Original Article

The Potential Inflammatory Role of IL-6 Signalling in Perturbing the Energy Metabolism Function by Stimulating the Akt-mTOR Pathway in Jurkat T Cells

(IL-6 / Jurkat cell line / glycolysis / OXPHOS / Akt / mTOR)

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Abstract. Numerous studies have reported that increased interleukin 6 (IL-6) and soluble IL-6 receptor (sIL-6) levels induce inflammatory conditions. However, the exact mechanisms by which IL-6 drives inflammatory conditions remain unclear. Therefore, we investigated the potential role of IL-6/sIL-6R in inducing energy metabolism, including glycolysis, oxidative phosphorylation, lactate secretion and Akt/mTOR phosphorylation, in Jurkat cells, and whether IL-6 would increase the risk of developing inflammatory conditions due to the high metabolic profile of the T cells. Jurkat CD4 T-cell lines were stimulated with IL-6/sIL-6R for 24 h prior to 48-h stimulation with anti-CD3/CD28. Lactate secretion, glycolysis and oxidative phosphorylation levels were characterized using the Seahorse XF analyser. The Akt and mTOR phosphorylation status was detected using Western blotting. IL-6/sIL-6R significantly induced glycolysis and oxidative phosphorylation and their related parameters, including glycolytic capacity and maximal respiration, followed by significantly increased lactate secretion. Akt and mTOR phosphorylation were increased, which could have resulted from energy metabolism. Here we show that IL-6 enhanced the metabolic profile of Jurkat cells. This effect could have consequences for the metabolism-related signalling pathways, including Akt and mTOR, suggesting that IL-6 might promote T-cell energy metabolism, where T-cell hyperactivity might increase the inflammatory disease risk. The findings should be validated using studies on primary cells isolated from humans.

Introduction

Energy metabolism is key in cell signalling, differentiation and proliferation. Glucose is metabolized to produce adenosine triphosphate (ATP), which is essential to provide cells with sufficient energy through the glycolysis or oxidative phosphorylation (OXPHOS) pathway (Rigoulet et al., 2020). As immunity and metabolism are crucial biological processes, they are important in homeostasis balance. Immunometabolism has recently increased the understanding of how metabolic pathway activity regulates immune cells (Makowski et al., 2020). Lactate is a glycolysis by-product and is considered as waste. However, lactate can be an important supplementary energy source to support brain activity when blood glucose levels decrease (Dienel, 2019). Furthermore, lactate is protective by supporting neuronal activity, suggesting that it is an important candidate to support the body energy balance (Lhomme et al., 2021). Metabolism imbalances trigger autoimmunity by stimulating the cytokine network, which includes interleukin 6 (IL-6).

IL-6 is one of the most critical cytokines directly involved in immune cell metabolism by modulating innate and adaptive immunity (Jones, 2005). It regulates OXPHOS, glycolysis and glucose uptake, suggesting that IL-6 can be anti-inflammatory by promoting cell proliferation and inhibiting apoptosis. IL-6 can also be pro-inflammatory by promoting the progression of rheu-

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Abbreviations: AMPK – AMP-activated protein kinase, ATP – adenosine triphosphate, ECAR – extracellular acidification rate, ERK – extracellular signal-regulated kinase, ETC – electron transport chain, GLUT 4 – glucose transporter 4, IL-6 – interleukin 6, OCR – oxygen consumption rate, OXPHOS – oxidative phosphorylation, PI3K-Akt-mTOR – phosphoinositide 3-kinase-protein kinase B-mammalian target of rapamycin, RA – rheumatoid arthritis, STAT3 – signal transducer and activator of transcription 3, TCR – T-cell receptor, TSC2 – tuberous sclerosis complex 2.

matoid arthritis (RA) and cancers (Helge et al., 2003; Marasco et al., 2018; Boyapati et al., 2020). IL-6 signalling activation involves the formation of a complex comprising IL-6 and the soluble IL-6 receptor (sIL6R) (*trans*-signalling), a classic signalling transmembrane IL-6 receptor (mIL-6R), or subunit molecule glycoprotein 130 (gp130) signalling to form two complexes: the IL-6–sIL-6R gp130 complex or IL-6–IL-6R gp130 complex (Uciechowski and Dempke, 2020). The membrane-bound form of IL-6R is mainly restricted to hepatocytes and myeloid cells, whereas sIL-6R can be found in most cells and is more frequently linked to inflammation induction (Nowell et al., 2003). The effects of IL-6 on energy metabolism affect immune cell signalling pathways. Consequently, the signalling pathway alteration increases immune cell activity and promotes autoimmunity. This led us to investigate the Jurkat CD4 T-cell line to understand the mechanism by which IL-6 alters energy metabolism and signalling pathways to induce inflammation.

Jurkat cells are widely used *in vitro* to study intracellular signalling, novel treatments, viral diseases and T-cell leukaemia (Yang et al., 2019). Jurkat cells are a robust and good tool to optimize and establish a methodology to investigate specific mechanisms or treatments before progressing to more sensitive cells, such as primary $CD4^+$ T cells, which include naïve and memory cells. T-cell receptors (TCR) trigger T-cell signalling, which is involved in various biological processes. However, the lack of TCR signalling has been linked to human diseases. One mechanism that regulates TCR signalling involves protein kinases, such as those participating in the phosphoinositide 3-kinases-protein kinase B-mammalian target of rapamycin (PI3K-Akt-mTOR) pathway, which is important in controlling T-cell metabolism and proliferation (Hawse et al., 2017; Jeong et al., 2023). Accordingly, we targeted Akt and mTOR with IL-6 to investigate whether IL-6 would alter the energy metabolism through this pathway.

Material and Methods

Cell culture and treatment

The Jurkat cell line, clone E6-1 was obtained from ATCC (TIB-152, ATCC, Manassas, VA). The cells were maintained in RPMI 1640 medium (R0883, Sigma-Aldrich, Dorset, UK) supplemented with 10 % foetal bovine serum (FBS, F7524, Sigma-Aldrich) and 1 % glutamine-penicillin-streptomycin (GPS) (G6784, Sigma-Aldrich) at 37 °C in a 5 % CO_2 incubator. The cells were collected from the incubator, washed, counted and incubated for 24 h with range concentrations of IL-6 (11340066, ImmunoTools, Friesoythe, Germany) and equimolar sIL-6R (11346064, ImmunoTools): 0, 0.2, 0.5 and 1 ng/ml. The concentrations were selected based on their physiological relevance. Between 2.0×10^6 and 3.0×10^6 cells/ml were seeded in 6-well plates (3516, Corning, Corning, NY). After 24 h, the cells were washed with culture medium, counted and stimulated for 48 h with anti-CD3 (2.5 µg/ml) (21850030, ImmunoTools) and CD28 (1 µg/ml) (21270281, ImmunoTools). The cells (2.0×10^5 cells/well) were seeded in a 96-well plate (3598, Corning). After 48 h, the cells were used based on the experiment design.

Seahorse XF flux analysis

Glycolysis and OXPHOS were determined using a Seahorse XFe96 analyser (Agilent, Santa Clara, CA), which measured the oxygen consumption rate (OCR, an indicator of the OXPHOS) and extracellular acidification rate (ECAR, an indicator of the glycolysis). Briefly, the Agilent calibrant solution was added to the cartridge plate and incubated in a non-CO_2 incubator at 37 °C one day before the experiment. The next day, a Seahorse cell culture plate was coated with Cell-Tak (CLS354240, Sigma-Aldrich) to allow the cells to attach to the bottom of the well. The cells were collected from the incubator, washed, counted and resuspended in the Seahorse XF medium, pH 7.4 (103576, Agilent). The Cell-Tak was washed off the Seahorse cell culture plate using sterile water, then the cells were added to each well except the wells in the corners (for background correction), which were topped up with the Seahorse XF medium. The plate was centrifuged for 2 min at 200 × *g*, then the Seahorse XF medium was added to the wells.

Glycolysis, mitochondrial functions and other related parameters were assessed by supplementing the medium with inhibitor drugs to induce the measurements of those parameters. The drug concentrations used were guided by previously published work (van der Windt et al., 2016). The concentrations used were as follows: 1 µM oligomycin (ATP synthase inhibitor, O4876, Sigma-Aldrich), 1 µM FCCP (oxidative phosphorylation uncoupler in mitochondria, C2920, Sigma-Aldrich), 1 mM glucose (to induce the glycolytic pathway, G6021, Sigma-Aldrich), 5 mM 2-deoxy-D-glucose (2-DG, glucose analogue and glycolysis inhibitor, D8375, Sigma-Aldrich), 1 µM antimycin A (complex III inhibitor of the electron transport chain, A8674, Sigma-Aldrich) and 0.5 µM rotenone (complex I inhibitor of the electron transport chain, R8875, Sigma-Aldrich). The calibrant plate was loaded into the machine for 20 min before loading the cell culture plate to run the assay.

Lactate assay

The lactate concentration in the Jurkat cells was determined using a commercial lactate assay kit (MAK064-1KT, Sigma-Aldrich). Briefly, the cell supernatants were centrifuged for 10 min at 12,000 × *g*. Next, proteins, including lactate dehydrogenase (LDH), were eliminated from the sample using ultrafiltration with a 10-kDa spin column (ab93349, Abcam, Cambridge, UK). Using a microplate, the absorbance was measured at 570 nm. The standard curve was used to obtain the concentration of the lactate.

Western blotting

The protein concentrations were measured using the bicinchoninic acid assay (BCA), then the cells were lysed using radioimmunoprecipitation assay (RIPA) buffer supplemented with protease and phosphatase inhibitors (1 : 100 dilution, Abcam). Briefly, the cell lysates were mixed with loading buffer (4× Laemmli sample buffer, 1610747, Bio-Rad, Watford, UK), transferred to 2–20 % precast protein gel (4561094, Bio-Rad) and separated using sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) (1658005, Bio-Rad). The proteins were transferred to a Trans-Blot Turbo PVDF transfer membrane (1704157, Bio-Rad), which was placed for 7 min in a Trans-Blot machine (Bio-Rad). Then, the membrane was blocked for 1 h with 5 % milk in Tris-buffered saline with Tween-20 (TBST). The membrane was washed and primary antibodies against phosphorylated (p)Akt (4056S, Cell Signaling, London, UK), total Akt (9272S, Cell Signaling), pmTOR (2971S, Cell Signaling), total mTOR (2972S, Cell Signaling) and beta-actin (4967S, Cell Signaling) were added and incubated overnight at 4 °C. Subsequently, horseradish peroxidase (HRP)-conjugated anti-rabbit or anti-mouse antibody (7074P2 or 7076P2, Cell Signaling) was added to the membrane and incubated for 1 h. The protein bands were visualized by adding Enhanced Chemiluminescence reagents (ECL, 1705061, Bio-Rad) to the membrane. Finally, the membrane was scanned using the ChemiDoc imaging system (Bio-Rad). The bands obtained from the machine were analysed using ImageJ version 1.54.

Statistical analysis

The data were analysed using GraphPad Prism 8 (GraphPad Software Inc., San Diego, CA). The concentrations were compared using one-way analysis of variance (ANOVA). The difference between treated cells and stimulated or untreated cells was analysed using the Mann-Whitney test. All data are reported as the mean ± SEM and were considered statistically significant when $P < 0.05$.

Results

IL-6/sIL-6R increases Jurkat cell glycolysis, glycolytic capacity and basal ECAR but not glycolytic reserve and non-glycolytic acidification rate

Metabolism is important for immune cell functions; therefore, we investigated the effect of IL-6 on the Jurkat cell glycolytic metabolism and signalling. Glycolysis is a potential immune response regulatory pathway. However, dysregulation leads to more energy production, which hyperactivates cells and causes autoimmune diseases. Therefore, whether IL-6 would augment the glycolysis pathway was investigated by stimulating Jurkat cells with or without 0, 0.2, 0.5 and 1 ng/ml IL-6/sIL-6R for 24 h prior to 48-h stimulation with or without anti-CD3/CD28. The ECAR and other related parameters were assessed in real time using the Seahorse XFe96 analyser.

Figure 1A is a representative graph of all changes in all parameters (N = 5). IL-6 (0.2 ng/ml) significantly increased the basal ECAR level by up to 70 % ($P < 0.01$) compared to the cells that did not receive IL-6/sIL-6R (0 ng/ml) and unstimulated cells (no anti-CD3/CD28). The trend was obvious at 0.5 ng/ml IL-6 but was not statistically significant, whereas 1 ng/ml IL-6 did not exert any effect (Fig. 1B). Addition of glucose and oligomycin significantly increased the glycolysis and glycolytic capacity levels by up to 65 % and 40 %, respectively ($P < 0.01$, $P < 0.05$), following 0.2 ng/ml IL-6 stimulation as compared to 0 ng/ml IL-6 and unstimulated cells (no anti-CD3/CD28) but not at 0.5 and 1 ng/ml IL6 (Fig. 1C, 1D). However, addition of 2-DG did not result in an obvious effect on the glycolytic reserve and non-glycolytic acidification rate in response to IL-6 stimulation (Fig. 1E, 1F).

IL-6/sIL-6R increases Jurkat cell ATP-linked respiration, glycolytic capacity and basal OCR but not glycolytic reserve and non-mitochondrial respiration

The mitochondrial function was assessed by investigating the OCR and other related parameters following IL-6 stimulation. OXPHOS produces approximately 15 times more ATP than glycolysis, indicating that OXPHOS is more efficient than the glycolytic pathway (du Plessis et al., 2015). Therefore, following our observation that IL-6 enhanced glycolysis, we examined mitochondrial respiration in real time using the Seahorse analyser to assess the mitochondrial function and whether IL-6 would enhance the OXPHOS pathway to generate abundant ATP. The Jurkat cells were stimulated with or without 0, 0.2, 0.5 and 1 ng/ml IL-6/sIL-6R for 24 h before 48-h stimulation with or without anti-CD3/CD28.

Figure 2A depicts the OCR and related parameters following the addition of mitochondrial inhibitor drugs (N = 5). The basal OCR, ATP production and maximal respiration levels were significantly enhanced following 0.2 ng/ml IL-6 stimulation ($P < 0.01$, $P < 0.05$, $P < 0.01$, respectively) but not 0.5 and 1 ng/ml IL-6 compared to 0 ng/ml IL-6 and unstimulated cells (no anti-CD3/CD28) (Fig. 2B–D). However, IL-6 did not affect the reserve capacity (Fig 2E). Non-mitochondrial respiration demonstrated an increased trend at 0.2 and 1 ng/ml IL-6 compared to 0 ng/ml IL-6 and unstimulated cells (no anti-CD3/CD28), but it was not statistically significant, although there was an obvious concentration progression (Fig. 2F). This result suggested that low IL-6 concentrations, but not high IL-6 concentrations, increased the OCR, which reflects OXPHOS.

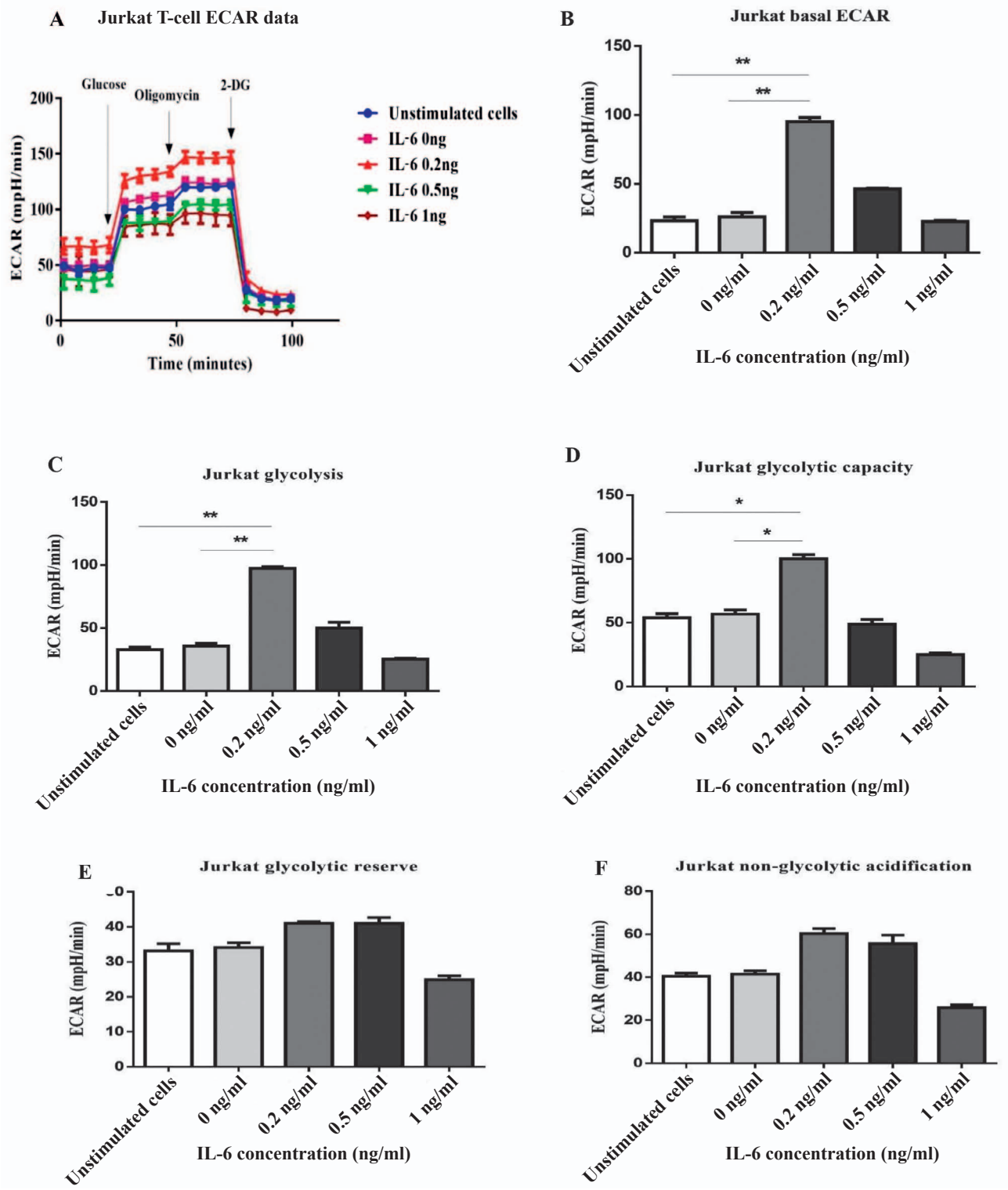

Fig. 1. IL-6 increased the Jurkat cell glycolytic metabolism. **(A)** Jurkat cells pre-treated with 0, 0.2, 0.5 and 1 ng/ml IL-6/sIL-6R and stimulated with anti-CD3/CD28 (N = 5) had elevated aerobic glycolysis compared to untreated (0 ng/ml) and unstimulated cells with anti-CD3/CD28. **(B)** Basal ECAR, **(C)** glycolysis and **(D)** glycolytic capacity were significantly increased following 0.2 ng/ml IL-6 stimulation. **(E)** Glycolytic reserve and **(F)** non-glycolytic acidification did not show any effect. Statistical analysis was tested using nonparametric ANOVA, and the Mann-Whitney test was used when comparing treated cells to untreated cells (0 ng/ml) and unstimulated cells (no anti-CD3/CD28). $^{*}P < 0.05$, $^{**}P < 0.01$. Data are the mean ± SEM of five separate experiments.

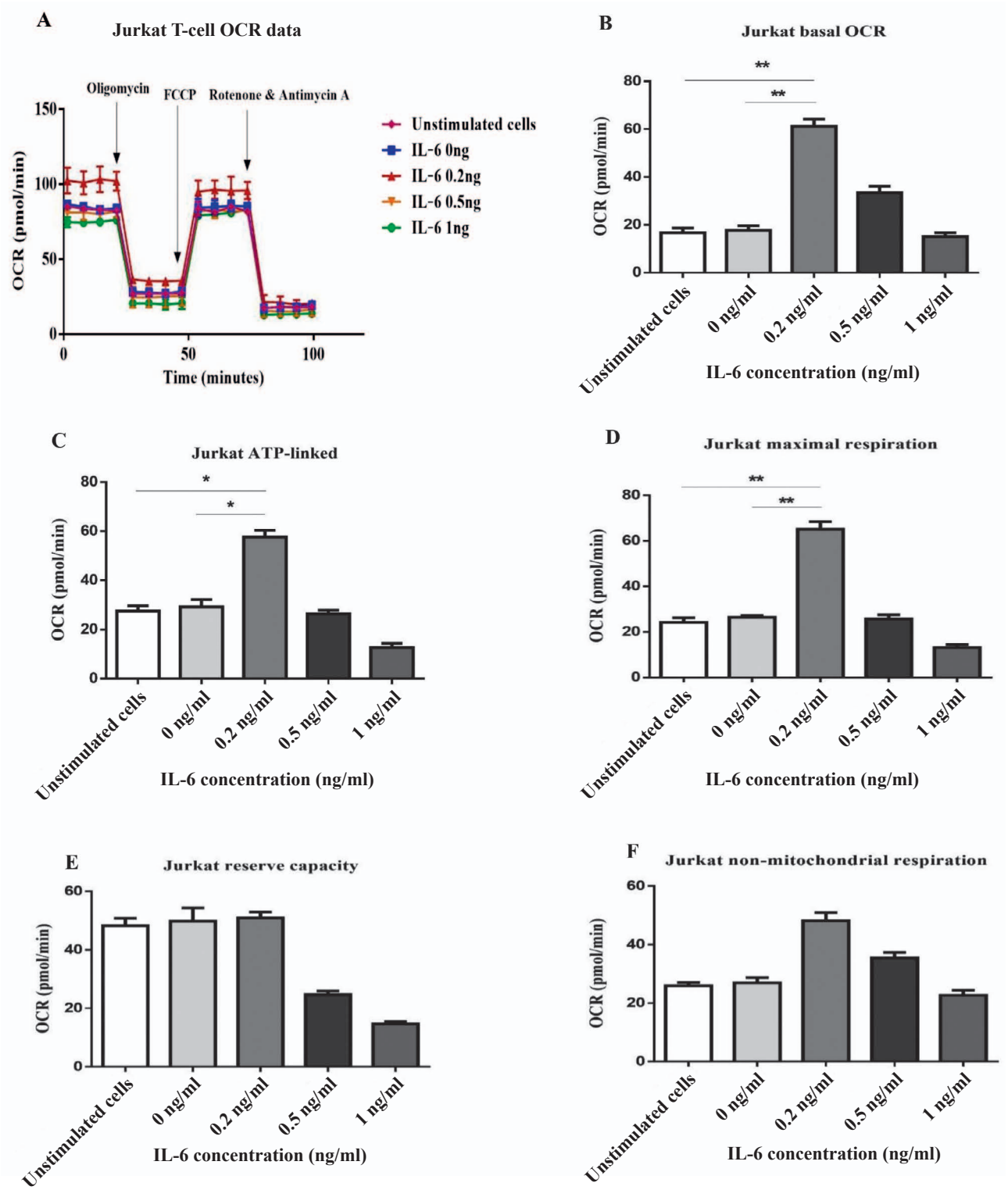

Fig. 2. IL-6 increased Jurkat cell mitochondrial respiration. **(A)** Jurkat cells pre-treated with 0, 0.2, 0.5 and 1 ng/ml IL-6/sIL-6R and stimulated with anti-CD3/CD28 (N = 5) exhibit increased mitochondrial respiration compared to untreated (0 ng/ml) and unstimulated cells with anti-CD3/CD28. **(B)** Basal OCR, **(C)** ATP production and **(D)** maximal respiration are significantly increased post-IL-6 stimulation (0.2 ng/ml). **(E)** Reserve capacity and **(F)** non-mitochondrial respiration were not affected. Statistical analysis was conducted using nonparametric ANOVA, and the Mann-Whitney test was used when comparing treated cells to untreated (0 ng/ml) cells and unstimulated cells (no anti-CD3/CD28). $^{*}P < 0.05$, $^{**}P < 0.01$. All data are the mean ± SEM of five separate experiments.

IL-6/sIL-6R induces lactate production in Jurkat cells

As lactate is a metabolism by-product of inflamed tissue, its accumulation in the tissue microenvironment triggers inflammatory diseases such as RA (Pucino et al., 2019; Khatib-Massalha et al., 2020). We determined that IL-6 stimulation significantly increased the glycolytic pathway levels. Subsequently, we investigated whether lactate would increase in response to IL-6 and support our earlier observation. Lactate production was measured in Jurkat cells (N = 4) that had been cultured for 24 h with or without IL-6 and stimulated for 48 h with or without anti-CD3/CD28. The cells exposed to 0.2 and 0.5 ng/ml IL-6 had significantly increased lactate production ($P < 0.01$) compared to 0 ng/ml and unstimulated cells (no anti-CD3/CD28) but not when exposed to high-concentration (1 ng/ml) IL-6 (Fig. 3A). This result indicated that the significantly increased lactate secretion following IL-6 stimulation could be related to significantly increased glycolysis, suggesting that lactate generated an inflammatory environment that increased the risk of inflammatory diseases.

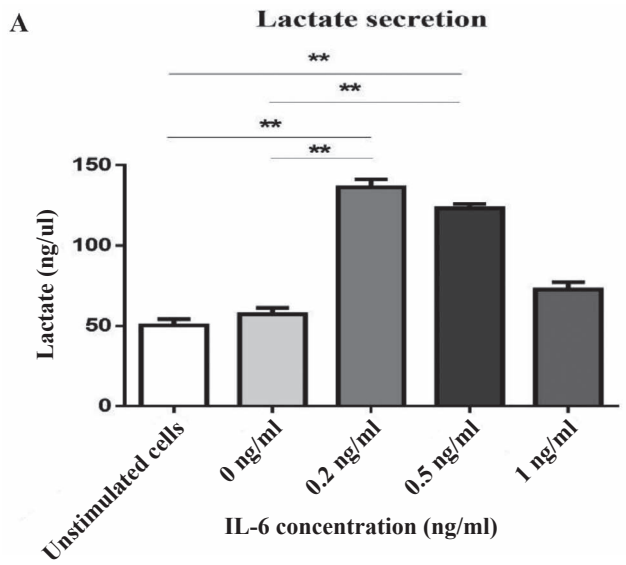

Fig. 3. IL-6 increased lactate production in Jurkat cells. **(A)** Jurkat cells pre-treated with 0, 0.2, 0.5 and 1 ng/ml IL-6/IL-6R and stimulated with anti-CD3/CD28 (N = 4) had significantly increased lactate secretion at 0.2 and 0.5 ng/ml IL-6 compared to untreated (0 ng/ml) and unstimulated cells with anti-CD3/CD28. Statistical analysis was conducted using nonparametric ANOVA, and the Mann-Whitney test was used when comparing treated cells to untreated cells (0 ng/ml) and unstimulated cells (no anti-CD3/CD28). $^{**}P < 0.01$. Data are the mean ± SEM of four separate experiments.

IL-6/sIL-6R increases Akt phosphorylation at the threonine 308 residue in Jurkat cells

We determined that IL-6 stimulation enhanced glycolysis, OXPHOS and lactate secretion. However, the mechanisms by which IL-6 perturbs the Jurkat cell energy metabolism and increases the risk of inflammatory diseases remain unclear. Therefore, we examined the energy metabolism-related signalling pathway to elucidate whether the energy metabolism modulation post-IL-6 stimulation would alter signalling in Jurkat cells. We investigated Akt at threonine 308 [Akt (T308)], which is a part of the PI3K-Akt-mTOR pathway and cell metabolism. We used Akt (T308) as it is a more reliable marker for assessing Akt phosphorylation than Akt serine 473 (Ser473) (Vincent et al., 2011). Jurkat cells were stimulated for 24 h with or without 0, 0.2, 0.5 and 1 ng/ml IL-6/sIL-6R and then stimulated for 48 h with or without anti-CD3/CD28. The Akt phosphorylation status (N = 4) was assessed using Western blotting. Akt expression and phosphorylation was significantly increased following stimulation with 0.2 ng/ml IL-6 ($P < 0.05$) but not 0.5 and 1 ng/ml IL-6 compared to 0 ng/ml and unstimulated cells (no anti-CD3/CD28) (Fig. 4A, 4B).

IL-6/sIL-6R increases mTOR phosphorylation at the Ser2448 residue in Jurkat cells

Akt directly activates mTOR by inhibiting the negative regulator of mTOR, namely tuberous sclerosis complex 2 (TSC2) (Hahn-Windgassen et al., 2005). Therefore, we next examined mTOR phosphorylation following IL-6 stimulation to investigate whether IL-6 would activate mTOR and whether Akt is involved in mTOR activation. Jurkat cells were treated with or without 0, 0.2, 0.5 and 1 ng/ml IL-6/sIL-6R for 24 h and then stimulated for 48 h with or without anti-CD3/CD28. The mTOR phosphorylation status (N = 4) was assessed using Western blotting. At 0.2 and 0.5 ng/ml, IL-6 significantly increased mTOR expression and phosphorylation ($P < 0.05$) compared to 0 ng/ml and unstimulated cells (no anti-CD3/CD28) but not 1 ng/ml IL-6 (Fig. 5A, 5B). This result suggested that the IL-6-induced metabolic process alteration affected Akt, which significantly affected mTOR activity in the Jurkat cells.

Discussion

In this study, we investigated the effect of IL-6 on the Jurkat cell metabolic activity and signalling. Jurkat cells were treated with IL-6/sIL-6R prior to stimulation with anti-CD3/CD28. We determined that IL-6 increased the glycolytic and OXPHOS pathways in the cells and significantly increased lactate secretion, which could have resulted from the increased glycolysis. IL-6 stimulation also significantly increased Akt and mTOR phosphorylation, indicating that IL-6 affected the PI3K-Akt-mTOR pathway, which is one of the main metabolism regulation pathways.

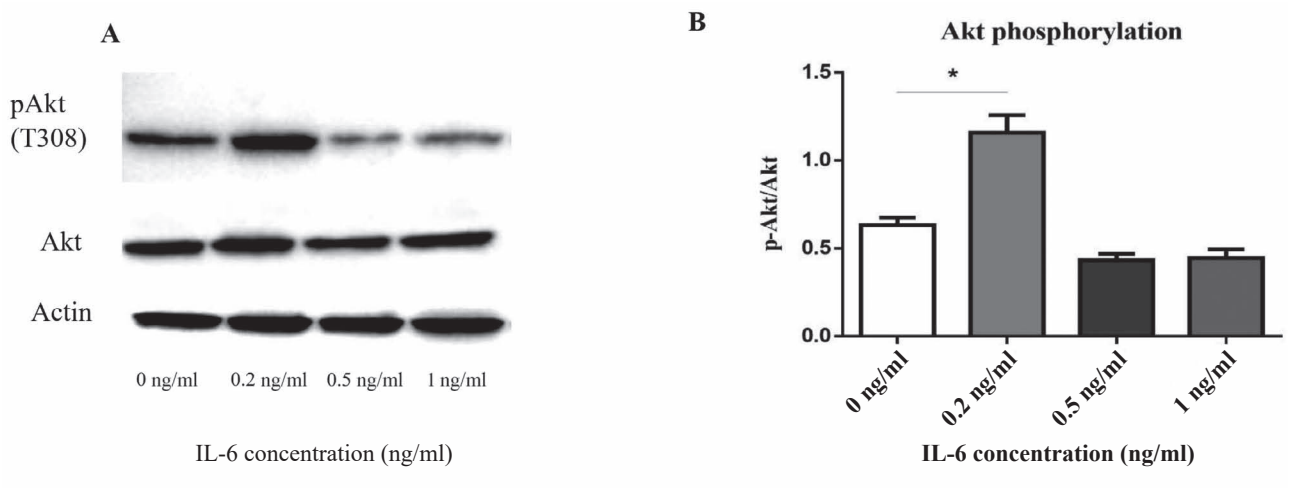

Fig. 4. IL-6 increased Akt phosphorylation in Jurkat cells. **(A** and **B)** Jurkat cells pre-treated with 0, 0.2, 0.5 and 1 ng/ml IL-6/IL-6R and stimulated with anti-CD3/CD28 (N = 4) had significantly increased Akt activity at 0.2 ng/ml IL-6 compared to untreated (0 ng/ml) and unstimulated cells with anti-CD3/CD28. Statistical analysis was conducted using nonparametric ANOVA, and the Mann-Whitney test was used when comparing treated cells to untreated cells (0 ng/ml) and unstimulated cells (no anti-CD3/CD28). $^{*}P < 0.05$. Data are the mean ± SEM of four separate experiments.

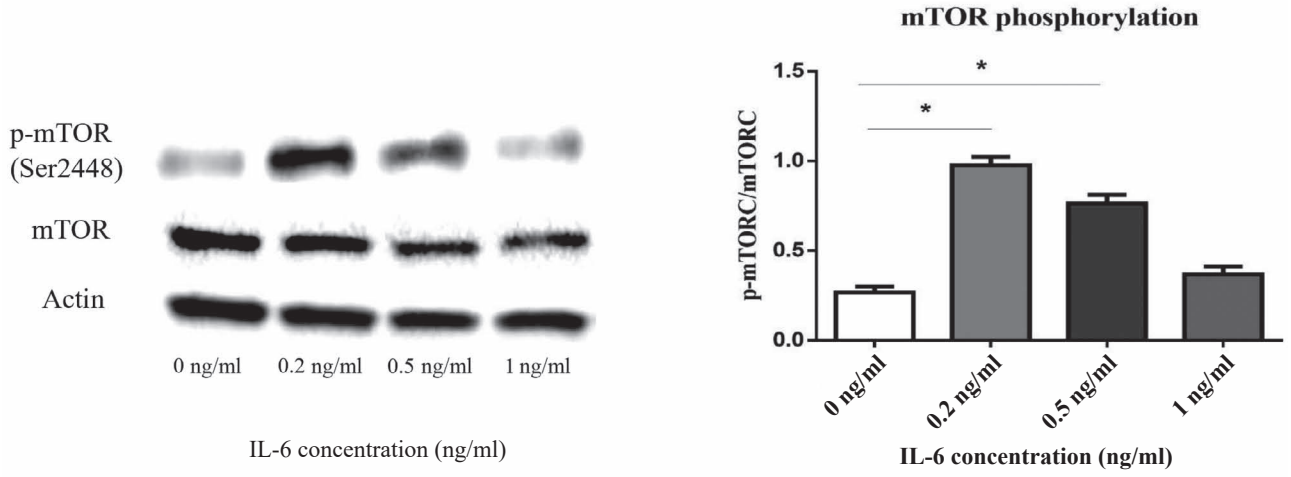

Fig. 5. IL-6 increased mTOR phosphorylation in Jurkat cells. **(A** and **B)** Jurkat cells pre-treated with 0, 0.2, 0.5 and 1 ng/ml IL-6/IL-6R and stimulated with anti-CD3/CD28 (N = 4) had significantly increased mTOR activity at 0.2 and 0.5 ng/ml IL-6 compared to untreated (0 ng/ml) and unstimulated cells with anti-CD3/CD28. Statistical analysis was conducted using nonparametric ANOVA, and the Mann-Whitney test was used when comparing treated cells to untreated cells (0 ng/ml) and unstimulated cells (no anti-CD3/CD28). $^{*}P < 0.05$. Data are the mean ± SEM of four separate experiments.

In the present study, we demonstrated that IL-6 enhanced the glycolytic pathway. However, the exact mechanism remains unclear. IL-6 directly increased glucose metabolism by increasing glucose transport and glucose oxidation in human skeletal muscles, and enhanced glycolytic enzymes, such as hexokinase 2 and 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase-3 (PFKFB3), in human cell lines and mouse fibroblasts (Glund et al., 2007; Ando et al., 2010). Furthermore, IL-6 significantly increased glucose transporter 4 (GLUT 4) translocation to the plasma membrane and basal and insulin-stimulated glucose uptake. Additionally, IL-6 rapidly enhanced AMP-activated protein kinase (AMPK), which is a master regulator of cellular and glucose metabolism, suggesting that AMPK mediates this increase in the glucose metabolism (Carey et al., 2006). However, the link between the increased metabolism due to IL-6 stimulation and inflammation ini-

tiation remains unclear. One study reported that IL-6 is involved in myasthenia gravis pathogenesis by increasing Akt phosphorylation, suggesting that IL-6 directly affects the Akt–mTOR pathway involved in regulating metabolism (Maurer et al., 2015). Furthermore, IL-6 stimulation increased Akt phosphorylation, which increased fibroblast migration during wound injury, indicating that IL-6 is involved in persistent inflammation, which impairs healing progression (Nishikai-Yan Shen et al., 2017). This PI3K-Akt-mTOR signalling activation was involved in RA development; therefore, suppressing this pathway with an anti-inflammatory agent, such as cinnamaldehyde, attenuated RA progression by decreasing IL-6 induction (Li and Wang, 2020).

We demonstrated that IL-6 successfully increased Akt and mTOR phosphorylation, which are crucial in inflammation by activating other pro-inflammatory cytokines. Many studies have proved this aspect by demonstrating that activated Akt and mTOR inhibited autophagy and participated in T-cell and fibroblast-like synoviocyte (FLS) abnormal proliferation by promoting production of cytokines, including TNF-α, IL-17 and IL-22, which are involved in RA pathogenesis (Mitra et al., 2012; Shoda et al., 2017; Dinesh and Rasool, 2018). The Akt- and mTOR-driven abnormal T-cell and FLS activation exacerbates RA and autoimmune diseases, suggesting that Akt and mTOR can be targeted for treating these diseases (Feng and Qiu, 2018; Du et al., 2019; Ba et al., 2021). Interestingly, one study used a similar treatment approach to the present study and demonstrated that recombinant IL-6/sIL-6R induced osteoclast differentiation, which resulted in bone and cartilage destruction by altering the extracellular signal-regulated kinase (ERK) and NF-κB pathways (Feng et al., 2017).

Although lactate is a dead-end waste product of glycolysis due to dysoxia, some believe that it has beneficial protective roles for the body (Berthet et al., 2009; Taher et al., 2016), while others believe that it can create inflammatory environments (Pucino et al., 2017; Zou et al., 2017). In the present study, we demonstrated that IL-6 significantly increased lactate secretion, which was accompanied by elevated glycolysis, suggesting that IL-6 drives lactate to create an inflammatory environment. Our observation was supported by a recent study that demonstrated that lactate increased fibroblast basal glycolysis, glycolytic capacity and cell migration, which was accompanied by elevated IL-6. This finding has proved that macrophages and fibroblasts contribute to RA pathogenesis in the presence of IL-6 and high lactate levels (Pucino et al., 2023). Independent of glycolysis, the accumulated extracellular lactate in T cells can enter the mitochondria and promote electron transport chain (ETC) activity, increasing oxidative phosphorylation, which stimulates ATP production by increasing respiratory substrate and pyruvate use (Cai et al., 2023). Our results support the notion that IL-6 stimulation causes extracellular and mitochondrial lactate accumulation, which supports inflammation induction due to the high metabolism profile.

In the present study, we demonstrated that IL-6 significantly enhanced OXPHOS, which could also be involved in inflammation initiation. IL-6 increased the mitochondrial fusion and OXPHOS by up-regulating mitofusin 1 (MFN1), which led to chemoresistance in acute myeloid leukaemia (AML). However, knocking down *MFN1* impaired the IL-6 effect on OXPHOS and mitochondrial function, suggesting that IL-6 facilitates mitochondrial respiration and induces AML chemoresistance (Hou et al., 2023). Furthermore, the acute exposure of skeletal muscle mitochondria to IL-6 increased the levels of OCR, mitochondrial hyperfusion and mitochondrial reactive oxygen species, which are signal transducer and activator of transcription 3 (STAT3) pathway dependent. However, addition of antioxidant MitoQ partly attenuated the STAT3 pathway. This evidence supported the interplay between IL-6 stimulation and increased mitochondrial respiration (Abid et al., 2020). Nonetheless, it should be noted that IL-6 can act as an anti-inflammatory agent; therefore, the literature may contain contradictory results. Although some studies reported that IL-6 is involved in mitochondrial metabolism as discussed earlier, others reported opposite findings. For example, IL-6 *trans*-signalling decreased OXPHOS and mitochondrial respiration, preventing mitochondrial dysfunction (Hoffman et al., 2023**)**. The conflicting IL-6 activities might depend on different cell types, the presence of other pro-inflammatory cytokines and the concentration levels (Borsini et al., 2020).

This study had limitations. First, we used 24-h incubation rather than time points to investigate the expression and phosphorylation of the targeted proteins. As several proteins can reach maximum activity early in a few hours, the phosphorylation status might have recovered after 24 h. Second, we did not target other signals involved in metabolism, including the AMPK, calcium (Ca^{2+}) and lymphocyte-specific protein tyrosine kinase (Lck) signals. Targeting these signals would strengthen our conclusion.

Conclusion

This study revealed that IL-6 significantly increased energy metabolism, including glycolysis, OXPHOS and lactate secretion, and the related signalling pathways (Akt and mTOR) in Jurkat cells. Although the exact mechanisms that drive high metabolism remain unclear, our results prove that IL-6 enhances energy metabolism by targeting Akt–mTOR signalling. This finding explains a part of the mechanism by which IL-6 participates in the development of inflammation and autoimmune diseases by increasing the immune cell metabolic profile. Therefore, future studies might be needed to provide therapeutic strategies to treat inflammatory diseases by targeting Akt-mTOR. However, this study was conducted using a cell line. Therefore, the results should be validated using primary human T cells. However, given that we investigated the effect of IL-6 on the energy metabolism and related signalling pathways for the

first time, it was reasonable to use a cell line to establish robust data and methodology before using primary human T cells.

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Conflict of interests

No potential conflict of interest was reported by the authors.

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