiroishi, K., Takeda, K., Tagawa, Y., Iwakura, Y., Kayagaki, N., Kurimoto, M., Okamura, H., Hada, T., Yagita, H., *et al.* (1999) IL-18 up-regulates perforin-mediated NK activity without increasing perforin mes-senger RNA expression by binding to constitutively expressed IL-18 re-ceptor. *J. Immunol.* **162**, 1662–1668 [Medline](#)
48. Haining, W. N., Ebert, B. L., Subrmanian, A., Wherry, E. J., Eichbaum, Q., Evans, J. W., Mak, R., Rivoli, S., Pretz, J., Angelosanto, J., Smutko, J. S., Walker, B. D., Kaech, S. M., Ahmed, R., Nadler, L. M., *et al.* (2008) Identi-fication of an evolutionarily conserved transcriptional signature of CD8 memory differentiation that is shared by T and B cells. *J. Immunol.* **181**, 1859–1868 [CrossRef Medline](#)
49. Luckey, C. J., Bhattacharya, D., Goldrath, A. W., Weissman, I. L., Benoist, C., and Mathis, D. (2006) Memory T and memory B cells share a transcrip-tional program of self-renewal with long-term hematopoietic stem cells. *Proc. Natl. Acad. Sci. U.S.A.* **103**, 3304–3309 [CrossRef Medline](#)
50. Wherry, E. J., Ha, S. J., Kaech, S. M., Haining, W. N., Sarkar, S., Kalia, V., Subramaniam, S., Blattman, J. N., Barber, D. L., and Ahmed, R. (2007) Molecular signature of CD8+ T cell exhaustion during chronic viral in-fection. *Immunity* **27**, 670–684 [CrossRef Medline](#)
51. Lian, G., Arimochi, H., Kitamura, A., Nishida, J., Li, S., Kishihara, K., Maekawa, Y., and Yasutomo, K. (2012) Manipulation of CD98 resolves type 1 diabetes in nonobese diabetic mice. *J. Immunol.* **188**, 2227–2234 [CrossRef Medline](#)
52. Cantor, J., Slepak, M., Ege, N., Chang, J. T., and Ginsberg, M. H. (2011) Loss of T cell CD98 H chain specifically ablates T cell clonal expansion and protects from autoimmunity. *J. Immunol.* **187**, 851–860 [CrossRef Medline](#)
53. Sinclair, L. V., Rolf, J., Emslie, E., Shi, Y. B., Taylor, P. M., and Cantrell, D. A. (2013) Control of amino-acid transport by antigen receptors coordinates the metabolic reprogramming essential for T cell differentiation. *Nat. Im-munol.* **14**, 500–508 [CrossRef Medline](#)
54. Romee, R., Schneider, S. E., Leong, J. W., Chase, J. M., Keppel, C. R., Sullivan, R. P., Cooper, M. A., and Fehniger, T. A. (2012) Cytokine activa-tion induces human memory-like NK cells. *Blood* **120**, 4751–4760 [CrossRef Medline](#)
55. Romee, R., Rosario, M., Berrien-Elliott, M. M., Wagner, J. A., Jewell, B. A., Schappe, T., Leong, J. W., Abdel-Latif, S., Schneider, S. E., Willey, S., Neal, C. C., Yu, L., Oh, S. T., Lee, Y. S., Mulder, A., *et al.* (2016) Cytokine-in-duced memory-like natural killer cells exhibit enhanced responses against myeloid leukemia. *Sci. Transl. Med.* **8**, 357ra123 [CrossRef Medline](#)

**Interleukin-18 up-regulates amino acid transporters and facilitates amino acid–induced mTORC1 activation in natural killer cells**

Saeedah MUSAED Almutairi, Alaa Kassim Ali, William He, Doo-Seok Yang, Peyman Ghorbani, Lisheng Wang, Morgan D. Fullerton and Seung-Hwan Lee

*J. Biol. Chem.* 2019, 294:4644-4655.

doi: 10.1074/jbc.RA118.005892 originally published online January 29, 2019

---

Access the most updated version of this article at doi: [10.1074/jbc.RA118.005892](https://doi.org/10.1074/jbc.RA118.005892)

Alerts:

- [When this article is cited](#)
- [When a correction for this article is posted](#)

[Click here](#) to choose from all of JBC's e-mail alerts

This article cites 55 references, 26 of which can be accessed free at <http://www.jbc.org/content/294/12/4644.full.html#ref-list-1>